Hematopoietic Cell Transplant and Gene Therapy for Non-Malignant Blood Disorders Biobank Resource (the 'HOPE Resource')
Version 1.0

Study Chairpersons
Leslie Kean, M.D., Ph.D.[4]
Jim Connelly, M.D.[3]

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Protocol Team:

Shan Chandrakasan, M.D.[1]
Jane Churpek, M.D., M.S.[2]
Elizabeth Krieger, M.D.[5]
Eric Leifer, Ph.D.[12]
Troy Lund, M.D.[6]
Michael Martens, Ph.D.[10]
Ryotaro Nakamura, M.D.[7]
Mary Horowitz, M.D., M.S.[10]
Nancy DiFronzo, Ph.D.[12]
Kristin Peyton[11]
Megan Scott[11]
Mark Watson, M.D.[9]
Ashley Spahn[13]
Stephanie Waldvogel[13]
Larisa Broglie, M.D.[10]

[1]Children's Healthcare of Atlanta
[2]University of Wisconsin-Madison
[3]Vanderbilt University Medical Center
[4]Boston Children's Hospital
[5]Children's Hospital of Richmond at VCU
[6]University of Minnesota
[7]City of Hope
[9]Washington University
[10]Medical College of Wisconsin
[11]The Emmes Company
[12]National Heart, Lung, and Blood Institute
[13]NMDP

Leslie Kean
Electronically signed by: Leslie Kean
Reason: I am Approving this document
Date: Dec 27, 2025 20:08:28 EST

Approval Signature (Protocol Chair)

Electronically signed by: James Connelly
Reason: I am Approving this document
Date: Dec 23, 2025 11:08:53 CST

Approval Signature (Protocol Chair)

Electronically signed by: Larisa Broglie
Reason: I am Approving this document
Date: Dec 23, 2025 13:27:02 CST

Approval Signature (Protocol Officer)

PROTOCOL SYNOPSIS

Hematopoietic Cell Transplant and Gene Therapy for Non-Malignant Blood Disorders Biobank Resource (the 'HOPE Resource')

Co-Principal Investigators:	**Leslie Kean, M.D., PhD and Jim Connelly , M.D.**
Study Design:	This is a prospective, multicenter study that will establish a repository of biospecimens and clinical data from patients undergoing hematopoietic stem cell transplant (HCT) or gene therapy (GT) for treatment of non-malignant blood diseases. The enrollment goal is 375 participants and all consenting related donors (approximately 100 are anticipated), accrued over 4 years. Participants will be followed until the end of study, but continued collection of specimens and clinical data may continue to allow up to 15 years of follow-up for all enrolled participants if funds are available.
Eligibility Criteria:	Participants with a diagnosis of Aplastic Anemia, Hemoglobinopathies or bone marrow failure undergoing HCT or GT for management of their underlying disease.
Treatment Description:	There is no treatment intervention in this study. Treatment will be at the provider's discretion. Participants will provide blood and other biospecimens pre and post-intervention at specified intervals.
Accrual Objective:	375 participants and approximately 100 related donors will be enrolled from 35 sites
Accrual Period:	The estimated accrual period is 4 years.
Study Duration:	Participants will be followed until the end of study, at a minimum through March 30, 2031, but continued collection of specimens and clinical data may continue to allow up to 15 years of follow-up for all enrolled participants if funds are available. Additional long-term clinical data will be collected through usual procedures of the Center for International Blood and Marrow Transplant Research (CIBMTR).

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ABBREVIATION	TERM
AA	Aplastic anemia
AML	Acute myeloid leukemia
ANC	Absolute Neutrophil Count
ATG	Anti-thymocyte globulin
BioLINCC	Biologic Specimen and Data Repository Information Coordinating Center
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BMF	Bone Marrow Failure
CH	Clonal Hematopoiesis
CIBMTR	Center for International Blood and Marrow Transplant Research
CRF	Case Report Form
CSA	Cyclosporine
DLI	Donor Lymphocyte Infusion
FFPE	Formalin-fixed, Faraffin-embedded
FISH	Fluorescence in Situ Hybridization
GPI	Glycosylphosphoinositiol
GT	Gene Therapy
GVHD	Graft-versus-host disease
HbS	Hemoglobin S
HCT	Hematopoietic Stem Cell Transplant
HOPE	Hematopoietic Cell Transplant and Gene Therapy for Non-Malignant Blood Disorders Biobank Resource
HLA	Human leukocyte antigen
IBMFS	Inherited bone marrow failure syndrome
IEI	Inborn Errors of Immunity
IRB	Internal Review Board
IST	Immunosuppression
KG	Kilogram
LDH	Lactate Dehydrogenase
LN_2	Liquid Nitrogen
MDS	Myelodysplastic syndrome
MSD	Matched Sibling Donor
NIH	National Institutes of Health
NMD	Non-malignant hematologic disease
PNH	Paroxysmal nocturnal hemoglobinuria
PTCy	Post-transplant cyclophosphamide
SAA	Severe aplastic anemia
SCD	Sickle cell disease
TBD	Telomere biology disorders
TCR	T-cell Receptor
T-LGL	T-cell large granular lymphocytosis
TMA	Thrombotic Microangiopathy

CHAPTER 1

1 BACKGROUND AND RATIONALE

1.1 Introduction

While hematopoietic stem cell transplantation (HCT) offers a cure for many patients with non-malignant hematologic diseases (NMD), this treatment is associated with significant risks, leading to high rates of morbidity and mortality. In addition to HCT, Gene Therapy (GT) is now a reality for patients with hemoglobinopathies and expected to be available for other NMD in the future. As with HCT, GT is also associated with a unique set of risks, many of which are still being discovered and rigorously defined; these are likely influenced by both the disease treated with GT and the specific methodologies used for conditioning the patient and creating the gene-modified product.

The most serious complications of HCT, especially for patients transplanted for NMD, include organ failure, infections, dysfunctional immune reconstitution or graft failure, graft-versus-host disease (GVHD), and post-HCT malignancies, which can be both donor- and recipient-derived. For patients treated with GT, organ failure, infections, and malignancies (arising both from the recipient bone marrow and the gene-modified stem cell product) are the most important post-therapy risks identified to date. Although some clinical variables (e.g., recipient age, donor-recipient human leukocyte antigen [HLA] mismatch for HCT) predict higher risk of specific events (e.g., GVHD, infection, dysfunctional immune reconstitution), no diagnostic tests exist that reliably predict occurrence, severity, or response to therapy of any of the prevailing complications for either HCT or GT. Moreover, because most HCTs (and no GTs) are performed for patients with malignant diseases, targeted strategies to understand the mechanisms underlying toxicities of HCT and GT for NMD are lacking. The rarity of these diseases makes studying these mechanisms difficult at the single center level. Here we describe a novel NMD **H**emat**op**oietic Cell Transplant and G**e**ne Therapy for Non-Malignant Blood Disorders Biobank Resource (the 'HOPE Resource'), which will provide a critical bio- and data-repository to understand the biology of patients undergoing HCT and GT for NMD.

The HOPE Resource will address a major gap in the field. There are currently no multicenter biospecimen collections that contain multi-tissue or body fluid specimens with paired, detailed, rigorously reviewed clinical data that can be used to perform state-of-the-art molecular and cellular studies on patients with NMD undergoing HCT and GT. As a collaboration of the BMT CTN and the Center for International Blood and Marrow Transplantation (CIBMTR), this study fills that gap by uniformly collecting and storing high-quality critical biological specimens and clinical data from a large, prospective cohort of patients with NMD undergoing HCT and GT. The HOPE Resource will be made available to the biomedical community and is expected to facilitate high quality research.

The goals of the HOPE Resource are:

- To facilitate studies that will establish mechanistic insights and biologic correlates to the key causes of failure of HCT and GT for NMD – graft rejection, infection, organ toxicity, post-treatment malignancies, delayed immune reconstitution, GVHD, and death.

- To facilitate mechanistic insights into the biologic causes of NMD that ultimately require HCT or GT.

- To identify new therapeutic targets for NMD and their HCT- or GT-related complications leading to development of more targeted and effective therapies.

To achieve these goals, patients and their related donors (for HCT) will be recruited, and consent obtained prior to start of conditioning, to contribute samples and data to the HOPE Resource. Samples and data will be collected: 1. from patients and related donors at baseline before HCT and GT; 2. from patients after HCT and GT at scheduled timepoints; and 3. from patients at the time of key post-HCT or post-GT events (detailed in disease-specific chapters, below).

1.2 Study Rationale

Various NMDs can be treated with HCT including aplastic anemia, hemoglobinopathies, inherited bone marrow failure syndromes (IBMFS), inborn errors of immunity (IEI), and metabolic diseases. In 2022, 1076 HCTs were performed in the United States for a NMD, accounting for about 6% and 53% of adult and pediatric allogeneic HCT, respectively. The most common non-malignant indications for HCT are aplastic anemia (44%) and hemoglobinopathies (19%), which will comprise the majority of cases in this biorepository[1]. HCTs for aplastic anemia and hemoglobinopathies share common and poorly understood complications such as high rates of graft failure, disrupted immune reconstitution, and clonal hematopoiesis (CH). Inclusion of both disease categories offers the opportunity to study these complications within each disease and across disease categories to better understand common and disorder-specific contributions to these morbidities. Since most studies using the HOPE resource will likely focus on these two groups of disorders, they are discussed in detail below. However, the protocol will also include specimens and data from patients with IBMFSs and iIEIundergoing HCT or GT for BMF. Though the numbers will be smaller, they will constitute a unique resource for investigators focused on these very rare diseases.

1.3 Aplastic Anemia (AA)

1.3.1 Overview

Aplastic anemia (AA) is characterized by severe peripheral blood pancytopenia resulting from decreased bone marrow production. Without definitive treatment, mortality from AA approaches 70% at 2 years. The underlying etiology of decreased bone marrow production is thought to be due to an acquired T cell-mediated destruction of hematopoietic progenitor cells[2]. At the time of diagnosis, it is critical to differentiate patients with severe (SAA) from those presenting in a similar fashionwith decreased bone marrow production due to an underlying IBMFS or IEI as these conditions are treated differently[3,4,5]. Patients with SAA are treated with either front-line HCT or immunosuppression (IST) depending on their age, comorbidities, and available donors. Historically, IST using anti-thymocyte globulin (ATG) in combination with cyclosporine (CSA), was used for patients with SAA who are older, infirm, or who lack a suitable donor, with HCT reserved for younger patients with HLA-matched sibling donors (MSD). The addition of eltrombopag, a thrombopoeitin receptor agonist, improved hematologic responses[7,8]. However, failure-free survival (survival without relapse or secondary clonal disease) after IST is less than 50%[9].

Recent improvements in conditioning regimens, graft-versus-host disease (GVHD) prophylaxis, and supportive care now allow wider access to HCT, which is increasingly offered for SAA patients without MSDs based on successful results using alternative donors (matched or mismatched unrelated donors, haploidentical donors) and to older patients [5,6,10,11]. However, HCT is associated with significant risks, including acute and chronic GVHD, graft failure, infections, regimen-related toxicities, and late complications including post-transplant malignancy[12]. Therefore, further research efforts are needed to understand which patients are at increased risk for these toxicities as well as their underlying mechanisms to achieve optimal outcomes in patients with SAA.

1.3.2 Current Understanding of the Pathophysiology of AA

AA is associated with a profound deficit in hematopoietic stem and progenitor cells primarily due to T cell-mediated suppression[13]. Studies of patients with AA demonstrate clonally or oligoclonally expanded cytotoxic T cells, which are phenotypically activated, skewed towards Th1 cytokines, and capable of inducing apoptosis via Fas/FasL[14-19]. Somatic mutations involving *STAT3,* which can result in constitutively activated T cells, have been implicated in immune pathogenesis of AA, similar to T-cell large granular lymphocytosis (T-LGL).[17] Regulatory T cells are decreased in patients with AA and may increase with a response to IST[21-23].

As further evidence of immune-mediated pathogenesis, AA is associated with specific HLA, namely, serologic HLA DR2, subsequently resolved as HLA DR15 (or HLA DR16) by higher-resolution HLA typing.[24,25,26]

Recent studies identify acquired copy number neutral loss of heterozygosity on the short arm of chromosome 6 (6p CN-LOH) as a recurrent clonal abnormality uniquely present in AA[27,28]. These findings support the possibility of escape hematopoiesis that survives the autoimmune insult by genetically deleting the relevant HLA species that are required for antigen presentation. Immune escape has also been hypothesized to explain the frequent finding of clonal expansion of cells deficient in glycosylphosphoinositiol (GPI)-anchored proteins in AA, similar to that seen at much higher levels in classical paroxysmal nocturnal hemoglobinuria (PNH) [29]. The GPI anchor itself has been suggested to be a target of the immune response.[27]

1.3.3 Clonal Hematopoiesis (CH)

Patients with AA are at risk for progression to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Among patients receiving IST, progression to MDS or AML occurs in about 13% of patients. Acquired genetic variants, including large chromosomal events such as deletion of all or part of chromosome 7 as well as single nucleotide or small insertion/deletion events, are often identified at the time of progression[30]. However, some of these same acquired genetic variants are found at the time of AA diagnosis or during treatment and do not necessarily indicate an MDS diagnosis or alter outcomes to therapy[31]. In fact, with next generation sequencing, CH is observed in about 1/3 of patients with AA[35]. Although the genes involved overlap with those seen in MDS and AML, in AA, a more limited set of genes (*DNMT3A, ASXL1,* and *BCOR*) is involved and the clone size is generally smaller[36,37]. Individual studies suggest that mutations of specific genes may be associated with AA outcomes (e.g., B*COR* and *PIGA*: favorable prognosis, *DNMT3A* and *ASXL1*: unfavorable prognosis)[36]. However, findings to date are not definitive and their relative roles with IST versus HCT are unknown. Thus, at present, which single or combination of acquired alterations accurately predict outcomes remains an area of active research. Biologically, the underlying immune destruction creates hematopoietic stress,

increasing cell cycling of remaining hematopoietic stem cells and facilitating selection of clones with a proliferative, immune escape, or other advantage. The observations that similar chromosome abnormalities occur in both AA and IBMFS suggest that the marrow failure environment itself may predispose to their selection[33,34]. Studies in IBMFS have begun to elucidate the biological underpinnings and specific advantages that individual acquired mutations confer. Notably, the patterns and types of genetic variants are often specific to the individual IBMFS context, revealing unique mechanisms to bypass the underlying cause leading to bone marrow failure. These data suggest that similar, context specific mechanisms yet to be elucidated may operate in AA as well.

1.3.4 Telomeres

Telomere shortening or dysfunction may also contribute to both acquired and inherited forms of bone marrow failure. In the germline setting, telomere maintenance defects cause telomere biology disorders (TBD), a spectrum of conditions falling within IBMFS and in which individuals are at risk for bone marrow failure and other organ dysfunction. In AA, telomere length may also be decreased due to increased mitotic demand on a limited pool of stem cells[38]. Telomere length at diagnosis has correlated with outcomes including response to immunosuppression and evolution to MDS and AML[39-42]. Accelerated telomere attrition has been observed to precede progression to monosomy 7[42]. Lastly, both donor lymphocyte and recipient short telomere lengths are associated with poorer outcomes after HCT[43-45]. Given the lack of uniform germline genetic testing for TBD and difficulty in diagnosing TBD in adults especially, it remains to be determined whether these adverse outcomes are driven by a subset of patients with undiagnosed TBD or are due to telomere shortening or dysfunction from cell turnover or other mechanisms.

Beyond TBD, numerous other IBMFS and, more recently, IEI have been identified among series of patients thought to have sporadic AA[46-48]. Although multigene germline genetic testing is not yet standard for all patients presenting with bone marrow failure or thought to have AA, multiple guidelines recommend evaluating for clinical signs and symptoms of these disorders and pursuing laboratory-based screening including telomere length measurement by Flow FISH and chromosome breakage analysis for TBD and Fanconi anemia, respectively[46-48]. Given the lack of germline tissue banks for large numbers of unselected patients with AA and lack of uniform germline testing at diagnosis, the prevalence of each IBMFS or IEI in patients thought to have sporadic AA remains to be defined but undoubtedly could greatly contribute to our understanding of the biology and differences in toxicities and outcomes.

1.3.5 Graft Failure and GVHD in AA

The autoimmune attack on the bone marrow in AA creates an inflammatory milieu that may impact stable donor hematopoietic stem cell homing to and engraftment in the bone marrow niche. This may manifest as acute immunologic graft rejection or poor donor cell engraftment leading to poor graft function or secondary graft failure. The result is cytopenias due to impaired hematopoiesis. Graft failure occurs in approximately 10-15% of HCTs for AA. This often necessitates additional therapy with either an infusion of donor lymphocytes (DLI), a second HCT, or IST to prevent potentially lethal complications of prolonged cytopenia.

Historically, HCT was primarily offered in the setting of an MSD due to the low rates of GVHD. With MSD, the cumulative incidence of GVHD is about 2% at 5 years for acute and 6% at 5 years for chronic GVHD[49]. Recent advances in alternative donor HCT have broadened the donor pool and increased availability of this treatment option for patients with AA, especially the use of post-transplant cyclophosphamide (PTCy) for GVHD prophylaxis in the unrelated and haploidentical related donor setting. With PTCy, recent reports show acute GVHD rates of approximately 10%

for both unrelated donors and haploidentical donors. Similarly, chronic GVHD rates are low, 20% or less with both donor sources, when PTCy is used[50].

Other post-transplant complications of particular interest in AA include infection, impaired immune reconstitution, organ toxicity, CH, and posttransplant malignancies. Although the incidence and prevalence of these post-HCT outcomes have been reported in HCT, much remains to be understood about pathogenesis, prevention and biomarkers for prediction.

1.3.6 Rationale for AA biorepository

The current protocol will establish a unique and annotated biorepository from unselected patients with AA undergoing allogeneic HCT. It will include sample types not included in currently available biobanks, particularly non-blood-based germline tissues from both recipient and related donors to aid in germline genetic and CH studies and stool samples for microbiome investigations. Both calendar-based longitudinal samples and event-based samples will allow investigators to conduct translational correlative biomarker/immunobiology/mechanistic studies to better understand these clinical events post-HCT. The HOPE resource has the potential to aid AA research in the following ways:

1. Role of Germline Genetics: The non-blood-based germline samples collected in this protocol are a rare and unique aspect that will afford investigators the opportunity to address key questions that are unanswered to date, including, but not limited to:

a. Defining the proportion of sporadic AA cases due to an inherited disorder across the age spectrum as well as unique toxicities or outcomes post-HCT in these subsets.

b. Determining if peripheral blood or a non-blood germline tissue is adequate for diagnosis of inherited syndromes in patients presenting with AA.

c. Discovering new IBMFS and IEI.

d. Identifying constitutional genetic polymorphisms that may impact disease outcomes such as genes associated with T-cell activity and regulation and drug metabolism.

2. Understanding AA pathogenesis: The combination of non-blood-based germline samples, germline and donor blood samples, as well as longitudinal samples will be a valuable resource to:

a. Determine specific cell types and epitopes targeted by immune cells leading to marrow failure.

b. Determine how and why specific acquired genetic variants are selected for and whether these are adaptive, helping to improve marrow function, or maladaptive, improving marrow function but increasing risk of progression to MDS/AML.

c. Test which single or combinations of acquired genetic variation impact specific transplant outcomes.

d. Identify immune phenotypes associated with AA and persistence or elimination of such phenotypes post-HCT and correlation with HCT outcomes.

3. Identifying biomarkers or inflammatory signatures that predict graft failure: Serial testing at defined timepoints post-therapy will provide the opportunity to identify novel biomarkers or define an inflammatory signature that predicts graft failure.

4. Determining dynamics and predictors of normal versus delayed immune reconstitution: Serial testing at defined timepoints post-therapy can create a longitudinal account of immunologic recovery that can be correlated with infectious complications and facilitate development of clinical tests to detect abnormalities of immune reconstitution for early interventions.

5. Delineating donor versus recipient cell roles in the etiology of MDS/AML or malignancies post therapy: Having both donor and recipient samples, including non-blood germline samples, will allow investigator to characterize the timing and cell-of-origin for acquired clonal diseases post-therapy.

1.4 Hemoglobinopathy

1.4.1 Overview

Hemoglobinopathies, including sickle cell disease (SCD) and beta-thalassemia, affect millions of individuals worldwide and lead to considerable morbidity and a diminished quality of life[51]. Recent advancements in cellular therapies, including HCT and GT, offer promising potential for cures[52]. However, these therapies also present unique risks and complications that require ongoing research and monitoring to fully understand their long-term impacts and benefits[53].

Historically, MSD transplants have been the standard of care for young patients with hemoglobinopathies. Unfortunately, only about 10% of patients with these disorders can access an unaffected HLA-matched sibling[54-56]. Consequently, alternative donor transplants are increasingly being explored[57]. Ongoing clinical trials are investigating graft manipulation techniques to prevent GVHD and graft failure and refining conditioning regimens to improve patient outcomes[58-60]. Gene therapy has also emerged as a revolutionary option[52,61]. Innovations in *ex vivo* autologous gene editing, using lentiviral vectors and CRISPR-based methods, led to the FDA approval of two curative therapies, with several other products progressing through clinical trials at the time of this protocol development[52,61].

1.4.2 Current Understanding of the Pathophysiology of Hemoglobinopathies

Hemoglobinopathies are a group of inherited disorders characterized by either structural abnormalities or impaired production of hemoglobin. The two most common hemoglobinopathies to receive cellular therapy are SCD and transfusion dependent beta thalassemia. In SCD, a point mutation in the β-globin gene leads to the production of hemoglobin S (HbS). Under deoxygenated conditions, HbS polymerizes, causing red blood cells to assume a rigid, sickled shape. These misshapen cells are prone to hemolysis and have impaired deformability, leading to vaso-occlusion, tissue ischemia, and chronic inflammation. Repeated cycles of sickling and unsickling cause irreversible membrane damage and contribute to acute and chronic complications, including pain crises, stroke, infection, and end-organ damage[62]. These complications of sickle cell disease contribute to a reduced life expectancy 20 years below the general population, despite advances in supportive care[63].

Beta thalassemia results from mutations in the β-globin gene that reduce or eliminate β-globin chain production, leading to an imbalance in globin chain synthesis. The relative excess of α-

globin chains precipitates within erythroid precursors, causing ineffective erythropoiesis and apoptosis of developing red blood cells. This ineffective erythropoiesis, and increased hemolysis, lead to chronic anemia and compensatory bone marrow expansion. Patients with beta thalassemia may develop extramedullary hematopoiesis, causing skeletal deformities without sufficient transfusion support as well as iron overload due to frequent transfusions and increased intestinal iron absorption[64]. The resulting iron overload in vital organs such as the liver, heart, and endocrine glands, increases the risk of life-threatening complications if not adequately managed with chelation therapy. Although advances in treatment and supportive care have improved outcomes for patients with transfusion dependent β-thalassemia, life expectancy remains lower than the general population and the lifelong need for transfusions and iron chelation can significantly impact health-related quality of life[65].

1.4.3 Limitations to the Success of cellular therapy in Hemoglobinopathies

Success of HCT/GT in patients with hemoglobinopathies is often limited by abnormal immune reconstitution (leading to infection, graft failure/rejection and GVHD) and concern for secondary malignancies. The successful implementation and risk stratification of treatment modalities depend on our understanding of patient responses and potential complications, most notably delayed or abnormal immune reconstitution, graft failure, and GVHD.

1.4.4 Graft Failure

In efforts to reduce transplant-related mortality (TRM) in patients with SCD, non-myeloablative conditioning regimens are increasingly utilized due to their lower toxicity profiles. In a recent multicenter collaborative study of adult and pediatric patients with SCD undergoing haploidentical HCT, a total of 70 participants were evaluable. Graft failure occurred in 8 of the 70 participants (11.4%), all of whom were under 18 years of age, corresponding to a graft failure rate of 25% in the pediatric cohort[60]. Further, data from a large CIBMTR analysis show that the use of non-myeloablative conditioning is significantly associated with a higher risk of late graft failure compared to myeloablative conditioning (OR 2.3, p=0.025). This highlights a potential trade-off between reducing transplant toxicity and maintaining long-term graft durability, underscoring the need for optimized conditioning strategies that balance safety and sustained engraftment[66].

1.4.5 Immune Reconstitution

Immune reconstitution after HCT varies significantly between malignant and non-malignant conditions. Patients with SCD often experience delayed or abnormal immune recovery, which can negatively impact their overall immunity, increasing infection risk and reducing response to vital vaccinations[67,68]. Most SCD patients have low or absent splenic function post-HCT and the impact of hyposplenism on humoral function is poorly understood. Conducting systematic analyses of immune cell subsets and functional assays is crucial to identify the factors influencing immune reconstitution, infection risk, and susceptibility to GVHD.

1.4.6 Post-Therapy Malignancies and Clonal Hematopoiesis (CH)

The risk of CHand subsequent malignancies is an area of growing concern for hemoglobinopathy patients undergoing cellular therapies. The presence of CH, originating from clones existing at levels below typical detection thresholds in either donor or recipient populations, is associated with an increased risk of MDS or AML after HCT. Exposure to chemotherapy significantly increases the incidence of CH, with prevalence rates estimated between 25% and 30%. In

patients with SCD, CH tends to occur at a younger age, likely due to increased cell turnover, the specific bone marrow microenvironment, and chronic inflammation[69]. Cases of AML and MDS are reported following both GT and HCT for SCD, particularly in patients experiencing graft failure or mixed chimerism. Currently, both HCT and GT require chemotherapy conditioning, which may further elevate the risk of these complications[70]. This crucial aspect of health for hemoglobinopathy patients has not been thoroughly studied.

1.4.7 Rationale for biorepository

Patients with hemoglobinopathies have valid concerns that influence their decisions regarding curative therapies. A survey of adults with SCD revealed that most would be open to considering a curative option like HCT, despite non-relapse mortality and graft failure rates exceeding 10%. However, the majority viewed chronic GVHD and infertility as unacceptable consequences[71]. Currently, the rates and risks of infertility related to HCT and GT for hemoglobinopathies are not well studied[53]. Additional prognostic factors and biomarkers that can better predict risk of complications in this unique population are needed to help guide patients and families in considering treatment options.

To address these complex challenges, the HOPE Resource aims to fill a significant void in the field by establishing a multicenter biospecimen collection that integrates a large volume of high-quality biological specimens and meticulously reviewed clinical data in NMDs including hemoglobinopathy patients. This coordinated effort will allow for state-of-the-art molecular and cellular studies, helping to identify biologic correlates associated with treatment complications and ultimately improving risk stratification and therapeutic interventions. The HOPE resource has the potential to aid SCD and thalassemia research in the following ways:

1. Role of germline genetics and CH: The collection of donor and recipient pretreatment blood samples as well as non-blood germline tissues may be instrumental in:

    a. Evaluating the presence of donor- or recipient-derived CH, and its potential role in the development of post-HCT or GT-related myeloid malignancies, particularly in patients with mixed chimerism or graft failure.

    b. Exploring whether germline factors influence the risk of treatment-related toxicities such as infertility or malignancies post-therapy, which remain underexplored in this population.

2. Understanding the pathophysiology of treatment outcomes:

    a. Identifying immune cell subsets and molecular pathways responsible for delayed or abnormal immune reconstitution in hemoglobinopathy HCT and GT.

    b. Clarifying the relationship between immune recovery, susceptibility to infection, and the risk of GVHD in patients with hemoglobinopathies, who may have unique baseline inflammatory or marrow microenvironmental features.

    c. Exploring the impact of mixed chimerism on immune function post-therapy.

3. Identifying biomarkers predictive of graft failure, GVHD, and other complications:

    a. Discovery of predictive biomarkers for graft failure and GVHD, critical events that currently limit the success of HCT in patient with hemoglobinopathies.

b. Characterization of inflammatory or cytokine signatures associated with graft failure or graft loss, which may be targets for early intervention or novel prophylaxis.

4. Describing the dynamics and predictors of immune reconstitution:

a. Serial immunophenotyping and functional assays will enable correlation of immune reconstitution with infection risk, vaccine response, and long-term immune competency.

b. Development of prognostic models for delayed or abnormal immune recovery to guide personalized supportive care and vaccine strategies.

5. Long-term surveillance:

a. Studies on how chemotherapy-based conditioning, chronic inflammation, and previous high erythroid turnover contribute to clonal selection and malignant transformation.

b. Understanding the molecular and cellular mechanisms that lead to adverse late effects, such as infertility, or organ toxicity.

CHAPTER 2

2 STUDY DESIGN

2.1 Study Overview

This is a prospective, multicenter study that will establish a repository of biospecimens and clinical data from patients undergoing HCT or GT for treatment of NMD. The enrollment goal is 375 participants and all consenting related donors (approximately 100 are anticipated), accrued over 4 years. HCT and GT participants will be followed until the end of study, at a minimum through March 30, 2031, but continued collection of specimens and clinical data may continue to allow up to 15 years of follow-up for all enrolled participants if funds are available.

2.2 Recipient Inclusion/Exclusion Criteria

2.2.1 HCT and GT Recipient Inclusion Criteria

1. Patients with a diagnosis of Aplastic Anemia (AA), hemoglobinopathies or bone marrow failure from other causes except for malignant diseases will be eligible for enrollment on this protocol:
    a. AA will be defined as having peripheral blood cytopenias with a hypocellular bone marrow for age and a clinical diagnosis of aplastic anemia as determined by their treating physicians.
    b. Hemoglobinopathies include sickle cell disease or thalassemia. Patients receiving potentially curative therapy with HCT or GT for hemoglobinopathies will be eligible for this study.
    c. Individuals with bone marrow failure due to clinical or molecularly diagnosed iIBMFS, IEI or other cause will be eligible for the study.
2. Patients must receive an allogeneic HCT or GT for management of their underlying disease. Allogeneic transplants including all conditioning regimens, donors, and GVHD prophylaxis regimens are eligible. This study does not define how the transplant or transplant-supportive care will be performed.
3. Patients or their legal guardian must consent to participate in the CIBMTR “Protocol for a Research Database for Hematopoietic Cell Transplantation and Marrow Toxic Injuries” (NCT 1166009) to allow linkage with the longitudinal clinical data collected by CIBMTR.
4. All ages, minorities, sexes and genders are eligible for the study, but participants must weigh at least 10 kilograms (kg) at the time of study enrollment given the volume and number of blood draws required.
5. All participants or a parent/legal guardian must sign an informed consent for this study. If there are questions regarding a patient’s eligibility for the study, contact the Protocol Team for review and discussion by emailing bmtctn2402@emmes.com.

2.2.2 HCT and GT Recipient Exclusion Criteria

1. Patients with aplastic anemia or hemoglobinopathies who are not pursuing allogeneic HCT or GT.
2. Active malignancy.
3. Hematologic malignancy or therapy for a prior hematologic malignancy in the previous 5 years.
4. Weight < 10.0 kg at time of study enrollment.
5. Prior autologous or allogeneic transplant.

2.3 Related Donor Inclusion/Exclusion Criteria

2.3.1 HCT Related Donor Inclusion Criteria

- All related donors for eligible recipients undergoing allogeneic HCT for AA, hemoglobinopathies, or bone marrow failure as defined in the recipient eligibility criteria above are eligible.
- All ages, minorities, sexes and genders are eligible for the study, but donors must weigh at least 10 kilograms (kg) at the time of study enrollment given the volume and number of blood draws required.
- All donors or a parent/legal guardian must sign an informed consent for this study. If there are questions regarding a donor's eligibility for the study, contact the Protocol Team for review and discussion by emailing bmtctn2402@emmes.com.
- All related donors must be evaluated, consented, and research sample collected at an IRB-approved protocol site.

  Note: HCT recipient participants will remain eligible if the related donor declines or is ineligible to participate in the study.

2.3.2 HCT Related Donor Exclusion Criteria

- Donor weight < 10.0 kg at time of study enrollment

CHAPTER 3

3 THE HOPE RESOURCE OUTCOMES AND DATA COLLECTION

The study is designed to build a biorepository of participant and donor samples, capturing longitudinal clinical data and specimens at specified timepoints as well at the time of specific events (see below). The timing and type of data and specimens collected will allow novel research to be performed and clinical questions to be addressed, as noted above. Although no pre-defined analyses are planned as part of this biorepository, the protocol aims to collect the appropriate samples (timing and power) to address the post-treatment complications described below.

Clinical data collection will focus on fields necessary for rigorous scientific study of the described endpoints. All participants will have data collected on the standard comprehensive report forms (CRFs) of the CIBMTR and additional supplemental data through the Advantage eClinical Electronic Data Capture System (eClinical). For the first 100 days post-therapy, highly detailed data are collected. After day 365, data are collected annually. Initial plans are for the study to end at the completion of the current BMT CTN grant cycle (August 31, 2031) but continued collection of specimens and clinical data may continue to allow additional follow-up if funds are available.

Additional samples are collected at the time of graft failure, GVHD or TMA diagnosis, donor lymphocyte infusion, or post-therapy malignancy diagnosis. The definitions of outcomes and “events” which require additional data and/or sample collection are noted below:

3.1 Graft Failure, Poor Graft Function and Donor Cell Infusions

Graft failure will be determined by the treating physician. Common definitions of graft failure include failure to recover neutrophils (persistent absolute neutrophil count (ANC) < 500/mm^3), irreversible decline (without additional cell infusions) to ANC < 500/mm^3 following an initial recovery, or less than 5% donor cells identified by standard institutional chimerism analysis (Lineage-specific chimerism [split or sorted chimerism] is recommended if available, including T cells and myeloid).[81,82] Graft failure should not be declared prior to Day 28 for marrow or peripheral blood transplants or prior to Day 42 for cord blood transplants. An exception to this rule is if the treating center determines graft failure occurred earlier than these timepoints AND additional cells are infused (i.e., subsequent HCT). Late graft failure (neutropenia or loss of donor cells following initial engraftment) will be determined by the treating physician and may be associated with decline in donor chimerism or recurrence of the underlying disease phenotype (autologous recovery), irrespective of the participant's ANC.

Poor graft function suggestive of inadequate donor hematopoiesis to support sufficient production of blood cells or reverse the underlying disease phenotype, without meeting the defining of graft failure, may be diagnosed by the treating physician and will be recorded.

Participants who receive a donor lymphocyte infusion or donor CD34+ cell infusion to enhance engraftment, improve hematopoiesis, or for management of graft rejection will be considered to have graft failure even if other criteria are not met.

GT failure will be determined by the treating physician. Common definitions of GT failure include inadequate engraftment of genetically engineered hematopoietic cells to reverse the disease phenotype, decline of gene-corrected hematopoietic cells resulting in recurrence of disease phenotype, or poor delivery or activation of the gene therapy to correct the disease phenotype.

3.2 Acute GVHD

The date of onset, severity, target organ involvement, treatment, and evolution over time of acute GVHD will be collected through calendar-based and 'for cause' samples and data forms for HCT participants. Acute GVHD will be graded based on the criteria published by Przepiorka et al., *Bone Marrow Transplant* 1995; 15(6):825-8 (Appendix D). For each assessment period all medications given for GVHD prevention and/or treatment are collected.

3.3 Chronic GVHD

The date of onset, severity, target organ involvement, and evolution of chronic GVHD will be collected for HCT participants. Chronic GVHD will be graded according to the NIH Consensus Criteria as described in the CIBMTR Manual (Appendix D).

3.4 Cause of Death

Post-therapy complications that can possibly result in mortality include but are not limited to GVHD, infection, graft failure, post-therapy malignancy, and organ toxicity.

3.5 Organ Toxicity

Assessment of clinical organ toxicity will be collected using the CIBMTR CRFs which are routinely collected 100 days, 6 months, and yearly (through year 6) after cellular therapy (HCT or GT). Forms collect whether an organ toxicity ever occurred, the date of onset, and the date of resolution as well as late effects data. A summary of clinical data collected on the CRF forms can be found in Appendix C.

3.6 Thrombotic Microangiopathy (TMA)

TMA is defined per physician discretion. Suggested diagnostic criteria from the CIBMTR Manual include:

- Lactate Dehydrogenase (LDH) greater than the center-specific upper limit of normal
- Serum creatinine > 2 mg/dL or >50% rise over baseline
- Bilirubin greater than twice the center-specific upper limit of normal

3.7 Renal Dysfunction

Renal dysfunction is considered as either a doubling of serum creatinine from baseline (where baseline is the serum creatinine before hydration and conditioning) or 50% decrease in creatinine clearance from baseline.

3.8 Recurrent or Persistent Disease

Recurrence or persistence of the disease for which HCT or GT is administered will be tracked.

3.9 Post-HCT or GT Malignancies

Any malignancy, myelodysplastic, myeloproliferative or lymphoproliferative disorder or clonal cytogenetic abnormality arising after HCT or GT will be reported, including, for HCT, whether the cells are donor or recipient in origin if known.

CHAPTER 4

4 PATIENT EVALUATION

A summary of data collection (clinical and biospecimens) is noted in Table 1.

4.1 Pre-HCT/GT Evaluations

Patients will be registered using the Advantage eClinical Electronic Data Capture System (eClinical). After a participant is consented for the study, an authorized user at the transplant center enters the patient demographics, consent date, and proposed start date for infusion into eClinical.

A planned infusion date will be provided on the baseline form. Baseline specimens should be collected no more than fourteen days prior to initiation of the conditioning regimen therapy. For this study, the conditioning regimen is defined as the combination of treatment agents given just prior to the hematopoietic cell infusion with the purpose of providing sufficient myelosuppression and for HCT, immunosuppression, to ensure adequate engraftment of the infused cells. The actual infusion date will be captured on the CIBMTR infusion form and the Infusion Day 0 form in eClinical which will dictate when post-therapy samples and clinical data are collected. If an eligible participant enrolls but then an HCT or GT is not completed or pursued following enrollment for any reason, this participant will be withdrawn in eClinical and will have no additional data or samples collected. If baseline samples were already collected, these will be stored in the HOPE resource and utilized for studies where appropriate.

Required pre-therapy data and specimens should not be collected sooner than 14 days prior to the start of conditioning regimen.

1. A specimen collection form will be collected pre-conditioning and pre-infusion (Day 0)
2. Specimen Collection:
    a. Blood, stool, germline, and bone marrow (only expected if collected as part of standard of care) samples will be collected pre-conditioning
    b. Blood will be collected pre-infusion (Day 0)
3. A CBC will be collected pre-conditioning
4. Infection and medication data will be collected pre-conditioning and pre-infusion (Day 0)

4.2 Post-Infusion Evaluations

1. For all enrolled HCT and GT participants, the empty bag or syringe in which the stem cell product was infused will be retrieved by the study team and sent to the BMT CTN Repository refrigerated where it will be washed to obtain donor cells for analysis.
2. A toxicity form will be collected on days 14, 28, 60, 100, 180, 365, and then annually.
3. The specimen collection form will be collected on days 7, 14, 28, 60, 100, 180, 365, and then annually.
4. Specimen collection
    a. Blood/CBC will be collected on days 7, 14, 28, 60, 100, 180, 365, and then annually.

b. Stool will be collected pre-conditioning and on days 7, 14, and 28. Stool collection will be optional on days 60, 100, and 180.
c. Bone Marrow will be collected if done for any clinical indication.

5. Limited Infection and medication data (Appendix C) will be collected on day 0 and days 14, 28, 60, 100, 180, 365, and then annually.
    a. Infection data collected: Infection type, Organism, Anatomical Location(s), Severity, Start and Stop Date
    b. Medication Data collected: medication name, dose (corticosteroid only), frequency (corticosteroid only), and indication

4.3 Collection of Research Samples and Justification

Blood, stool, biopsy, bone marrow, and emptied product bag/syringe research samples will be collected in this study as outlined in Table 1. The majority of study samples will be collected on a calendar driven schedule.

4.3.1 Study Blood Draws

For HCT or GT participants, the blood sample collections will occur at baseline (defined as pre-conditioning). Samples will then be collected on Days 0, 7, 14, 28, 60, 100, 180, 365 and yearly through year 5 or end of study, and at specified events as outlined in Table 1. Related donor samples will be drawn prior to the stem cell collection.

4.3.1.1 Serum

Serum will be collected by venipuncture or central access in a Serum Separator tube at regular intervals to allow investigations of proteins, antibodies, hormones, antigens, or other serum components that may serve as biomarkers of immune reconstitution, GVHD, graft failure, or other outcomes of interest. Tubes will be shipped at ambient temperature to the BMT CTN Repository within 24-48 hours of collection. At the BMT CTN Repository, the Serum Separator tube will be processed according to standard protocol to obtain 0.5 mL aliquots for storage at -80 degrees Celsius until use. Peripheral blood is easily obtained in sufficient quantities to allow a robust biobank resource of serum for many future investigations. Reference Appendix B, Protocol-Based Laboratory Procedures

4.3.1.2 Plasma

Plasma will be collected by venipuncture or central access in a Sodium heparin tube at regular intervals to allow investigations of proteins, antibodies, hormones, antigens, or other serum components that may serve as biomarkers of immune reconstitution, GVHD, graft failure, or other outcomes of interest. Tubes will be shipped at ambient temperature to the BMT CTN Repository within 24-48 hours of collection. At the BMT CTN Repository, the Sodium Heparin tube will be processed according to standard protocol to obtain 0.5 mL aliquots for storage at –80 degrees Celsius until use. Peripheral blood is easily obtained in sufficient quantities to allow a robust biobank resource of frozen plasma for many future investigations.

4.3.1.3 Whole Blood for PBMCs

Peripheral blood samples will be collected via venipuncture or central access in EDTA tubes at regular intervals as outlined in Table 1. Tubes will be shipped at ambient temperature to the BMT CTN Repository within 24-48 hours of collection. At the BMT CTN Repository, peripheral blood mononuclear cells will be isolated by Ficoll gradient separation or RBC lysis and viably frozen in aliquots to allow diverse studies of proteins, RNA, DNA, and/or studies of live PBMCs (e.g., cell surface markers, telomere lengths, cellular functions, etc.) Multiple viably frozen aliquots of approximately 5x 10^6 cells per aliquot from each scheduled interval will be maintained at –140 degrees Celsius (vapor phase of liquid nitrogen) until utilized. Peripheral blood is easily obtained in sufficient quantities to allow a robust biobank resource of viably frozen mononuclear cells for many future investigations.

4.3.1.4 Stool Samples

Stool samples will be collected in OMNIgene-GUT DNA and RNA tubes (for stabilization of microbial DNA and RNA from fecal samples for up to 10 days at ambient temperature prior to processing) and OMNImet-GUT tubes (for stabilization of metabolites from fecal samples for up to 7 days at ambient temperature prior to processing). Tubes will be shipped at ambient temperature to the BMT CTN Repository within 24-48 hours of collection. At the BMT CTN Repository, the samples will be aliquoted and stored at -80^{o}C. Samples will be collected to enable downstream DNA, RNA and mass spectrometry (metabolite and metaproteome) analysis. The stool sample collections will occur at baseline (defined as pre-conditioning, pre-antibiotic prophylaxis) at day -7 to day -14 prior to start of conditioning. Samples will then be collected on Days 7, 14, and 28. Collection of stool will be optional for Days 60, 100, and 180, as outlined in Table 1. Stool collection kits will be configured to allow for inpatient or outpatient/at home collections. At home collections will be performed within 24-48 hours prior to the patients' next clinic visit to allow for the shipment to the BMT CTN Repository.

4.3.1.5 Skin Biopsy

A 3-mm skin punch biopsy should be obtained at the site of a pre-planned bone marrow biopsy, central line placement, or at another skin site acceptable to the recipient (e.g. inner arm, scapula, etc.) and placed into High Glucose DMEM media and shipped at cooled temperature to the laboratory of Dr. Troy Lund (University of Minnesota) to obtain fibroblast cultures per a previously established protocol.[72] Fibroblast cultures will be expanded until nine aliquots of 1 x 10^6 cells per aliquot are viably frozen with cell passage numbers recorded are obtained. These aliquots will then be transferred to The HOPE Biorepository for storage. Viable skin fibroblast samples will be utilized for studies of germline genetic factors implicated in risk for AA, bone marrow failure, or hemoglobinopathies or in risk for specific complications post HCT or GT. Viably frozen skin fibroblasts can be utilized to make induced pluripotent stem cells for future investigations of germline genetic variant functional consequences or to create patient-specific disease models. In addition, DNA from cultured skin fibroblasts is the currently widely accepted clinical standard for a non-blood-based germline sample that can be used as a comparison with blood-based DNA studies to determine which alterations identified are germline (e.g. in blood and skin) versus acquired (e.g. only present in blood and not skin). These types of comparisons are essential for CH studies as well as for investigations to determine if DNA extracted from blood is suitable for germline genetic testing in these NMDs. This is because cases of somatic reversion, in which a germline genetic variant is no longer detectable in blood due to a genetic correction event, are

estimated to occur in blood from ~8-10% of patients with IBMFS. Thus, the ideal tissue for germline genetic testing remains unknown and comparisons between blood, skin fibroblasts, and hair or nails as additional alternatives sources are crucial and will be highly valued samples in this HOPE resource. Skin fibroblasts are an expandable resource, providing plentiful, high-quality DNA, RNA, protein, and other cellular components for investigations. Thus, they are an ideal source of non-blood germline tissue for a robust biobank for the future.

4.3.1.6 Hair Collection

Approximately 10-15 hair plucks from the eyebrows or scalp per participant preference will be collected to maximize collection of the hair follicle. These hair plucks will be immediately placed in a sterile cryovial. These cryovials will be shipped at ambient temperature to the BMT CTN Repository and then stored frozen at –80 degrees Celsius until utilized. Collecting hair samples is a key alternative non-blood based germline tissue that is being considered as a future tissue type for germline genetic testing in NMDs. However, prior studies in malignant diseases such as MDS in which acquired genetic variants are frequent in blood, have found low level contamination of hair follicle DNA with blood-based acquired genetic variants. It is unknown if acquired alterations that can be seen in AA, hemoglobinopathies, or other causes of BMF are found in DNA made from hair follicles. These samples may be more acceptable for some participants rather than undergoing a skin biopsy procedure so will allow for collection of a non-blood based germline tissue in some recipients who decline a skin biopsy as well as for related donors whom we will not ask for a skin biopsy. For recipients, hair follicles must be collected pre-therapy due to studies showing that donor DNA is detectable in a significant proportion of samples post-HCT. Lastly, 10 hair follicles yields ~300 ng of DNA, limiting the number of times this sample can be utilized for research studies. Thus, collection of multiple non-blood based germline samples whenever possible is warranted to facilitate a robust, biobank for many future studies

4.3.1.7 Nail Clippings

After instructing the participant to use a nail brush to remove any visible dirt or debris from the nails, nail clippings from 10 fingernails or toenails will be collected using a standard nail clipper by either the participant or research coordinator. These will be immediately placed into a sterile cryovial. These cryovials will be shipped at ambient temperature to the BMT CTN Repository and stored at –80 degrees Celsius until utilized. Collecting nail samples is a key alternative non-blood based germline tissue that is being considered as a future tissue type for germline genetic testing in NMDs. However, prior studies in malignant diseases such as MDS in which acquired genetic variants are frequent in blood, have found low level contamination of nail DNA with blood-based acquired genetic variants. It is unknown if acquired alterations that can be seen in AA, hemoglobinopathies, or other causes of bone marrow failure (BMF) are found in DNA made from nails. Nail samples may be more acceptable for some participants rather than undergoing a skin biopsy procedure so will allow for collection of a non-blood based germline tissue in some recipients who decline a skin biopsy as well as for donors whom we will not ask for a skin biopsy. For recipients, nail clippings must be collected pre-therapy due to studies showing that donor DNA is detectable in a significant proportion of samples post-HCT. Lastly, 3 slivers (20 mg) of nails yields ~500 ng of DNA, limiting the number of times this sample can be utilized for research studies.[73] Thus, collection of multiple non-blood based germline samples whenever possible is warranted to facilitate a robust, biobank for many future studies

4.3.1.8 Bone Marrow Aspirate and Core

At the time of a bone marrow biopsy performed for clinical purposes, an extra pull of up to 6 ml of bone marrow aspirate will be taken and stored in an EDTA blood tube. This tube will be shipped at ambient temperature to the BMT CTN Repository within 24-48 hours of collection. At the BMT CTN Repository, the bone marrow sample will be processed to obtain bone marrow mononuclear cell aliquots to be viably frozen for storage in cryovials. If a bone marrow core sample of adequate length is obtained for clinical purposes and embedded in formalin-fixed, paraffin-embedded (FFPE) per usual clinical procedures, a portion of this core biopsy specimen in FFPE residual after clinical needs are complete, will be shipped at ambient temperature to the BMT CTN Repository for storage. Bone marrow samples are critical for understanding the cellular components in the bone marrow pre- and post-HCT or –GT that can inform disease mechanisms and adverse outcomes. Viably frozen BM mononuclear cells provide a robust source of live cells, DNA, RNA, proteins, and other components for investigations. An FFPE core sample would allow studies of cellular and non-cellular components and organization within the bone marrow, including spatial transcriptomics, immunohistochemistry and other studies that will inform disease mechanisms and complications.

4.3.1.9 Hematopoietic Stem Cell Product Bag Wash Samples

After the infusion of all stem cells into the recipient is complete, the otherwise discarded infusion bag will be shipped at refrigerated temperature to the BMT CTN Repository. At the BMT CTN Repository, the product bag/syringe will be rinsed to collect leftover cells for viable frozen storage in cryovials. These cells will be utilized for multiple investigations, including stem cell and immune cell profiling, and T-cell receptor sequencing, in order to rigorously characterize the infused cell product. To enable these studies, mononuclear cells obtained from the bag will be viably cryopreserved. These will provide a robust source of live cells, DNA, RNA, proteins, and other components for investigations.

4.4 Data Capture

Participant data will be captured in CIBMTR FormsNet with supplemental study-specific information captured in eClinical via calendar and event-based forms which will be integrated with the participant's data from FormsNet.

4.4.1 Criteria for Forms Submission

Criteria for timeliness of submission for all study-specific forms are detailed in the CRF Completion Guidelines. Forms that are not entered within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered an exception is granted.

4.4.2 CIBMTR Data Reporting

Centers participating in BMT CTN trials must register pre- and post-HCT and GT outcomes on all consecutive cellular therapies and HCTs done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms from the CIBMTR. (Note: Federal legislation requires submission of these forms for all United States [US] allotransplant recipients.) Enrollment on BMT CTN 2402 must be indicated on the CIBMTR Pre-Transplant Essential Data (Pre-TED/F2400) form. Additionally, CIBMTR pre- and post-HCT/GT CRFs must also be submitted for all

participants enrolled on this trial. CIBMTR forms and data will be provided at the standard CIBMTR data collection timepoints.

4.4.3 Advantage eClinical Data Reporting

All participant evaluation data not included in the CIBMTR Clinical Data Collection (Appendix C) will be captured in eClinical. This includes demographics, study eligibility, medications, infection data, specimen collection and tracking, and event-based data. Comprehensive details regarding the data to be collected will be provided in the EDC system User's Guide and CRF Completion Guidelines. Only authorized individuals shall have access to eCRFs.

4.4.3.1 Advantage eClinical Training and Certification

Advantage eClinical site users will be required to at minimum review training materials (user's guides and video demonstrations of the system functionality and specific protocol functionality). They will demonstrate their understanding of this review by completing quizzes and a protocol-specific practicum. When they have completed these, the Emmes team will provide the team with certification.

4.4.3.2 Data Linkage

In eClinical, along with the randomly assigned participant identification number, the site users will be required to enter the patient's CIBMTR ID in the system. This will link the eClinical ID to the CIBMTR ID. All specimens collected (donor and recipient) will be linked to the associated eClinical participant ID. A Specimen ID is linked to the associated eClinical participant ID through programming in the specimen tracking module. This information on the site training and data linkage will be added to the protocol.

4.5 Data and Safety Monitoring Board (DSMB)

Study data will be reviewed annually by the DSMB. The DSMB is responsible for safeguarding the interests of study participants, assessing the safety of study procedures, ensuring data quality, evaluating clinical equipoise, and for monitoring the overall conduct of the study. Policies and composition of the DSMB are described in the BMT CTN Manual of Procedures.

4.6 Processes for Sample Disbursement and Data Sharing

Specimens will be available for analysis at the completion of accrual and follow-up samples will be transferred to Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). As an incentive for participating, investigators and centers enrolling on this protocol will have the opportunity to submit proposals to receive specimens free of charge before the transfer to BioLINCC. Investigators will be required to submit a scientific proposal with a pre-specified analysis plan that clearly demonstrates the requested data and samples (either alone or in combination with existing samples) are sufficient to answer the proposed scientific question. The proposals will be reviewed and approved by the BMT CTN Biomarker Committee. The exact time that specimens will be made available is not yet determined pending discussions with NHLBI but, at a minimum, patients must have had their 1-year post-HCT or GT visit and with a reviewed data set locked for investigator use. A detailed plan for releasing data will be developed and reviewed by the DSMB before implementation. Once samples are transferred to BioLINCC, investigators must submit an application using BioLINCC's standard processes for requesting

access to data sets and/or biospecimens. The investigators will be responsible for supplying funding to support the administrative cost of identifying samples of interest, shipping of the sample to their institution, and collecting corresponding clinical data. Data sharing requirements should follow standard NHLBI guidelines.

4.7 Participant Evaluations

Table 1: Patient and Donor Assesments

			Study Day (Window)										
Data/ Sample Source	**Specimen**	**Specimen subtype**	**Baseline/ Pre-Conditioning** (-14 Days - pre-conditioning start)	**Day 0 Infusion**	**Day 7** (+ 3 Days)	**Day 14** (+ 3 Days	**Day 28** (+ 7 Days)	**Day 60** (+ 7 Days)	**Day 100** (+ 7 Days)	**Day 180** (+ 28 Days)	**Day 365** (+ 28 Days)	**Years 2, 3, 4, 5** (+ 60 Days)	**Event Driven Collection**[2,16]
Patient Data	**Demographics**		X										
	Eligibility Review		X										
	CBC		X		X	X	X	X	X	X	X	X	X[13]
	Infections[14]		X	X		X	X	X	X	X	X	X	
	All Medications[15]		X	X		X	X	X	X	X	X	X	X
	Toxicities					X	X	X	X	X	X	X	
	Date of Engraftment[1]				Report date(s) of occurrence as applicable through Year 5								
	Date off Immunosuppression												
	Date of Immunizations												
Patient Samples	**Peripheral Blood**	PBMC	X			X	X	X	X	X	X	X	X
		Plasma	X	X	X	X	X	X	X	X	X	X	X
		Serum	X	X	X	X	X	X	X	X	X	X	X
	Stool		X		X	X	X	X[4]	X[4]	X[4]			
	Hematopoietic Stem Cell Product Cells[11]			X									
	Germline Samples	Skin biopsy for cultured skin fibroblasts	X										

		Nail clippings (8-10) **AND/OR** Hair Plucks (10-15 hairs)	X										
	Bone Marrow[5]	BM-MNCs	If BM biopsy done for any clinical indication, collect research sample										
		BM – part of FFPE BM core	If BM biopsy done for any clinical indication, collect research sample										
	Event Driven Samples & Data (see footnotes for required data and samples)	Donor Lymphocyte Infusion[6]	Sample in footnote[6] to be collected at time of event and prior to initiating therapy for DLI with planned study sample collection when possible[16]										
		Post-Therapy Malignancy Diagnosis[7,9]	Sample in footnote[7,9] to be collected at time of event and prior to initiating therapy for malignancy with planned study sample collection when possible[16]										
		Graft Failure[8,9]	Sample in footnote[9] to be collected at time of event and prior to initiating therapy for graft failure with planned study sample collection when possible[16]										
		TMA[10]	Sample in footnote[10] to be collected at time of event and prior to initiating therapy for TMA with planned study sample collection when possible[16]										
		Graft versus Host Disease[3,10]	Sample in footnote[10] to be collected at time of diagnosis and prior to initiating therapy for acute or chronic GVHD with planned study sample collection when possible[16]										
		Poor Graft Function[11]	Sample in footnote[11] to be collected at time of event and prior to initiating therapy for poor graft function < with planned study sample collection when possible[16]										
Related Donor Samples	**CBC**		X										
	Peripheral Blood	PBMC	X										
		Plasma	X										
		Serum	X										
	Germline Sample	Nail clippings (8-10) **AND/OR** Hair Plucks (10-15 hairs)	X										

[1]For engraftment for AA, use standardized definition per BMT CTN 2207 trial
[2]Events: Graft failure, poor graft function GVHD diagnosis, Donor lymphocyte infusion, Transplant Associated Microangiopathy (TMA) diagnosis, other cell infusion, post-therapy malignancy diagnosis.
[3]For GVHD event, GVH cumulative scoring will be collected
[4]Stool collection required through D30 and is optional from D60 – D180
[5]Bone marrow only expected if collected as part of standard medical care
[6]PBMC, serum, and plasma; Hematopoietic stem cell product bag/syringe(s)

[7]For post-therapy malignancy event, pathology report form biopsy, any genomic studies performed on tumor, viral studies in tumor or PB at times of event if relevant (HPV, EBV, CMV, etc.)
[8]Graft failure event, CBC with diff, chimerism/engraftment (blood and marrow), hempathology report from bone marrow biopsy
[9]PBMC, serum and plasma; Bone marrow aspirate and FFPE block portion (if marrow done)
[10]PBMC, serum and plasma
[11]PBMC, serum and plasma; Bone marrow aspirate and FFPE block portion (if marrow done)
[12]Sample from washed bag, serum, plasma and PBMC frozen viably prior to infusion
[13]CBC to be collected for graft failure, TMA, and poor graft function.
[14] Infection type, Organism, Anatomical Location(s), Severity, Start and Stop Date
[15] Medication name and indication
[16]If a study sample has been collected within the past 7 days prior to the event, an additional sample is not needed; however, if the event occurs before the next scheduled sample collection, collect the sample at the time of the event . If the event driven sample is collected before the scheduled sample collection and within the collection window for that visit, an additional sample of the same type is not needed (see Appendix B for additional details).

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 Study Design

This is a prospective, multicenter study that will establish a repository of biospecimens and clinical data from participants undergoing HCT or GT for treatment of nonmalignant blood diseases. The enrollment goal is 375 participants and approximately 100 related donors, accrued over 4 years. Participants will be followed until the end of study. Initial plans are for the study to end at the completion of the current BMT CTN grant cycle (August 2031), but continued collection of specimens and clinical data may continue to allow up to 15 years of follow-up for all enrolled participants if funds are available.

The objective of this biorepository is to obtain the appropriate samples required to perform novel research and answer clinical questions important to patients with non-malignant blood diseases. Although the exact research analyses are not defined as part of this biorepository, the protocol will provide adequate power to answer prominent questions in the field.

5.2 Sample Size and Power Considerations for Future Studies

Because no specific research is specified for this biorepository, we illustrate sample size / power justification for hypothetical future studies that may use this resource.

5.3 Biomarker Discovery Study for Graft Failure

Graft failure after HCT is a serious complication that portends poor outcomes due to high morbidity and mortality from prolonged cytopenia and higher risks of toxicity with a second HCT, if the latter is possible. For NMDs, graft failure rates after HCT vary from 10-20% depending on the underlying disease, donor type, graft source, and the overall transplant approach (myeloablative versus reduced intensity, serotherapy, and GVHD prophylaxis used). For patients who are at elevated risk of graft failure, it is desirable to detect this risk early so that preemptive therapy can be administered to prevent graft failure or mitigate its adverse outcomes. Previous small studies reported that two plasma biomarkers, CXCL9 and interferon-gamma, are associated with graft failure.[74.75.76] Plasma biospecimens from the HOPE biorepository could be used to evaluate these and other candidate markers for actionable predictive markers for graft failure.

A case-control study can evaluate the prognostic value of biomarkers by comparing their mean levels between patients who experience graft failure versus those who do not. The power to detect a change in mean biomarker levels between graft failure cases and controls depends on the total sample size, the targeted fold-change in mean levels, and the coefficient of variation (CV), which is the ratio of the standard deviation of biomarker levels to their mean value. With a graft failure rate of 10-20%, the biorepository should yield at least 37 cases and thus a case-control study of at least 74 patients Table 2 displays the power provided to test 1, 10, and 20 candidate markers with a two-sided 5% significance level for CV values of 0.3 and 0.4 and targeted fold-change in means of 1.3, 1.4, and 1.5, corresponding to 30%, 40%, and 50% relative differences in levels

between cases and controls.[77] A Bonferroni adjustment is used to compute power for testing multiple markers.

Table 2: Power to Detect Fold-change in Mean Biomarker Levels for Case-Control Study of 74 Patients

		Number of Markers Tested		
CV	**True Fold-change in Mean Level**	**1**	**10**	**20**
0.3	1.5	>99.9%	>99.9%	99.8%
0.3	1.4	99.9%	98.3%	97.2%
0.3	1.3	97.0%	85.0%	79.4%
0.4	1.5	99.5%	95.7%	93.4%
0.4	1.4	96.4%	82.9%	76.8%
0.4	1.3	83.4%	54.9%	46.2%

Biomarker levels for cases and controls are assumed to follow lognormal distributions.

The power levels are high, at over 80% for most scenarios. Power is deflated for scenarios when multiple markers are tested targeting a fold-change of 1.3 with CV of 0.4. Generally, the study is well-powered to detect relative differences in mean biomarker levels of 30-50% between graft failure cases and controls.

5.4 Biomarker Validation Study for Graft Failure

The HOPE repository could also be used to evaluate the prediction accuracy of biomarkers for graft failure. After discovering associations of an individual biomarker or biomarker panel with graft failure, a validation study may be conducted to evaluate its ability to predict future graft failure, as described by its sensitivity and specificity. Table 3 displays the widths of 95% confidence intervals for these metrics for hypothetical validation studies using a case-control design, assuming a 10% graft failure rate among the cohort and considering true sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) of 0.70 and 0.80.[83,84] The confidence intervals are less than 0.30 in width, showing that the HOPE biorepository will provide sufficiently large studies to estimate these prediction metrics precisely.

Table 3: Precision of Estimation for Sensitivity and Specificity of Graft Failure Biomarker for Case-Control Study of 74 Patients

Prediction Metric's True Value	Width of 95% Confidence Interval
Sensitivity = 0.70	0.295
Specificity = 0.70	0.295
PPV = 0.70	0.222
NPV = 0.70	0.222
Sensitivity = 0.80	0.258
Specificity = 0.80	0.258
PPV = 0.80	0.213
NPV = 0.80	0.213

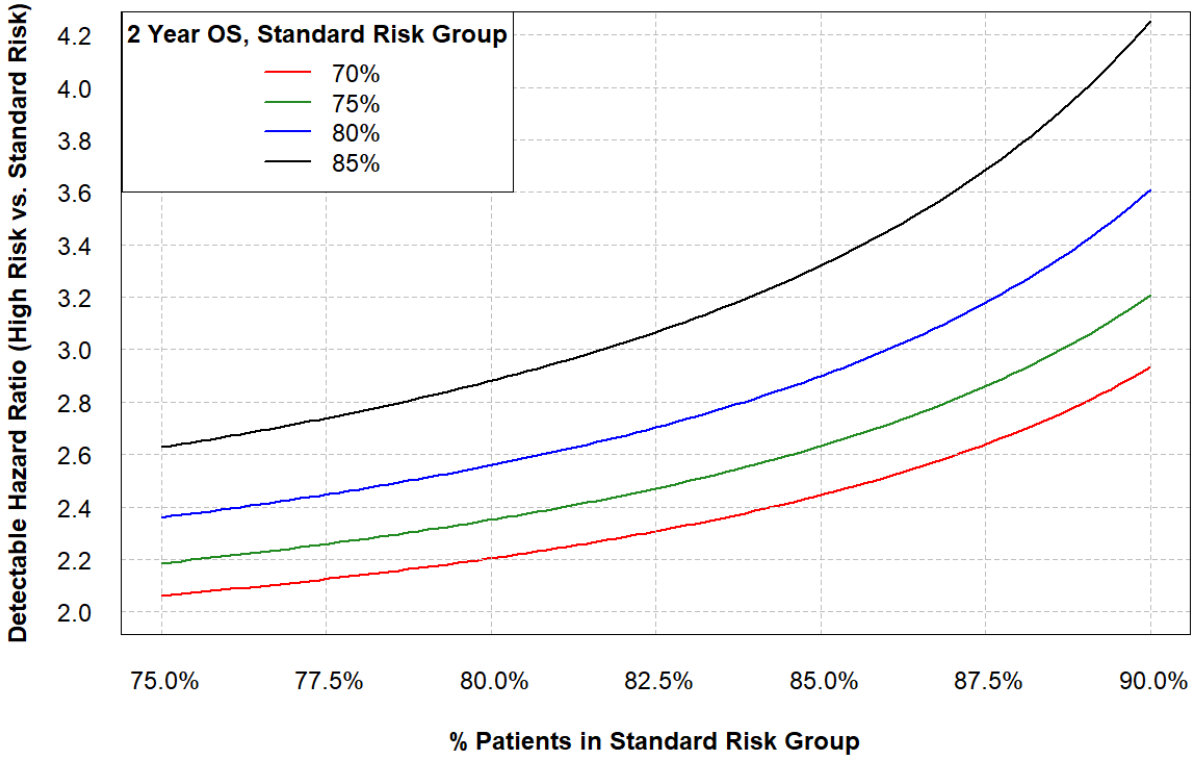
* PPV and NPV confidence intervals were calculated assuming that the true sensitivity and specificity have equal values.

5.5 Impact of Somatic Mutations on Mortality Risk in Aplastic Anemia

In AA, patients are at risk for progression to MDS or MAL), with a cumulative incidence of progression of 13% estimated among patients receiving IST in a recent study.[78] CH has been observed in about 1/3 of patients with AA. Prior studies suggest that somatic mutations of the genes *BCOR* and *PIGA* indicate a favorable prognosis for AA patients, while those in *DNMT3A* and *ASXL1* signify an unfavorable prognosis; this has not been definitively determined, though.[79] It is unknown whether these mutations also affect HCT outcomes. Thus, there is interest in assessing the impact of somatic mutations on HCT outcomes for AA patients, such as survival and progression to MDS and AML. Further, in the setting of an HCT, CH in the donor cells infused at the time of HCT has also been observed in small case series to impact outcomes such as GVHD and risk of donor-derived MDS or AML. The HOPE biorepository will collect bone marrow and PBMCs on recipients prior to transplant as well as residual stem cell product from paired related donors that can both be analyzed for somatic mutations, permitting future investigations of correlations of these mutations with HCT outcomes for AA patients. Importantly, non-blood-based germline samples will also be collected. These germline samples are a critical tissue source that will allow a more precise determination of which variants found in the blood, bone marrow, or stem cell product samples are indeed somatic mutations (i.e., they are not present in the paired non-blood germline tissue). Prior studies using blood, bone marrow, or stem cell product only samples have defined CH/acquired mutations in these tissues using assumptions such as variant allele frequency cut-offs due to the lack of these critical non-blood-based germline tissues.

As an example, consider a hypothetical study that will compare overall survival (OS) between patients with specific mutations hypothesized to constitute “high risk”, such as *DNMT3A* and *ASXL1*, to patients free of these mutations (“standard risk”). It is expected that approximately 60% of enrolled participants will have AA or about 225 patients. Figure 1 displays the hazard ratios between high and standard risk patients that can be detected with 80% power by a log rank test with a two-sided 5% significance level.[80] Scenarios considered include plausible 2-year OS rates of 70-85% for the standard risk group and proportions of patients in the standard risk group of 75-90%.

Figure 1: Hazard Ratios Detected with 80% Power for a Log Rank Test Comparing High and Standard Risk Groups in a 225 Patient Cohort



Hazard ratios of 2.2 - 2.8 may be detected with high power when there are 80% or fewer patients in the standard risk group, depending on the value of the 2-year OS rate in this group. When 90% of the patients are in the standard risk group, the hazard ratios detectable with 80% power are 2.9 - 4.2. This study would be well-powered to identify a hazard ratio of 3.5 in all settings considered except when the standard risk group has a 2-year survival rate of 85+% and comprises 90% or more of the study population.

APPENDIX A: HUMAN SUBJECTS PROTECTION

1. Subject Consent

Candidates for the study will be identified by the research team at their institution. The Principal Investigator or his/her designee at each transplant center will contact the candidates or parent/legal guardian, provide themwith information about the purpose of the study and obtain voluntary consent if the candidate or parent/legal guardian agrees to participate. Related donors or their parent/legal guardian of consenting HCT participants will be approached for study participation by the research team. The Principal Investigator or his/her designee at each transplant center will contact the dono r or their parent/legal guardian, provide them with information about the purpose of the study, and obtain voluntary consent if the related donor or their parent/legal guardian agrees to participate. The BMT CTN will provide a template of the assent and the consent forms to each center. Each center will add their NMDP Internal Review Board (IRB)-approved boiler-plate language to these documents and submit it for review by the NMDP IRB. The Data Coordinating Center will verify the adequacy of the assent and consent forms prior to submission to the IRB. The NMDP IRB will provide evidence of IRB approval.

For participants who are minors at the time of enrollment, assent will be obtained in accordance with local and federal regulations. Upon reaching the age of majority (typically 18 years), these participants must be re-consented using the adult consent form. The research team at each center is responsible for tracking participant age and ensuring timely re-consent. Continued participation in the study is contingent upon obtaining informed consent from the now-adult participant. Documentation of the re-consent process must be maintained in the study records.

2. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relaying the patient's identity with the ID code will be kept separately at the institution caring for the patient. The ID code will be transmitted to the network.

3. GCP

This study will be conducted in accordance with standards of Good Clinical Practice (as defined by the International Council for Harmonization) and all applicable national and local regulations.

APPENDIX B: PROTOCOL-BASED LABORATORY PROCEDURES

Participant samples will be collected both prior to the initiation of conditioning therapy (up to -14 days pre-infusion), on the day of infusion (Day 0), and post-therapy Day 7, Day 14, Day 28, Day 60, Day 100, Day 180, Day 365, and then annually as specified in the table on the next page. Event-driven sample collection will also be requested in cases of Donor cell (lymphocyte or CD34+) infusions, post-therapy malignancy, graft failure, poor graft function, GVHD (acute and chronic), and TMA. Length of participation in research sample collections will be dictated by the timing of patient enrollment on the protocol. Each blood collection time point will involve a draw of 35 mL or the maximum blood volume allowable based on age and weight by each center's institutional guidelines (whichever amount is smallest). and Whenever possible, research blood samples will be co-drawn with clinical laboratory collections and through central venous catheters to minimize additional venipunctures. Please refer to the BMT CTN 2402 Research Sample Information Guide for more information.

Donor samples will be collected prior to graft collection.

Event driven samples should be collected as close as possible to the onset of the event (Day of Event) and prior to intervention. If the Day of Event aligns with already scheduled (time-driven) sample collections, collected samples for the Day of Event will also count for the time-driven specimens. If the Day of Event is greater than 7 days after a time-driven sample collection, a new collection is necessary for the Day of Event. If the Day of Event collection is before the time-driven sample collection and outside the allowable time collection window, a new sample is required for the time-driven sample. . For example, a participant develops acute GvHD, which is an event requiring a sample collection. The following scenarios may occur depending on the Day of Event:

- Acute GvHD develops on Day 19. The last scheduled sample was collected on Day 14. A new sample is NOT required for the event as the Day of Event is within 7 days of a collected sample.
- Acute GvHD develops on Day 24. The last scheduled sample was collected on Day 14 so a new sample IS required for the event as it is greater than 7 days from development of acute GvHD. As Day 24 is within the time window of the scheduled Day 28 collection (Day 28 +/- 7 days), a new sample is NOT required for Day 28.
- Acute GvHD develops on Day 50. The last scheduled sample was collected on Day 28. A new sample IS required for the event. As Day 50 is outside the time window of the scheduled Day 60 collection (Day 60 +/- 7 days), a new sample IS required for Day 60.

Please email BMTCTN_NMDP@nmdp.org as soon as an event is suspected with any questions regarding sample types or collection timing.

All research samples (except skin biopsies) will be collected and shipped same day to the BMT CTN Repository at NMDP for processing and sample aliquot storage; skin biopsies will be shipped directly to the University of Minnesota for culturing fibroblasts prior to being transferred to the BMT CTN Repository at NMDP for long term storage.

Refer to the BMT CTN 2402 Research Sample Information Guide for more information on sample collection and shipping procedures.

Study Subject	Research Sample Type	Time Points	Sample Quantity	Shipping Temperature	Sample Collection or Shipping Container	Lab/ Repository Destination	Processed Research Sample Material	Research Sample Material Aliquots	Storage Temperature
Patient (N = 375)	**Peripheral Blood**	Pre-conditioning, Days 0 (no PBMC), 7 (no PBMC), 14, 28, 60, 100, 180, 365 and then annually. Event driven: DLI, Post-Transplant Malignancy, TMA, Poor Graft Function, GVHD, and Graft Failure	35 mL (15 mL only for Days 0 and 7) Follow institutional guidelines for max volumes	Insulated ambient	20 mL EDTA tube	BMT CTN Repository	PBMC	~6 - 5x10^6/ 1 mL	LN_2
					10 mL NaHep tube		Plasma	10 - 0.5 mL	-80°C
					5 mL SST clot tube		Serum	10 - 0.5 mL	-80°C
	Stool	Pre-conditioning, Days 7, 14, 28, 60, 100, and 180	~1 gram	Insulated ambient	OMNIgene OMR-205 tube	BMT CTN Repository	Stool - for DNA/RNA	3 – 1 mL	-80°C
					OMNImet GUT ME-200 tube		Stool - for metabolomics	2 – 1 mL	-80°C
	Germline	Pre-conditioning	1 3mm-skin punch	Insulated cooled	cryovial with high glucose DMEM media	U of MN Lab	cultured fibroblasts	9- 1x10^6/ vial	LN_2
		Pre-conditioning	10-15 hairs	Insulated ambient	Cryovial	BMT CTN Repository	Hair plucks	1	-80°C
		Pre-conditioning	8-10 nail clippings		Cryovial		Nail clippings	1	-80°C
	Bone Marrow	BM to be collected for any clinical indication	6 mL BM aspirate	Insulated ambient	6 mL EDTA tube	BMT CTN Repository	Bone Marrow Mononuclear Cells	~6 - 5x10^6/ 1 mL	LN_2
			Portion of FFPE BM core		Portion of FFPE BM core		FFPE Bone Marrow Core	1	Temperature/humidity-controlled ambient
	Hematopoietic Stem Cell Product Cells	Day 0 and if event-driven DLI occurs	Product Bag or Syringe	Refrigerated 2-8°C	Product Bag or Syringe	BMT CTN Repository	Washed cells from product bag	~7 - 5x10^6/ 1 mL	LN_2
Related Donors	**Peripheral Blood**	Pre-stem cell collection	35 mL Follow institutional guidelines for max volumes	Insulated ambient	20 mL EDTA tube	BMT CTN Repository	PBMC	~6 - 5x10^6/ 1 mL	LN_2
					10 mL NaHep tube		Plasma	10 - 0.5 mL	-80°C
					5 mL SST clot tube		Serum	10 - 0.5 mL	-80°C
	Germline	Pre-stem cell collection	10-15 hairs	Insulated ambient	Cryovial	BMT CTN Repository	Hair plucks	1	-80°C
		Pre-stem cell collection	8-10 nail clippings		Cryovial		Nail clippings	1	-80°C

APPENDIX C: CLINICAL DATA COLLECTION

Clinical data will be collected to correlate clinical status with biorepository samples. Data collection will occur through 2 avenues:

- CIBMTR Comprehensive Report Forms (CRF) – obtained at baseline (Form 2000), post-therapy at 100 days, 6 months, yearly through 6 years, then every other year (Form 2100). Submission of the CRF forms will be through FormsNet with standard CIBMTR reporting expectations. Routine reviews of CIBMTR form completion for this study will be completed by CIBMTR at least annually. Monitoring of CIBMTR data will be conducted per CIBMTR standards.

1) Advantage eClinical CRFs – obtained at each study visit to collect demographics, study eligibility, specimen acquisition information, outcomes at timepoints when CIBMTR CRFs are not due (D0, D14, D28, and D60), and additional details not collected on CIBMTR CRFs on concomitant medications, infections, and other events. Routine reviews of Advantage eClinical data will be conducted by Emmes data managers per the Data Management Plan and monitoring of the data will be conducted by Emmes clinical research associates per the Clinical Monitoring Plan.

The format of the forms will be aligned so that consistent data can be tracked longitudinally for patients. This will be achieved by using the CIBMTR form question template; definitions of post-therapy outcomes/complications will follow the recommendation of the CIBMTR Forms Instruction Manual (Comprehensive Baseline & Follow-up Manuals - Forms Instruction Manual - 1). The responsibility for form collection and monitoring of form completion will be different for each form type, as noted above, with CIBMTR CRF forms managed by CIBMTR and Advantage eClinical forms managed by Emmes.

CIBMTR data includes baseline assessment, reporting of post-infusion complications, and follow-up of disease status post-infusion. Below represents a table of post-infusion events, complications, and late effects that are captured on CIBMTR forms. This sample does not represent the comprehensive list of data collection elements – the forms in their entirety can be viewed here: CIBMTR Data Collection Forms . Comprehensive details regarding the data to be collected in eClinical will be provided in the CRF Completion Guidelines.

Outcome	Definition	Form Assessment	Timepoints Assessed
Engraftment		CIBMTR CRF	D100, 6 mo, 1yr, 2 yr
Neutrophil Recovery	The first day of ANC ≥500/mm^3 (or 0.5x10^9/L) for three consecutive days		
Initial Platelet Recovery	The first day of platelet count ≥20x10^9/L and ≥50x10^9/L for three consecutive days, with no platelet transfusions administered for the previous 7 days		

Immune Reconstitution Immunoglobulin Assessment Lymphocyte Assessment	 Quantification and date of assessment of IgG, IgA, IgM Quantification and date of assessment of CD3, CD4, CD8, CD19/CD20, CD56	CIBMTR CRF	D100, 6mo, 1yr, 2yr
Gene Therapy Persistence*	Were tests performed to detect persistence of the gene therapy product	CIBMTR CRF	D100, 6mo, yearly
Chimerism*	Method, cell source, cell type (whole or sorted) with date and attached documentation	CIBMTR CRF	D100, 6mo, 1yr
Acute GVHD*	Onset: stage of organ involvement, overall grade, date of onset (see Appendix D) Maximum severity: max stage of organ involvement, max overall grade (see Appendix D)	CIBMTR CRF eClinical CRF	D100 D28, D60
Chronic GVHD*	Date of onset of chronic GVHD will be captured and organ involvement assessed based on NIH criteria Maximum grade of chronic GVHD as mild, moderate, severe, or unknown will be reported (see Appendix D) Chronic GVHD will be categorized as limited or extensive based on organ involvement (see Appendix D)	CIBMTR CRF eClinical CRF	D100, 6mo, yearly Event driven timepoints
Infection	Any clinically significant infection will be captured (site and organism). Clinically significant infections are defined as those requiring treatment. Infection data includes Infection type, Organism, Anatomical Location(s), Severity, Start and Stop Date	CIBMTR CRF eClinical CRF	D100, 6mo, yearly Pre-Conditioning, D0, D14, D28, D60
Malignant Neoplasm	Type of malignancy and documentation submitted to CIBMTR	CIBMTR CRF	D100, 6mo, yearly
Specimen Collection	Confirmation and date of specimen collection	eClinical CRF	All timepoints

CBC Results	Results of CBC assessments	eClinical CRF	Pre-Conditioning, D7, D14, D28, D60, D100, 6mo, yearly
Medication Review	All current medications at the time of assessment .Collection includes medication name, dose, frequency, and indication.	eClinical CRF	Pre-Conditioning, D0, D14, D28, D60, D100, 6mo, yearly

*as applicable to the therapy received

Organ Toxicities

The following Organ Toxicities are collected on CIBMTR CRF forms at D100, 6mo, yearly and data includes:

- Whether the event occurred
- date of onset
- date of resolution

Organ	Definition
Pulmonary Toxicity	
Non-infectious interstitial pneumonitis/ARDS/Idiopathic Pneumonia Syndrome (IPS)	All non-infectious lung injuries that occur within 120 days post-therapy
Bronchiolitis Obliterans	Airway obstruction as a result of inflammation, typically occurs late and can be a manifestation of cGVHD
Cryptogenic Organizing Pneumonia (COP/BOOP)	Idiopathic pneumonia affecting bronchioles and alveoli, typically occurs late
Diffuse Alveolar Hemorrhage	Bleeding into the alveolar space
Other non-infectious pulmonary abnormality	Any other non-infectious pulmonary complication not otherwise captured in other categories
Liver Toxicities	
Veno-occlusive Disease (VOD)/Sinusoidal obstruction syndrome (SOS)	Diagnosis fits criteria for VOD/SOS
Cirrhosis	Degenerative disease in which fibrous tissue forms and the lobes become filled with fat. Diagnosis can be based on liver biopsy, clinical symptoms (enlarged liver), blood tests, laparoscopy, or radiology imaging. The resolution date data field is disabled for this option.

Medication Toxicity	Liver abnormality is associated with drug initiation and improve with cessation
Other Toxicity	Other non-GVHD and non-infectious causes of liver abnormalities
Thrombotic Microangiopathy (TMA)	Clinical diagnosis of TMA with microangiopathic hemolytic anemia, thrombocytopenia, renal abnormalities. Other possible clinical findings may include: neurologic changes, pulmonary dysfunction Other laboratory features include: • LDH > than center-specific upper limit of normal • Serum creatinine >2mg/dL or >50% rise over baseline • Bilirubin greater than twice the center-specific upper limit of normal
Renal Impairment	Stage 2 or 3 acute kidney failure and/or chronic kidney failure. Whether event occurred and if dialysis was required
Stage 2 Acute Kidney Failure	Serum creatinine 2-2.9 x baseline
Stage 3 Acute Kidney Failure	Serum creatinine is 3x baseline, OR Serum creatinine is ≥4.0mg/dL, OR eGFR declines to <35ml/min per 1.73m^2 (recipients <18yo), OR renal replacement therapy is started
Chronic Renal Failure	GFR <60ml/min per 1.73m^2 for ≥3 months or requiring dialysis for > 3 months
Cardiac Impairment	
Arrhythmia	Clinically reported. Includes atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmia
Cardiomyopathy	Clinically reported decrease in cardiac function
Congestive Heart Failure	Inability for the heart to supply oxygenated blood to meet the body's needs, ejection fraction % specified
Coronary Artery Disease	Clinically reported damage or disease in major blood vessels of the heart
Unstable Angina	Clinically reported chest pain due to reduced blood flow and oxygen to the heart
Myocardial Infarction	Clinically reported obstruction in coronary arteries resulting in damage/necrosis of the cardiac muscle
Hypertension requiring therapy	Clinically reported if patient requiring medications for management

Pericarditis	Clinically reported if patient developed swelling and irritation of the pericardium
Heart Valve Disease	The presence of one or more of the following: • Moderate or severe degree of valve stenosis or insufficiency as noted on echo • Prosthetic mitral or aortic valve • Symptomatic mitral valve prolapse
Vascular Impairment	
Deep Vein Thrombosis	Development of a blood clot in a deep vein. Specify if catheter-related
Pulmonary Embolism	Development of a blood clot in the arteries of the lung. Specify if catheter-related
Hyperlipidemia	High total cholesterol, low HDL cholesterol, high LDL cholesterol, and/or high triglyceride levels. Levels for each collected
Neurologic Impairment	
CNS Hemorrhage	Bleeding within the central nervous system
Non-infectious Encephalopathy	Damage or disease in the brain. Symptoms may include memory loss, personality changes, or declining ability to concentrate and reason
Neuropathy	Nerve damage which causes pain, weakness and numbness
Seizure	Sudden, involuntary muscle contractions due to the hyperexcitation of neurons
Stroke/Transient Ischemic Attack	Loss of brain function due to a disturbance in the blood supply to the brain.
Endocrine Impairment	
Diabetes / hyperglycemia requiring chronic treatment	High blood glucose levels that develop after therapy and require insulin and / or oral medication for treatment
Growth hormone deficiency / short stature	Condition in which the body does not produce enough growth hormone / a reduced overall rate of growth and if therapy was given for treatment
Hypothyroidism requiring replacement therapy	Decreased activity of the thyroid gland with high levels of thyroid-stimulating hormone (TSH)
Pancreatitis	Inflammation of the pancreas
Genitourinary	

Gonadal Dysfunction	Requiring hormone replacement
Hemorrhagic Cystitis	Hemorrhagic cystitis or hematuria requiring medical intervention (medical interventions may include but are not limited to catheterization of bladder, extra transfusions, or urology consult for management)
Musculoskeletal	
Avascular Necrosis	If avascular necrosis occurred during the reporting period and is new or recurred
Osteonecrosis	If osteonecrosis of the jaw occurs for the first time, persists, or recurs within the reporting period
Osteoporosis	If osteoporosis is diagnosed for the first time within the reporting period or persists
Osteoporotic Fracture	If osteoporotic fracture occurred during the reporting period and is new or recurred
Psychiatric	Requires treatment beyond “counseling/therapy sessions”
Depression	Depressed mood disorder requiring medication management
Anxiety	Anxiety (feelings of worry, anxiety, or fear interfering with daily activities) requiring therapy and medication management
Post-traumatic stress disorder (PTSD)	PTSD is triggered by seeing or experiencing a traumatic event requiring medication management
Other	Cataracts Iron overload requiring therapy Mucositis requiring therapy Other impairment or disorder Solid organ transplant Pregnancy or Pregnant partner

APPENDIX D: ACUTE GVHD GRADING

Extent of Organ Involvement			
Stage	**Skin**	**Liver**	**Gut**
1	Rash on <25% of skin[1]	Bilirubin 2-3 mg/dl[2]	Diarrhea > 500 ml/day[3] or persistent nausea[4] *Pediatric*: 280-555 ml/m^2/day or 10-19.9 mL/kg/day
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dl	Diarrhea >1000 ml/day *Pediatric*: 556-833 ml/m^2/day or 20-30 mL/kg/day
3	Rash on >50% of skin	Bilirubin 6-15 mg/dl	Diarrhea >1500 ml/day *Pediatric*: >833 ml/m^2/day or > 30 mL/kg/day
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl	Severe abdominal pain, with or without ileus, and / or grossly blood stool

Grade[5]			
I	Stage 1-2	None	None
II	Stage 3	Stage 1	Stage 1
III	—	Stage 2-3	Stages 2-4
IV[6]	Stage 4	Stage 4	—

1. Use "Rule of Nines" (Table 4) or burn chart to determine extent of rash.
2. Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
3. Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if an additional cause of diarrhea has been documented.
4. Persistent nausea with or without histological evidence of GVHD in the stomach or duodenum.
5. Criteria for grading given as minimum degree of organ involvement required to confer that grade.
6. Grade IV may also include lesser organ involvement with an extreme decrease in performance status.

APPENDIX D (Continued) Chronic GVHD Grading

Organ	Score 0	Score 1	Score 2	Score 3
Skin %BSA[1]	No BSA involved	1-18% BSA	19-50% BSA	>50% BSA
Skin Features	No sclerotic features	N/A	Superficial sclerotic features, but not "hidebound"	Deep sclerotic features; "hidebound;" impaired mobility; ulceration
Mouth	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 with major limitation of oral intake
Eyes	No symptoms	Mild dry eye symptoms not affecting ADL (requirement of lubricant drops ≤ 3x/day)	Moderate dry eye symptoms partially affecting ADL (requiring lubricant drops > 3x/day or punctal plugs) WITHOUT new vision impairment due to keratoconjunctivitis sicca (KCS)	Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) OR unable to work because of ocular symptoms OR loss of vision due to keratoconjunctivitis sicca (KCS)
GI Tract	No symptoms	Symptoms without significant weight loss (< 5%)	Symptoms associated with mild to moderate weight loss (5-15%) within 3 months OR moderate diarrhea without significant interference with daily living	Symptoms associated with significant weight loss (> 15%) within 3 months, requires nutritional supplement for most calorie needs OR esophageal dilation OR severe diarrhea with significant interference with daily living.
Liver	Normal total bilirubin and ALT or AP < 3 x ULN	Normal total bilirubin with ALT ≥ 3 to 5 x ULN or AP ≥ 3 ULN	Elevated total bilirubin but ≤ 3 mg / dL or ALT >5 x ULN	Elevated total bilirubin > 3 mg / dL

APPENDIX D (Continued) Chronic GVHD Grading

Lungs Symptom score:	No symptoms	Mild symptoms (SOB after climbing one flight of steps)	Moderate symptoms (SOB after walking on flat ground)	Severe symptoms (SOB at rests; requires O2)
Lungs Lung score:	FEV1 ≥ 80%	FEV1 60-79%	FEV1 40-59%	FEV1 ≤ 39%
Joints and Fascia	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion AND not affecting ADL	Tightness of arms or legs OR joint contractures, erythema thought to be due to fasciitis, moderate decrease of range of motion AND mild to moderate limitation of ADL	Contractures WITH significant decrease of range of motion *AND* significant limitation of ADL (unable to tie shoes, button shirts, dress self, etc.)
Genital Tract[2]	No signs	Mild signs and females with or without discomfort on exam	Moderate signs and may have signs of discomfort on exam	Severe signs with or without symptoms
Other Features[3]	No GVHD	Mild	Moderate	Severe

NIH Consensus Criteria, 2014

1. Features to be scored by BSA: Maculopapular rash, lichen planus-like features, sclerotic features, papulosquamous lesions or ichthyosis, keratosis pilaris-like GVHD.
2. Scoring is based on severity of the signs instead of symptoms, based on limited available data and the opinions of experts. Female or male genital GVHD is not scored if a practitioner is unable to examine the patient.
3. May include ascites, pericardial effusion, pleural effusion(s), nephrotic syndrome, myasthenia gravis, peripheral neuropathy, polymyositis, weight loss without GI symptoms, eosinophilia > 500/μL, platelets < 100,000/μL, others.

Limited cGVHD – cGVHD that includes on localized skin involvement and/or liver dysfunction

Extensive cGVHD – cGVHD that includes any of the following:

- Generalized skin involvement and/or liver dysfunction
- Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis
- Involvement of the eye: Schirmer's test with < 5mm wetting, or
- Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy (labial biopsy not required), or
- Involvement of any other target organ.

APPENDIX E: REFERENCES

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