A Randomized, Double-Blind, Placebo-Controlled Multicenter Phase III Trial of Alpha 1 – Antitrypsin (AAT) Combined with Corticosteroids vs Corticosteroids Alone for the Treatment of High Risk Acute Graft-versus-Host Disease (GVHD) Following Allogeneic Hematopoietic Stem Cell Transplant

BMT CTN PROTOCOL 1705

CSL BEHRING PROTOCOL CSL964_5001

Version 5.0

PPD

PPD

Sponsored by the National Institutes of Health
National Heart, Lung, and Blood Institute
National Cancer Institute
CSL Behring
IND# 18195

[1]MD Anderson Cancer Center
[2]University of Michigan
[3]CSL Behring
[4]Levine Cancer Institute
[5]Fred Hutchinson Cancer Research Center
[6]National Heart, Lung, and Blood Institute
[7]Center for International Blood and Marrow Transplant Research, Medical College of Wisconsin
[8]Emmes
[9]Cleveland Clinic
[10]Baylor College of Medicine
[11]Dana-Farber Cancer Institute
[12]H. Lee Moffitt Cancer Institute
[13]Memorial Sloan-Kettering Cancer Center
[14]Children's Healthcare of Atlanta
[15]Washington University in St. Louis
[16]National Cancer Institute

PPD

PROTOCOL SYNOPSIS

A Randomized, Double-Blind, Placebo-Controlled Multicenter Phase III Trial of Alpha 1 – Antitrypsin (AAT) Combined with Corticosteroids vs Corticosteroids Alone for the Treatment of High-Risk Acute Graft-versus-Host Disease (GVHD) Following Allogeneic Hematopoietic Stem Cell Transplant

Co-Chairs: PPD

Study Design: This study is a phase III, multicenter, double-blinded, randomized, placebo-controlled trial designed to compare AAT and corticosteroids (CS) to placebo and CS as first line therapy for patients with high-risk acute GVHD.

Primary Objective: The primary objective of this trial is to compare the rate of complete response (CR) and partial response (PR) on Day 28 post-randomization between AAT and CS versus placebo to match (PTM) and CS in patients with high-risk acute GVHD.

Secondary Objectives: Secondary objectives are to assess the following:

1. Duration of response at 6- and 12-months post-randomization.
2. Cumulative incidence of non-relapse mortality (NRM) at 6- and 12-months post-randomization.
3. Overall survival (OS) and progression free survival (PFS) at 6- and 12-months post-randomization.
4. GVHD-free survival at Day 56 post-randomization.
5. Proportions of CR, very good partial response (VGPR), PR, and treatment failure (TF) at Days 7, 14, 21, 28, 56, and 86 post-randomizations.
6. Proportion of patients with CR, PR (including subset with VGPR), and treatment failure (TF) at Days 7, 14, 21, 28, 56, and 86 post-randomization who receive ruxolitinib or other second line therapies approved by the protocol Chairs as next-line therapy and remain on AAT/PTM.
7. Incidence of systemic infections to assess safety.
8. Incidence of Adverse Events (AEs) at 30 days post last dose of drug to assess safety.
9. Incidence of chronic GVHD at 6- and 12-months post-randomization.
10. Incidence of disease relapse at 6- and 12-months post-randomization.

Exploratory Objectives: Exploratory objectives are as follows:

1. AAT levels in serum at Days 1, 8, 16, 24, 28, and 56 post-treatment initiation.
2. Stool concentrations of AAT at baseline and at Days 8 and 28 post-treatment initiation.
3. Blood ratios of T regulatory to T effector cells (Treg/Teff), Natural Killer (NK) cells and cellular immune subsets at baseline and at Days 14, 28, and 56 post-treatment initiation.
4. Serum levels of inflammatory cytokines and biomarkers at baseline and at Days 8, 28 and 56 post-treatment initiation.
5. Overall and organ-specific response rates comparison based on Minnesota (MN) risk groups (Revised Minnesota High vs Standard) and organ-specific response rates comparison based on biomarker-based risk groups.
6. Corticosteroid dose at baseline, Days 7, 14, 21, 28, 56, 86, 6 months and 12 months post-randomization.
7. CMV reactivation requiring therapy by Day 56 post-randomization.
8. Change in patient-reported outcomes from baseline to Day 28, Day 56, and 6-months post-randomization.

Inclusion Criteria: Patients meeting the eligibility criteria should be enrolled as soon as possible after the start of CS, but no more than 72 hours afterwards.

1. Patients experiencing their initial presentation of acute GVHD requiring systemic therapy after allogeneic transplant for any malignant or non-malignant indication.
2. The clinical diagnosis of acute GVHD requiring systemic therapy with CS. Patients can be enrolled with only a clinically established diagnosis. Biopsy of involved organs with acute GVHD is encouraged but is not required and should not delay study entry. Enrollment/randomization includes commitment to continue steroids with PTM or AAT as specified in the protocol, as well as the required follow-up observations. If, according to institutional practice, the intention to treat is dependent upon biopsy results, the patient should not complete enrollment on the BMT CTN 1705 study until the biopsy results are available.
3. Acute GVHD must meet one (either A or B) of following clinical features within 72 hours prior to enrollment:
    (A) High-risk by Refined Minnesota Criteria (any one below):

    Single organ involvement

    a. Stage 4 skin
    b. Stage 3-4 lower GI
    c. Stage 1-4 liver

Multiple organ involvement

a. Stage 1-2 lower GI plus any liver
b. Stage 2 lower GI plus any skin
c. Stage 3-4 lower GI plus any liver or skin
d. Any three organ involvement

OR:

(B) Either of the below:

1. Isolated stage 2 involvement of the lower GI tract
2. Stage 1 lower GI tract disease with skin involvement

4. Acute GVHD developing after allogeneic hematopoietic cell transplantation using any graft or donor source or conditioning intensity.
5. Patients should not have received systemic immune suppressive therapy for treatment of active GVHD except for a maximum of 72 hours of prior CS therapy prior to enrollment. Topical skin and GI CS (such as budesonide and oral beclomethasone diproprionate) are allowed.
6. Patients 12 years of age or older at time of enrollment.
7. Ability to provide written informed consent from patient, parent or legal guardian, and assent if applicable.

Exclusion Criteria

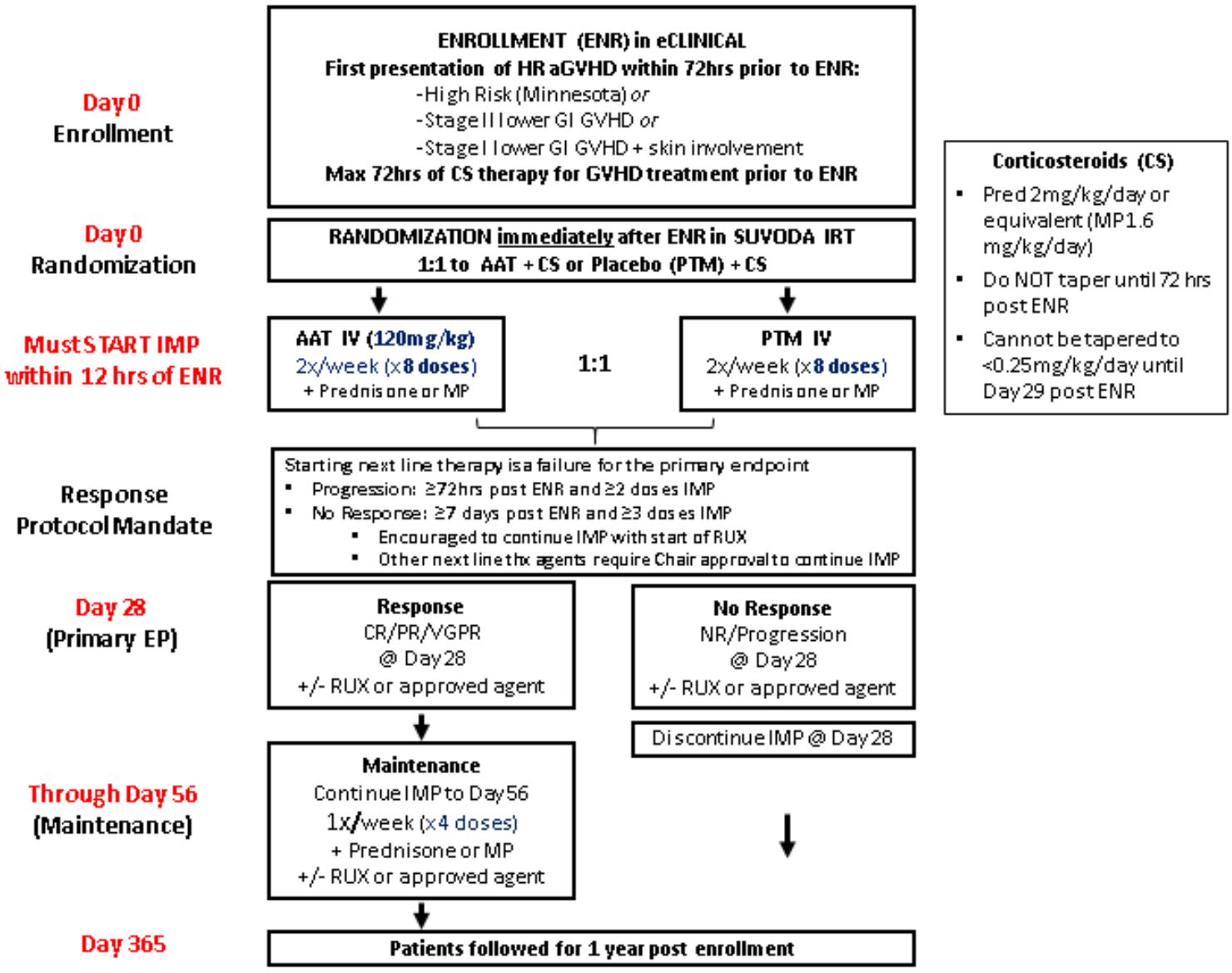
1. Patients with prior exogenous AAT exposure for GVHD prophylaxis.
2. Relapsed, progressing or persistent malignancy
3. Evidence of minimal residual disease (MRD) requiring withdrawal of systemic immune suppression.
4. Patients with acute GVHD developing after administration of a donor lymphocyte infusion (DLI) for relapse / progression of disease. Patients with acute GVHD after planned donor lymphocyte infusion or planned T cell or NK cell add back are eligible.
5. Patients with uncontrolled infections will be excluded. Infections are considered controlled if appropriate therapy has been instituted and, at the time of enrollment, no signs of progression are present. Progression of infection is defined as hemodynamic instability attributable to sepsis, new symptoms, worsening physical signs or radiographic findings attributable to infection. Persisting fever without other signs or symptoms will not be interpreted as progressing infection.

6. A clinical presentation resembling de novo chronic GVHD or overlap syndrome (as defined in Appendix C) developing before or present at the time of enrollment.
7. Patients receiving other drugs for the treatment of GVHD. Note, GVHD prophylaxis agents (e.g., calcineurin inhibitors) may be continued at local Investigator's discretion.
8. Patients receiving systemic CS for any indication within 7 days before enrollment, except the following:
    a. CS administered as premedication for supportive care (such as before transfusion of blood products or before intravenous medications to prevent infusion reactions, fever, etc.).
    b. If steroid therapy has been administered for treatment of a non-GVHD related condition and tapered to < 0.6 mg/kg/day prednisone (0.5 mg/kg/day methylprednisolone) for 7 or more days prior to enrollment.
    c. Treatment of active GVHD with CS is allowed for up to 72 hours prior to enrollment.
9. Patients who are pregnant or breastfeeding.
10. Females of childbearing potential (FCBP) or males who can get a FCBP pregnant and have sexual contact with FCBP and are unwilling to use 2 effective forms of birth control or abstinence from the start of study drug treatment through 30 days after the last dose of study drug, Effective forms of birth control are listed in Appendix D.
11. Patients on renal replacement therapy.
12. Patients requiring continuous supplemental oxygen (O2 requirement > 2L/min to maintain peripheral O2 saturation [SpO2] > 90%).
13. Patients with active hepatic sinusoidal obstructive syndrome (SOS) and/or clinical evidence of impaired hepatic function (ascites or encephalopathy related to liver disease) who in the judgment of the treating physician are not expected to have normalized bilirubin by Day 56 after enrollment.
14. Patients with a history of hypersensitivity to AAT or any component of the investigational product or PTM (albumin), including congenitally IgA-deficient patients with antibodies to IgA or PTM.
15. Patients unlikely to be adherent to study specific assessments at the transplant center.

Treatment Description: All patients will receive prednisone 2 mg/kg/day PO (or methylprednisolone (MP) at 1.6 mg/kg/day IV) divided into 1-2 daily doses for at least 72 hours after enrollment. CS may be

	tapered as tolerated according to institutional practice. However, corticosteroid taper may not start sooner than 3 days after enrollment and the prednisone dose cannot be less than 0.25 mg/kg/day prednisone (MP 0.2 mg/kg/day) at Day 28 post-enrollment. Patients will be randomized 1:1 to receive AAT or PTM. Patients will receive AAT/PTM on Days 0 or 1, 4, 8, 12, 16, 20, 24, and 28. Responding patients will continue to receive AAT/PTM on Days 35, 42, 49, and 56.
Accrual Objective:	136 total patients will be enrolled and randomized 1:1 to AAT vs PTM.
Accrual Period:	The estimated accrual period is 3 years.
Study Duration:	Patients will be followed for 12 months following randomization.
Interim Analysis:	The study will consist of one interim analysis for futility after 76 patients are evaluable, at a time coincident with a regularly scheduled meeting of the National Heart, Lung, and Blood Institute (NHLBI)-appointed Data and Safety Monitoring Board (DSMB) review, then a 12-month analysis after accrual and all patients follow up is complete. An additional analysis will be conducted after all randomized patients complete 6 months of follow-up. Multiplicity adjustment is not needed for this analysis as all the patients would have completed the primary endpoint visit at Day 28 by the time of analysis. Policies and composition of the DSMB are described in the BMT CTN Manual of Procedures.
Stopping Guidelines:	The stopping guidelines serve as a trigger for consultation with the DSMB for additional review and are not formal “stopping rules” that would mandate automatic closure of study enrollment. Safety monitoring will be based on Day 56 NRM. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each meeting.

STUDY SCHEMA



IMP and CS dosing is the same for adolescent and adult patients.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1 BACKGROUND AND RATIONALE

1.1 Introduction

Acute graft-versus-host disease (GVHD) is a frequent complication of allogeneic hematopoietic cell transplantation (HCT) involving activation of donor T-lymphocytes against host tissues[1]. Despite immune suppression prophylaxis, up to 30-50% of HCT recipients will experience acute GVHD of varying severity[1-3]. The syndrome of acute GVHD involves multiple target organs, including the skin (presenting most frequently as a maculopapular rash), intestinal tract (presenting as nausea/vomiting and/or diarrhea), and liver (presenting as cholestatic liver injury with or without transaminase elevation).

The mainstay of treatment of acute GVHD is high dose corticosteroids (CS), typically initially dosed at the prednisone equivalent of 1-2 mg/kg per day[4]. High dose CS have several shortcomings, including both limited efficacy as well as toxicity including infections, hyperglycemia, hypertension, hyperlipidemia, osteoporosis and osteonecrosis. Approximately 50% of patients respond to CS, while only 30% have a durable response[5-7]. Despite these limitations, no prospective study has demonstrated an advantage in response rate or survival for the addition of a second agent to CS for the treatment of steroid naïve acute GVHD[8-13].

1.2 Results of Previous Acute GVHD Therapy Trials

To date, two multicenter acute GVHD treatment trials have been conducted in the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The first, BMT CTN 0302, was a multicenter, randomized, four-arm phase II trial that was designed to identify the agent most promising for use in addition to CS for the front-line therapy of acute GVHD[8]. Patients with a new diagnosis of acute GVHD were randomized to receive either etanercept, mycophenolate mofetil (MMF), denileukin diftitox, or pentostatin in additional to high dose CS. The proportion of Day 28 CR/PR was highest in the MMF arm at 60%. Thus, MMF was selected for a randomized phase III trial of MMF versus placebo in addition to CS for first-line therapy of acute GVHD in the subsequent study, BMT CTN 0802[13]. In BMT CTN 0802, MMF did not meet the primary endpoint of extending GVHD-free survival at Day 56 at a planned interim analysis of 235 patients, and the trial was terminated for futility.

It has been suggested that the enrollment of all newly diagnosed cases without regard to the likelihood of response to steroids alone or GVHD severity diminished the ability of these trials to show improvement beyond standard therapy. Neither BMT CTN 0302 nor 0802 was risk-stratified according to clinical or biomarker-based strategies (nor any such trial published to date). To that end, application of risk-adaptive approaches targeting patients who have a lower likelihood of responding to steroids alone and; accordingly, a high-risk for non-relapse mortality (NRM), may identify cohorts capable of benefiting from intensification of immune suppression.

1.3 Risk Stratification of Acute GVHD

Identifying which patients are at increased risk of becoming steroid-refractory may help to better test novel agents in combination with CS or perhaps even alone. Different approaches to risk stratify patients with acute GVHD have been recently developed and refined. These strategies include models built upon initial clinical staging of acute GVHD target organs (e.g., skin, intestinal tract, and liver) and serum biomarker-based approaches. Both of these strategies hold

value in identifying patients who are likely to respond well (i.e., demonstrate a complete response [CR] or partial response [PR]) to CS and thus less likely to die due to complications of the transplant (NRM)[14-16].

1.3.1 Risk Stratification by Clinical Grading using Onset Organ Severity

In 1990, Weisdorf et al. identified in multivariate analyses that overall stage score (sum of each acute GVHD organ stage 0-4, plus 1 point for upper GI, for a maximum score of 13) was strongly associated with likelihood of CR[17]. Based upon this initial observation that single organ involvement was more likely to achieve a CR than multi-organ involvement, the GVHD Risk Score was subsequently developed by the Minnesota group[18]. Multivariate analysis of the outcomes of 864 consecutive patients from 1990-2007 yielded the following high-risk organ stages: skin stage 4, lower gastrointestinal stage 3/4, liver stage 3/4, or skin stage 3 plus lower gastrointestinal or liver stage ≥ 2 GVHD.

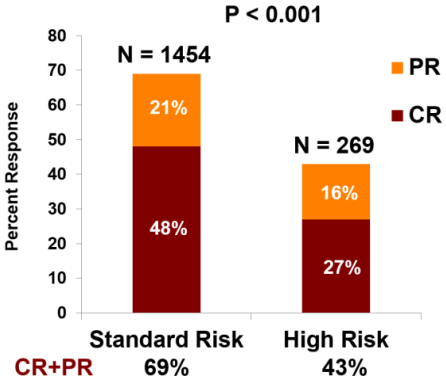


Figure 1-1 Comparison of Day 28 CR/PR in MN Standard vs

The GVHD Risk Score has recently been refined using data from multiple centers with a total of 1,723 patients used in modeling – the largest acute GVHD cohort analyzed for their characteristics and outcomes to date[19]. Developed using clinical grouping and recursive partitioning, this new Risk Score (http://z.umn.edu/MNAcuteGVHDRiskScore) [referred to as Minnesota (MN) Risk Scores] can classify patients into high-risk (HR) or standard risk at the onset of acute GVHD symptoms. In this model, 84% of patients are classified as standard risk, defined as single organ involvement (stage 1-3 skin or stage 1-2 GI) or two organ involvement (stage 1-3 skin plus stage 1 GI; or stage 1-3 skin plus stage 1-4 liver), with a Day 28 CR/PR rate of 69% (Figure 1-1). All others are considered HR, with a Day 28 CR/PR rate of 43%. This model can be used in real time at the time of acute GVHD diagnosis, making it practical for stratification in clinical trials. Similarly, this model predicted for 6-month NRM (Standard risk 22% vs High risk 44%, p-0.001).

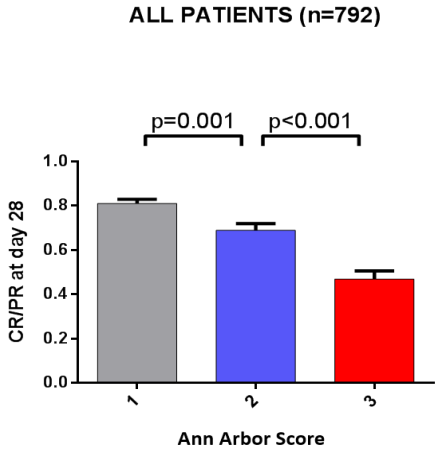


Figure 1-2 Comparison of Day 28 CR/PR in AA1, AA2, and AA3 biomarker-based risk stratified patients

1.3.2 Risk Stratification by Blood Biomarkers

Development of risk profiles for patients with newly diagnosed acute GVHD that are predictive of outcomes independent of clinical staging have been a major advancement in acute GVHD research. Serum proteomic patterns associated with acute GVHD were first published approximately a decade ago[20, 21]. The University of Michigan group has significantly expanded the proteomic profiling of patients with acute GVHD in recent years by developing a 3-level risk stratification system, Ann Arbor 1 (AA1) low-risk acute GVHD, Ann Arbor 2 (AA2) intermediate-risk acute GVHD, and Ann Arbor 3 (AA3) high-risk acute GVHD. The Ann Arbor score is based upon serum or plasma levels of tumor necrosis factor receptor-1 (TNFR1), regenerating islet-derived 3-alpha (REG3α), and suppression of tumorigenicity 2 (ST2) measured at diagnosis of GVHD, regardless of clinical severity (grades I-IV). Each Ann Arbor score corresponds to a distinct risk of six-month NRM, such that AA1 GVHD has < 10% NRM, AA2 GVHD ~25% NRM, and AA3 GVHD has > 40% NRM. The scoring algorithm was validated in

an independent test set of patients with acute GVHD from the University of Michigan and the University of Regensberg, and in a separate validation set from the BMT CTN acute GVHD treatment trials 0302 and 0802[7]. Relapse rates do not differ between Ann Arbor scores and thus the differences in NRM translate into significant differences in survival. Differences in response to treatment account for the vast majority of differences in NRM. Patients with AA1 demonstrate 81% CR/PR at Day 28 and AA2 patients demonstrate 68% CR/PR at Day 28 (Figure 1-1 and Figure 1-2). In contrast, high risk AA3 patients demonstrate 46% CR/PR at Day 28. Furthermore, treatment responses for patients with AA3 GVHD are significantly less likely to be durable compared to AA1 and AA2 patients with only 21% of AA3 patients remaining in a CR without a flare at six months from diagnosis compared to 47% of AA1/2 patients ($p < 0.001$). Importantly, similar proportions of patients are assigned to each Ann Arbor score in each of the three standard groupings of Glucksberg grades (I vs II vs III/IV). Thus, approximately 20% of all newly diagnosed GVHD cases have a high-risk biomarker profile and have lower likelihood of response to primary treatment and experience high NRM even when the clinical presentation is not severe, such as a < 50% skin rash (grade 1 acute GVHD). Further, patients who present with a limited skin rash yet have a high-risk biomarker panel (AA3) have been shown to have high NRM due to subsequent development of steroid-refractory lower GI GVHD. As such, the biomarker panel appears to identify patients who have or will develop high risk lower GI GVHD. More recently, the same group has validated these results using two of the original three biomarkers (ST2 and REG3α)[22]. While promising to date, biomarker-based risk stratification has heretofore not been prospectively validated for determining eligibility in the context of a large multi-center GVHD treatment trial.

1.3.3 Impact of Lower GI GVHD on NRM: Further Refinement of the Revised Minnesota Prognostic

As confirmed by the Minnesota grading system, isolated acute GVHD of the skin is generally not life-threatening with the majority of patients responding to topical and/or systemic steroids. On the other end of the spectrum, patients who[19, 23] experience advanced stage 3-4 acute GVHD of liver (peak total serum bilirubin > 6 mg/dL) have poor outcomes but represent only 2% of the current transplant population[19, 24]. While the incidence of severe gastrointestinal GVHD (stage 3-4, or peak diarrheal volumes over 1.5 liters per day) has also decreased during the past decade, treatment remains unsuccessful in most cases, and the gastrointestinal tract is involved in virtually all fatal cases of acute GVHD[24]. Accordingly, reducing acute GVHD mortality is contingent upon improving responses in patients with lower GI GVHD.

Two distinct phenotypes of gut GVHD can be identified—upper gut (limited to stage 1) and mid-lower gut—that differ in presentation, natural history, response to therapy, and risk of mortality. The upper gut phenotype generally does not progress to the mid-lower gut phenotype and has been shown not to impact NRM[25, 26]. In contrast, the mid-lower gut phenotype of GVHD presents with secretory, protein-rich diarrhea[27, 28]. In severe cases, the entire small intestine and colon are edematous and inflamed, with diarrheal volumes in excess of 1.5 liters per day often associated with mucosal ulceration, sloughing and bleeding and severe abdominal pain. Most patients with severe mid-lower gut GVHD require prolonged hospitalization for supportive care including total parenteral nutrition and pain control. Although outcomes are typically poor, the standard initial therapy remains prednisone at 2 mg/kg/day, with addition of other immune suppressive therapies only when treatment with prednisone fails to control the disease. Despite this approach, outcomes are poor in the approximately 50% of patients who do not respond to high dose CS due to a high likelihood for treatment resistance to additional lines of immune suppression, infection, and considerable NRM. While de novo development of stage 3-4 lower

GI GVHD clearly represents high risk acute GVHD, only a small percent (8%) of patients present with this stage, with the vast majority developing advanced organ stage later in their disease course while already on primary treatment (termed steroid-refractory)[19]. Taking this into consideration, targeting any patient who presents with diarrhea (regardless of volume) for clinical trials would be one strategy to reduce GI GVHD-related mortality. Unfortunately, amongst patients who present with any lower GI tract involvement, a sizable fraction may do well with a steroid-alone approach, thereby diminishing the ability to detect a meaningful effect of investigational therapy.

Recently, a database of 309 consecutive patients with GI GVHD was analyzed from The University of Texas, MD Anderson Cancer Center between the years of 2009-2012[29]. Roughly half of the patients presented with isolated upper GI GVHD with minimal (< 500 cc in 24 hours) stool output. An analysis of all the remaining patients with stage 1 or higher lower GI GVHD (either isolated or combined with another organ) is shown in Table 1-1. From this analysis, patients with isolated lower GI stage 1 GVHD appear to have a higher Day 28 response rate with roughly 3/4 of the patients responding to first-line therapy. Similar results were seen for patients enrolled onto two consecutive acute GVHD trials within the Blood and Marrow Transplant Clinical Trials Network (BMT CTN 0302 and 0802 trials) with a 75% Day 28 response rate to first-line therapy for those presenting with isolated stage 1 lower GI GVHD (Table 1-1). However, from these datasets, patients with isolated lower GI stage 2 or stage 1 lower GI plus skin have a Day 28 response rate of 50-57% and despite being otherwise "standard risk" per the Minnesota Clinical Staging system define a population at risk for treatment resistance who may potentially benefit from a high-risk treatment protocol. Importantly, combining these subsets of GI GVHD with the Minnesota HR groups would increase study eligible patients from roughly 15% to 25-30%.

Table 1-1 Day 28 Response Rate for Patients with Lower GI GVHD

MD Anderson Cancer Center			
	N	Day 28 CR/PR (%)	6-month NRM (95% Confidence Interval)
Isolated Lower GI			
Stage 1	37	27 (73%)	33% (21-53)
Stage 2*	14	7 (50%)	29% (12-65)
Stage 3 - 4	23	11 (48%)	39% (23-65)
Lower GI + any liver			
Stage 1	9	5 (56%)	56% (31-99)
Stage 2	5	2 (40%)	40% (14-100)
Stage 3 - 4	12	2 (17%)	58% (36-94)
Lower GI + any skin			
Stage 1*	19	10 (53%)	33% (16-68)
Stage 2	10	5 (50%)	20% (6-69)
Stage 3 – 4	17	6 (35%)	53% (34-83)
BMT CTN 0302 / 0802 Trials			
	N	Day 28 CR/PR (%)	6-month NRM (95% Confidence Interval)
Isolated Lower GI			
Stage 1	8	75%	25%
Stage 2*	9	56%	44%
Stage 3 - 4	15	67%	27%
Lower GI + any liver			
Stage 1	7	43%	43%
Stage 2	8	50%	37.5%
Stage 3 - 4	5	0%	80%
Lower GI + any skin			
Stage 1*	30	57%	30%
Stage 2	17	41%	41%
Stage 3 - 4	17	41%	41%
***Patients designated as Minnesota Standard Risk considered appropriate for High-Risk Trial**			

1.4 Study Hypothesis and Design

Identification and novel treatment strategies for patients at risk for steroid resistance remains a major unmet clinical need. In this context it is hypothesized that administration of additional immune suppression capable of increasing response rates without toxicity will limit further progression of GVHD and improve outcomes. Reflective of the above advancements in risk stratification for patients with newly diagnosed acute GVHD, we propose enrolling patients with newly-diagnosed Minnesota HR, isolated lower GI Stage 2 or Lower GI stage 1 plus any skin onto a prospective, double-blind, randomized, phase III trial of prednisone at a dose of 2 mg/kg/day (or methylprednisolone- equivalent) plus α_1- Antitrypsin (AAT) or placebo.

Our hypothesis is that the addition of the AAT, Zemaira®, a therapy with a demonstrated safety record as well as pre-clinical and clinical data in the setting of acute GVHD, will improve Day 28 acute GVHD response when added to CS in patients with newly diagnosed, HR acute GVHD. Patients who achieve a CR or PR to GVHD treatment at Day 28 have improved long-term outcomes (survival and NRM) when compared to non-responders and serves as a validated primary endpoint for acute GVHD trials.

1.5 Alpha 1-Antitrypsin (AAT)

Alpha 1-Antitrypsin (AAT) is a naturally occurring 52-kDa circulating protease inhibitor produced by the liver that inactivates several serine proteases from neutrophils and macrophages and protects tissues from proteolytic degradation. Serum AAT concentrations in healthy individuals range from 1.5 to 2.5 mg/ml and can increase twofold during inflammation, coinciding with AAT's role as an acute phase reactant[30]. Congenital deficiency in AAT results in emphysema and cirrhosis of the liver[31, 32]. In addition, AAT deficiency has been associated with the development of various autoimmune diseases including necrotizing panniculitis, vasculitis, inflammatory bowel disease and glomerulonephritis[33]. AAT is licensed for long-term augmentation and maintenance therapy in adults with severe hereditary deficiency of AAT with clinically evident emphysema. Consistent safety of the investigational product when administered intravenously (IV) for AAT deficiency has been observed in more than 14 years of use, with 833,197.22 standard doses sold worldwide for the period between 01 July 2003 and 20 August 2017, corresponding to 16,023 patient-years. Post-marketing surveillance has shown that AAT is safe and well tolerated when used at the recommended dosage with no association with increased risk of infection. Recent research has elucidated immune regulatory roles of AAT, independent of protease inhibition, leading to growing interest in this therapy across various inflammatory and immune-mediated conditions.

1.5.1 Alpha 1-Antitrypsin (AAT) as an Immunomodulatory

A growing body of evidence has demonstrated AAT to not only possess the ability to inhibit serine proteases, such as elastase and proteinase-3, but also exert anti-inflammatory and tissue protective effects independent of protease inhibition. AAT appears to effectively interfere with inflammatory responses and protect from cell death in a large number of *in vivo* and *in vitro* experimental models reviewed in Table 1-2 and Table 1-3. A noteworthy example includes the blockade of inflammatory cytokine release from human peripheral blood mononuclear cells[34]. Specifically, AAT decreases the production of important inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1β, two prototypical upstream mediators of inflammation. AAT also lowers the levels of chemokines IL-8 and monocyte chemotactic protein (MCP)-1, two major cytokines important in the trafficking of inflammatory cells. While the activity of proinflammatory cytokines appears to consistently diminish in the presence of AAT, the release of anti-inflammatory mediators increases. For example, the endogenous inhibitor of IL-1 activity, IL-1 receptor antagonist, is upregulated by AAT in human blood cells[35]. Similarly, IL-10 levels have been shown to increase by AAT in various experimental conditions[34, 36-39]. An examination of the cellular targets of AAT, demonstrates it to be specific for members of the innate immune system, such as macrophages and neutrophils, as well as B-lymphocytes and dendritic cells. In contrast, responses of purified T-lymphocytes are consistently unaffected[37, 38, 40-43], allowing for a variety of responses to concanavalin A and anti-CD3/CD28 stimulation to persist. This cell-specific discretion, together with the ability to protect tissues from injury, sets AAT in a unique niche among modulators of the immune system and distinguishes it from classic immunosuppressants.

Table 1-2 Selected In Vivo Biological Activities of AAT[44]

	In vivo model	Outcome	Reference
Modulation of adaptive immunity	Islet allograft immune response	Graft survival prolonged, immune cell infiltration reduced, intragraft insulin content increased, intragraft vascular endothelial growth factor (VEGF) transcript levels elevated	40
		Grafts accepted, immune tolerance achieved, Tregs localized at graft sites, systemic and local IL-1RA elevated.	38
	Islet autoimmune response	Islet function preserved, immune tolerance achieved, auto-and alloreactive grafts accepted.	37, 45-47
	Cell allograft immune response	Day 1-5 immune cell infiltration reduced, including macrophages, neutrophils, T cells and NK cells	40
	Collagen-induced arthritis	Delayed disease onset, lower disease score	48, 49
	Experimental autoimmune encephalomyelitis	Decreased disease incidence, lower disease scores, Increased Treg proportions in lymphoid compartments	36
	GVHD (major histocompatibility complex [MHC] disparate bone marrow transplantation)	Attenuation (posttreatment) and prevention (pretreatment) of GVHD, reduced expansion of alloreactive T cells, enhanced recovery of Tregs, reduced serum levels of proinflammatory cytokines and superior survival	41, 42
In vivo leukocyte infiltration	Thioglycolate-elicited peritoneal infiltration	Infiltrating macrophages and neutrophils diminished	40
	Acute myocardial infarction	Myocardial leukocyte infiltration diminished	50
	Experimental autoimmune encephalomyelitis	Decreased Spinal leukocyte infiltration	36
In vivo innate responses	Systemic lipopolysaccharide (LPS) challenge (mice)	Anti-inflammatory serum cytokine profile including elevated IL-1Ra and IL-10 and greater levels of Foxp3FOXP3 Tregs	38
	Lung LPS challenge (rabbits)	Lung Function and arterial blood gas improved, bronchoalveolar neutrophil elastase, TNF-α and IL-8 reduced	51
In vivo cell injury	Toxic β-cell injury	48-h cell death reduced, insulin release preserved	40, 52
	Acute myocardial injury after Left anterior descending occlusion and myocardial infarction	Reduced infarct size, decreased caspase-1 tissue levels, reduced post-infarct remodeling	50

Table 1-3 Selected In Vitro Biological Activities of AAT[44]

In vitro assay	Cellular targets	Outcomes	Reference
Cell function			
Glucose-stimulated insulin secretion	• Mouse islets • Human Islets	• Cytokine-dampened insulin release restored • Impure islet culture insulin release improved	40 53
Collagen 1 production during wound healing	• B-cell lines • Skin Fibroblasts	• Insulin release improved • Production increased	54 55
Cell survival			
Proinflammatory cytokine toxicity	• Primary mouse islets • Rat INS-1 cell line	• Lactate dehydrogenase (LDH) release diminished • Cell death reduced	40 54
B-cell-specific toxin (steptozotocin)	• Murine MIN-6 cell line	• Apoptosis reduced	52
Casapase-3-induced apoptosis	• Primary lung alveolar endothelial cells	• Apoptosis reduced	56
LPS and ischemia-induced injury	• Adult cardiac myocyte cell line HL-1	• Cell death reduced	50
Immune cell cytokine production			
LPS stimulation	• Human peripheral blood mononuclear cells (PBMC)	• Reduced proinflammatory cytokine release	57
Heat-inactivated *S. epi* stimulation	• Human PBMC	• Reduced proinflammatory cytokine release	34
Steady-state	• Nonstimulated murine B cell • Human neutrophils	• Steady-state B cell activating factor production decreased • Reduced TNF-α release	48 58
LPS stimulation	• Human PBMC	• Reduced IL-32	41
Mixed lymphocyte reaction	• Murine OT-II T cells and ovalbumin (OVA-)-loaded Dendritic cell	• Reduced IL-6, elevated IL-2 and elevated IL-10	39
Immune cells not directly targeted by AAT			
• In vitro immunization • Concanavalin A stimulation • CD3/CD28 stimulation	• Mouse splenocytes • Mouse splenocytes • Purified mouse T cells	All 3 models: • Intact T-cell clumping, proliferation, response to IL-2 and IFNγ release	48 40 37, 42

1.5.2 Alpha-1 Antitrypsin in Pre-Clinical Models of GVHD

It is believed that AAT acts as an immunomodulator[59], allowing for expression of regulatory T-cell repertoires that support chimerism, immune tolerance, and protection of allografts. AAT was shown to inhibit cytokine release in a mouse model and is likely to have an important protective role against inflammatory responses in GVHD due to its function as an acute-phase reactant.

Studies suggest that inflammatory cytokines play a key role in the pathogenesis of GVHD[60-62]. Inflammatory cytokines promote end organ damage and so their suppression should be beneficial to patients at GVHD symptom onset. In multiple experimental models of allogeneic HCT, the administration of AAT appears to have a number of effects: induction of anti-inflammatory cytokines and suppression of anti-inflammatory cytokines; indirectly altering the *in vivo* ratios of donor effector T-cells (Teff) to donor regulatory T-cells (Tregs); interference with activation of dendritic cells; and suppression of the development of GVHD[42, 63, 64]. In three independent laboratories using several different murine models of GVHD, administration of AAT improved survival and GVHD-severity scores when compared to mice treated with control, human albumin. Despite altering these ratios, AAT exposure did not impact T-cell function (either Tcon or Treg) in vitro and in minimal residual disease (leukemia) models, AAT exposure did not appear to inhibit the graft-versus-leukemic effect. The impact of AAT on T-cell ratios was determined to result from the suppression of pro-inflammatory cytokine secretion, namely, TNF-α, IL-1β and IL-6 and increased secretion of anti-inflammatory cytokines IL-10 and IL-6 via inhibition of NF-κβ in dendritic cells.

More recently, the mechanisms through which excessive inflammatory responses contribute to the pathophysiology of GVHD have been studied and suggest that AAT may be able to attenuate immune responses amplified by tissue injury. Detection of tissue damage by innate immune cells using pattern-recognition receptors (PRRs) that sense noninfectious molecular signatures [called danger-associated molecular patterns (DAMPs)] is known to augment adaptive immunity[65]. Murine models have demonstrated that one such DAMP, heparan sulfate (HS), an extracellular matrix component, activates Toll-like receptor 4 on dendritic cells *in vitro*, leading to the enhancement of dendritic cell maturation and alloreactive T-cell responses. In addition, it was demonstrated that serum HS levels were acutely elevated at the onset of clinical GVHD in mice after allo-HSCT and treatment of mice with AAT decreased serum levels of HS, leading to a reduction in alloreactive T-cell responses and GVHD severity[63]. Conversely, an HS mimetic that increased serum HS levels accelerated GVHD. Further, in patients undergoing allo-HSCT for hematologic malignancies, serum HS levels were found to be elevated and correlated with the severity of GVHD. These results identify a critical role for HS, and more broadly DAMPs, in promoting acute GVHD after allogeneic HSCT and suggest that modulation of these inflammatory stimuli may have therapeutic potential for the control of clinical GVHD.

1.5.3 Safety and Efficacy of AAT in Patients with Steroid-Refractory Acute GVHD

An investigator-initiated phase 2 open-label study with Zemaira® was conducted in 40 steroid-refractory acute GVHD patients (median age 50 years, range 18-70) at a dose of 60 mg/kg twice weekly for 4 weeks[66]. One third of the patients had lower GI GVHD; all stage 3 or 4, while a quarter had liver-involvement (all stage 3/4). The overall response rate and complete response rate by Day 28 was 65% and 35%, respectively, and included responses in all GVHD target organs. At Day 60, responses were sustained in 73% of patients without intervening immunosuppression. Organ-specific responses were seen in skin, GI and liver. Importantly, 50% of patients with steroid-resistant lower GI GVHD achieved a Day 28 CR. The treatment was well tolerated, with no infusion reactions or drug-related Grade 3/4 adverse events. A total of 7 patients died within 30 days of the last infusion of AAT with 5/7 of these patients having progressive acute GVHD, 1 patient dying from gram negative sepsis and 1 patient from interstitial pneumonia syndrome. 6-month overall survival was 45% [95% confidence interval (CI), 32-63%].

In a second study, an open-label phase 1/2 clinical trial of Glassia® (Baxalta/Kamada) for steroid-refractory acute GVHD was undertaken in 12 patients following HCT[67]. Patients received

AAT over a 15-day course: In Cohort 1, the loading dose of AAT was 90 mg/kg IV on Day 1, followed by maintenance doses of 30 mg/kg/day IV on Days 3, 5, 7, 9, 11, 13, and 15. In Cohort 2, the loading dose was 90 mg/kg IV on Day 1, followed by 7 maintenance doses of 60 mg/kg/day IV on the same schedule (total weekly dosage of 270 mg/kg). Eight of the 12 subjects included in the study, who had received allogeneic HCT, showed improvement in GVHD manifestations, with four showing complete improvement. Treatment response was particularly apparent in terms of sigmoidoscopic appearance of the GI tract and GI-related symptoms. Survival at the last follow-up (> 104 to > 820 days) was 50%. In this study, no clinically relevant signs of toxicity attributable to treatment with AAT were reported.

1.5.4 Clinical Efficacy, Safety and Pharmacokinetic Data with AAT in Humans

Clinical efficacy, safety, and pharmacokinetic (PK) data are available for the investigational product from 6 completed studies in subjects with Alpha 1-antitrypsin (also called Alpha1-Proteinase Inhibitor (A1-PI)) deficiency and/or subjects with emphysema. The results of these studies are presented in this section. Summaries of the studies are provided in Table 1-4.

Table 1-4 Clinical Studies Conducted in Subjects with A1-PI Deficiency and/or Subjects with Emphysema

Study Number	No. of subjects enrolled	Route, regimen, duration	Dose (mg/kg/body weight)	Results
RPR118635-101 (phase 1)	19	IV, single-dose	15 30 60 120	The terminal half-life of AAT was estimated to be approximately 5 days, independent of dose. Concentrations of antigenic AAT in epithelial lining fluid were approximately 10% of the serum AAT concentrations and demonstrated diffusion into the lung. There were no clinically relevant safety findings that could be directly attributed to AAT.
RPR118635-201 (phase 2/3)	9	IV, weekly for 6 months, 3-5 months of compassionate use	60	Weekly serum AAT measurements showed that AAT was successful in maintaining trough serum concentrations well above the threshold of 0.6 mg/mL. AAT was safe and well tolerated.
CE1226/2-1002 (phase 1b) vs Prolastin	18	IV, single dose	60	Non-inferiority of AAT to Prolastin in bioavailability was shown, as measured by area under the curve of functional AAT after a dose of 60 mg/kg body weight functionally active AAT. AAT was well tolerated and similar to Prolastin with respect to the incidence and severity of AEs.

Study Number	No. of subjects enrolled	Route, regimen, duration	Dose (mg/kg/body weight)	Results
CE1226/2-2002 (phase 3) vs Prolastin	44	IV, weekly for 6 months	60	Mean steady-state trough concentration of serum antigenic AAT was shown to be non-inferior to that achieved with Prolastin and was above the threshold of 0.6 mg/mL. Antigenic assays also demonstrated that AAT was delivered to the lower lung and was able to complex with its substrate, neutrophil elastase. AAT was safe and well tolerated.
CE1226_4001 (phase 3/4) vs placebo	180	IV, weekly for up to 24 months	60	The rate of change in lung density, measured as computer tomography-measured lung volume adjusted 15th percentile of the lung density (Adjusted P15), consistently showed a slower decline in subjects treated with AAT, relative to placebo. AAT was safe and well tolerated.
CE1226_3001 (phase 3, open label extension study to CE1226_4001) Delayed Start vs Early Start group[a]	140	IV, weekly for up to 24 months	60	The rate of change in lung density, measured as computer tomography-measured Adjusted P15, consistently demonstrated: • A continued slower decline across 4 years for the Early Start group • A consistent advantage favoring the Early Start group over the Delayed Start group at all time points from baseline to Month 48, i.e., the lung tissue loss during the first 2 years of placebo exposure in the Delayed Start group was permanent • A statistically significant change in the rate of annual lung density decline temporal to the administration of AAT in the Delayed Start group • Modest, statistically significant 4-year correlations between annual lung density decline rates and FEV1, FEV1%, and FVC were established • Lower rates and proportions of fast decliners temporal to administration of drug

AAT = alpha-1 antitrypsin; Adjusted P15 = measured lung volume-adjusted 15th percentile of the lung density; AE = adverse event; FEV1 = Forced expiratory volume in 1 second; FVC = Forced vital capacity
[a] Subjects receiving AAT throughout the treatment period of studies CE1226_4001 and CE1226_3001 are termed "Early Start" subjects, whilst those who received placebo during study CE1226_4001 and were then reallocated to AAT in the open-label extension study CE1226_3001 are termed "Delayed Start" subjects.

1.6 Dose Rationale for Planned Study

In this trial we propose administration of AAT at a dose of 120 mg/kg twice weekly through Day 28. Responding patients (CR/PR) will continue to receive a once weekly dose of 120 mg/kg through Day 56. As is detailed below, there is a strong rationale for studying this dose in adults and adolescents for this disease population.

1.6.1 Acute Phase Reactant Properties of AAT

AAT is the most abundant, endogenous serine protease inhibitor in the circulation [30, 68, 69]. Serum AAT concentrations in healthy individuals range from 1.5 to 2.5 mg/mL and levels have been shown to further increase in inflammatory states [30, 70]. Similar to C-reactive protein (CRP), AAT is a known acute-phase reactant whose serum levels rise in response to injury, inflammation, or infection[71]. High levels of AAT are commonly seen in various physiologic or disease states including: patients with colorectal cancer (mean 206 mg/dL, range 459 mg/dL), pregnancy (mean 427 mg/dL; range 316–582 mg/dL), and pregnancy in diabetic women (mean, 786 mg/dL; range 523-1255 mg/dL) [72-74].

1.6.2 Pre-Clinical Experience with AAT

Multiple experimental models of allogeneic HCT have shown that administration of AAT both reduces the development of, as well, as provides effective therapy for established GVHD. These models implicate a reduction of inflammatory cytokines and an induction of anti-inflammatory cytokines as being integral to AAT's efficacy[42, 63, 64]. Additional models suggest that AAT's effect on cytokines may occur in a dose-response fashion. For example, in a human pulmonary cell culture model simulating ischemia- reperfusion processes in lung transplantation, AAT significantly inhibited cell death and inflammatory cytokine release in a dose-dependent fashion *in vitro*, with maximal cell viability observed at concentration of 5mg/ml[75]. In addition, *ex vivo* assays performed on fresh whole blood from adult subjects with new-onset type 1 diabetes, AAT suppressed expression of IL-6 and IL-1β dose-dependently, with > 50% inhibition achieved in the range of 2.5 -5 mg/mL AAT[76]. Based on the totality of the evidence available, it is hypothesized that targeting AAT peak levels above the normal range or ≥ 2.5 mg/mL should down-regulate inflammatory cytokines and therefore provide protection against cytokine-mediated damage.

1.6.3 Clinical Experience with AAT in HCT Setting

Clearance of AAT as a result of stool losses has been used as a diagnostic tool in patients with protein-losing enteropathies including inflammatory bowel disease and GI malignancies[77]. Not surprisingly, patients with lower GI GVHD (also a protein-losing enteropathy) have been found to have increased clearance (via stool losses) of AAT. The first report detailing the measurement of levels of AAT in stool in allogeneic HCT recipients was by Weisdorf and colleagues in 1983 where it was demonstrated that patients receiving conditioning chemotherapy for transplant experience increased clearance of AAT as a result of higher (than normal) stool concentrations of AAT[78]. Serial measurements of stool AAT showed that patients who did not go on to develop GI GVHD had a return to baseline levels whereas those who developed lower GI GVHD had a marked and persistent increased clearance of AAT. These finds were subsequently reproduced in two additional reports with one demonstrating that stool AAT levels at the time of GI GVHD onset could serve as an independent- predictor of GVHD response to therapy[79, 80]. Based on these reports, accounting for increased stool losses of AAT in patients with lower GI GVHD requires consideration when dosing this agent for this study population.

In additional support for a higher dosing regimen of AAT, a prospective analysis by Marcondes and colleagues showed an inverse relationship between donor AAT levels and the occurrence of GVHD in matched sibling allogeneic HCT recipients, with average donor endogenous AAT levels of approximately 4 mg/mL reported to result in no incidence of GVHD[64]. Further, in an open-label, single-center, phase 1/2 study of AAT in 12 heavily-treated patients with

steroid-refractory acute GVHD of the GI tract, an overall response rate of 66%, and complete response rate of 33% was seen[67]. In this study, a cumulative weekly dose of 270 mg/kg/week was shown to be safe. Based on PK measurements, the authors concluded that higher plasma levels for AAT via more extended/repeated courses of AAT and a longer maintenance regimen (beyond 15 days as was used in this study) may be necessary to allow for complete suppression of GVHD. In contrast, a second study of AAT for patients with newly diagnosed, steroid-refractory acute GVHD, AAT dosed at 60 mg/kg twice weekly resulted in a 65% overall response rate at Day 28[66]. In this study, although AAT trough levels increased relative to study entry AAT levels, these trough levels were still within the normal range at 4 weeks (2.15 mg/mL) suggesting that 60 mg/kg twice weekly (120 mg total weekly dose) did not reach the optimal levels reflected in the above cited pre-clinical models.

1.6.4 Clinical Experience with AAT in Adolescents

Similar to adults, adolescent children are at significant risk for mortality from GVHD and thus may derive potential benefit from equal access to promising investigational therapies. Efficacy and safety of AAT in pediatric populations have been explored in several published studies of its use in type 1 diabetes (T1DM), where doses of either 180 mg/kg/week or up to 90 mg/kg/week were administered with no concerning adverse events (AEs) reported (Gottlieb et al[81] (AAT product not specified), 2014; Rachmiel et al[82] (Glassia®), 2016; Brener et al[83] (Glassia®), 2018; Weir et al[84] (Aralast NP®), 2018). In these studies, AAT therapy was reported to be safe and well tolerated in children (Rachmiel et al[82], 2016), including out to 5 years of surveillance (Brener et al[83], 2018), and no safety differences were reported between adult and pediatric patients (Gottlieb et al[81], 2014; Weir et al[84], 2018). Presently, there is no data on the use of AAT treatment in acute GVHD or emphysema conditions in pediatric populations. As an endogenous protein with proposed weight-based dosing and without evidence of differential pharmacokinetics between adults and children where studied, no differential safety profile is anticipated with its use in treatment of aGVHD. Due to these considerations, a stepwise plan was developed by the sponsor and protocol team (approved by FDA) whereby adolescent patients would be enrolled only after first observing no adverse safety signals after enrolling the first 40 patients.

1.6.5 AAT Target Level for Proposed Dose in Treatment of GVHD

It is hypothesized that during acute GVHD, AAT may attenuate inflammation, inhibit development of T-memory cells and enhance Tregs and protect against damage and down-regulate inflammatory cytokines. The target level of AAT, based on both *in vitro* and clinical data suggest that targeting a level above the upper limit of normal values may provide the optimal regulation of T cells and cytokines to ameliorate GVHD symptoms. This target level of AAT is likely to provide sustained AAT levels, thereby enhancing Tregs and reducing the pro-inflammatory cytokines.

A PK model of 1000 subjects has been used to simulate a variety of AAT doses and dose regimens, with the intent to identify those which result in mean AAT peak level estimates around or above the upper limit of normal, i.e., ≥ 2.5 mg/mL. The simulated concentration–time profiles generated using AATD PK estimates was validated with data in GVHD patients from the literature and multiple doses were modeled.

Based on this simulation, the dose of 120 mg/kg twice weekly for 4 weeks achieved median trough levels above the upper limit of normal (ULN) and provided sufficient separation in median AAT levels when compared with a dose 60 mg/kg twice weekly. In addition, this dosing regimen shows

that > 50% of the simulated GVHD subjects at this dose regimen achieve serum AAT levels above normal physiologic levels almost immediately after initiation of treatment, and levels are maintained throughout the 28-day treatment period. The cumulative weekly dose (240 mg/kg/week) is similar to the dose regimen of 270 mg/kg/week shown to be safe in Marcondes et al trial[67].

1.6.6 Ruxolitinib for the Treatment of Steroid-Refractory Acute GVHD

The Janus kinase (JAK) and signal transducers and activators of transcription (STAT) signaling pathways play an important role in immune-cell activation and tissue inflammation during acute GVHD. Ruxolitinib is an oral selective inhibitor of JAK1 and JAK2 that reduces the incidence and severity of GVHD in vivo while preserving graft-versus-leukemia effects in preclinical models. Recently, ruxolitinib was granted FDA approval in the United States for patients 12 years and older who develop steroid-refractory or dependent acute GVHD grade II-IV based on the results of a prospective phase 2 trial (REACH1). At a protocol-specified dose of 10mg twice daily, 54.9% of the 36 enrolled patients achieved a response on Day 28 without additional therapy. Simultaneously, an open-label, phase 3 trial (based on the same study criteria) was performed outside of the United States with patients randomized to ruxolitinib 10mg twice daily versus best available therapy (BAT). The results on this study were recently published and showed a higher Day 28 and Day 56 response rate in the ruxolitinib arm when compared to BAT (62% vs. 39%; odds ratio, 2.64; 95% C.I. 1.43 to 3.94; P< 0.001) and (40% vs. 22%; odds ratio 2.38; 95% C.I. 1.43 to 3.94; P< 0.001), respectively. Adverse events were not significantly different between the study arms with thrombocytopenia, anemia and CMV infection being most common.

The availability of an FDA-approved agent for patients enrolled onto this trial may affect the frequency and timing of use of next-line therapy. Importantly, while the results of the REACH 1 and 2 trials are encouraging, a majority of patients will not achieve or maintain a response through Day 56 of treatment with ruxolitinib and outcomes remain poor for patients with steroid-refractory acute GVHD. In an effort to improve upon the outcomes seen thus far with ruxolitinib in a steroid-refractory setting, patients that progress or are non-responders on the current trial will have the option to stay on AAT/PTM when next-line therapy with ruxolitinib is initiated. These patients will be followed for secondary endpoints (while being considered a failure for the primary endpoint). Importantly, the wealth of experience with AAT outside of the study indication would suggest that AAT is unlikely to have drug interaction with ruxolitinib nor contribute additional safety concerns.

CHAPTER 2

2 STUDY DESIGN

2.1 Study Overview

This study is a phase III, multicenter, double-blinded, randomized, placebo-controlled trial designed to compare AAT and CS to PTM and CS as first-line therapy for patients with high-risk acute GVHD.

2.2 Hypothesis and Specific Objectives

2.2.1 Primary Hypothesis

The primary hypothesis is that the addition of AAT to standard therapy with CS will improve the Day 28 post-randomization complete response (CR)/partial response (PR) rate in patients with newly diagnosed high-risk acute GVHD.

2.3 Study Objectives

2.3.1 Primary Objective

The primary objective of this trial is to compare the rate of CR and PR on Day 28 post-randomization between AAT and CS versus PTM and CS in patients with high-risk acute GVHD.

2.3.2 Secondary Objectives

1. Evaluate the duration of response at 6- and 12-months post-randomization.
2. Cumulative incidence of non-relapse mortality at 6- and 12-months post-randomization.
3. Overall survival and progression-free survival at 6- and 12-months post-randomization.
4. GVHD-free survival at Day 56 post-randomization.
5. Proportions of CR, very good partial response (VGPR), PR, and treatment failure (TF) at Days 7, 14, 21, 28, 56, and 86 post-randomization.
6. Proportions of CR, very good partial response (VGPR), PR, and treatment failure (TF) at Days 7, 14, 21, 28, 56, and 86 post-randomization for patients who receive ruxolitinib or other second line therapies approved by the protocol Chairs as next-line therapy and remain on AAT/PTM.
7. Incidence of systemic infections to assess safety.
8. Incidence of Adverse Events at 30 days post last dose of drug to assess safety.
9. Incidence of chronic GVHD at 6- and 12-months post-randomization.
10. Incidence of disease relapse at 6- and 12-months post-randomization.

2.3.3 Exploratory Objectives

1. AAT levels in serum at Days 0, 8, 16, 24, 28, and 56 post-treatment initiation.
2. Stool concentrations of AAT at baseline and at Days 8 and 28 post-treatment initiation.

3. Blood ratios of T regulatory to T effector cells (Treg/Teff), Natural Killer (NK) cells and cellular immune subsets at baseline and at Days 16, 28, and 56 post-treatment initiation.
4. Serum levels of inflammatory cytokines and biomarkers at baseline and at Days 8, 28 and 56 post-treatment initiation.
5. Overall and organ-specific response rates comparison based on Minnesota (MN) risk groups (Revised Minnesota High vs Standard) and organ-specific response rates comparison based on biomarker-based risk groups.
6. Corticosteroid dose at baseline, Days 7, 14, 21, 28, 56, 86, 6 months and 12 months post-randomization.
7. CMV reactivation requiring therapy by Day 56 post-randomization.
8. Change in patient-reported outcomes from baseline to Day 28, Day 56, and 6-months post-randomization.
    a. MD Anderson Symptom Inventory (MDASI)
    b. Patient-Reported Outcome Measurements Information System (PROMIS)

2.4 Patient Eligibility

Patients meeting the eligibility criteria should be enrolled as soon as possible after the start of CS, but no more than 72 hours afterwards.

2.4.1 Inclusion Criteria

1. Patients experiencing their initial presentation of acute GVHD requiring systemic therapy after allogeneic transplant for any malignant or non-malignant indication.
2. The clinical diagnosis of acute GVHD requiring systemic therapy with CS. Patients can be enrolled with only a clinically established diagnosis. Biopsy of involved organs with acute GVHD is encouraged but is not required and should not delay study entry. Enrollment and randomization include a commitment to continue steroids with PTM or AAT as specified in the protocol, as well as the required follow-up observations. *If, according to institutional practice, the intention to treat is dependent upon biopsy results, the patient should not complete enrollment on the BMT CTN 1705 study until the biopsy results are available*.
3. Acute GVHD must meet one (either A or B) of following clinical features within 72 hours prior to enrollment:

    A. High-risk by Refined Minnesota Criteria (any one below):

    Single organ involvement

    a. Stage 4 skin
    b. Stage 3-4 lower GI
    c. Stage 1-4 liver

    Multiple organ involvement

    a. Stage 1-2 lower GI plus any liver
    b. Stage 2 lower GI plus any skin
    c. Stage 3-4 lower GI plus any liver or skin
    d. Any three organ involvement

OR:

B. Either of the below:

1. Isolated stage 2 involvement of the lower GI tract

2. Stage 1 lower GI tract disease with skin involvement

4. Acute GVHD developing after allogeneic hematopoietic cell transplantation using any graft or donor source or conditioning intensity.
5. Patients should not have received systemic immune suppressive therapy for treatment of active GVHD except for a maximum of 72 hours of prior CS therapy prior to enrollment. Topical skin and GI CS (such as budesonide and oral beclomethasone diproprionate) are allowed.
6. Patients 12 years of age or older at time of enrollment.
7. Ability to provide written informed consent from patient, parent or legal guardian, and assent if applicable.

2.4.2 Exclusion Criteria

1. Patients with prior exogenous AAT exposure for GVHD prophylaxis.
2. Relapsed, progressing or persistent malignancy
3. Evidence of minimal residual disease (MRD) requiring withdrawal of systemic immune suppression.
4. Patients with acute GVHD developing after administration of a donor lymphocyte infusion (DLI) for relapse / progression of disease. Patients with acute GVHD after planned donor lymphocyte infusion or planned T cell or NK cell add back are eligible.
5. Patients with uncontrolled infections will be excluded. Infections are considered controlled if appropriate therapy has been instituted and, at the time of enrollment, no signs of progression are present. Progression of infection is defined as hemodynamic instability attributable to sepsis, new symptoms, worsening physical signs or radiographic findings attributable to infection. Persisting fever without other signs or symptoms will not be interpreted as progressing infection.
6. A clinical presentation resembling de novo chronic GVHD or overlap syndrome (as defined in APPENDIX C) developing before or present at the time of enrollment.
7. Patients receiving other drugs for the treatment of GVHD. Note, GVHD prophylaxis agents (e.g., calcineurin inhibitors) may be continued at local Investigator's discretion.
8. Patients receiving systemic CS for any indication within 7 days before enrollment, except the following:
    a. CS administered as premedication for supportive care (such as before transfusion of blood products or before intravenous medications to prevent infusion reactions, fever, etc.).
    b. If steroid therapy has been administered for treatment of a non-GVHD related condition and tapered to $\leq$ 0.6 mg/kg/day prednisone (0.5 mg/kg/day methylprednisolone) for 7 or more days prior to enrollment.

c. Treatment of active GVHD with CS is allowed for up to 72 hours prior to enrollment.

9. Patients who are pregnant or breastfeeding.
10. Females of childbearing potential (FCBP) or males who can get a FCBP pregnant and have sexual contact with FCBP and are unwilling to use 2 effective forms of birth control or abstinence from the start of study drug treatment through 30 days after the last dose of study drug, Effective forms of birth control are listed in APPENDIX D.
11. Patients on renal replacement therapy.
12. Patients requiring continuous supplemental oxygen (O_2 requirement > 2L/min to maintain peripheral O_2 saturation [SpO_2] > 90%).
13. Patients with active hepatic sinusoidal obstructive syndrome (SOS) and/or clinical evidence of impaired hepatic function (ascites or encephalopathy related to liver disease) who in the judgment of the treating physician are not expected to have normalized bilirubin by Day 56 after enrollment.
14. Patients with a history of hypersensitivity to AAT or any component of the investigational product or PTM (albumin), including congenitally IgA-deficient patients with antibodies to IgA or PTM.
15. Patients unlikely to be adherent to study specific assessments at the transplant center.

2.4.3 Adolescent Population

The protocol was amended (v4.0), after DSMB review and approval, to include patients between the ages of 12 to 17.99 years after 40 adult patients had been enrolled onto the trial and completed the Day 56 follow-up assessment for safety.

2.5 Treatment Plan

2.5.1 Alpha 1 – Antitrypsin (AAT)

2.5.1.1 Drug Information – AAT

AAT is a lyophilized product for IV administration. Each product package contains 1 single-use vial of lyophilized powder and 1 single use vial of sterile water for injection.

AAT will be manufactured by CSL Behring (CSLB) in accordance with International Conference on Harmonisation (ICH) Good Manufacturing Practice (GMP) guidelines and local regulatory requirements.

Information on the preparation and administration of AAT is provided in the Investigational Medicinal Product (IMP) Handling Instructions.

2.5.1.2 Drug Information – PTM

The PTM solution will be a commercial albumin product (5% Alburex) diluted in 5% dextrose to a visual match to reconstituted AAT solution of 50 mg/mL. An unblinded pharmacist or designee will prepare the AAT solution or PTM solution. Once prepared, the solutions are a visual match, allowing the study team to be blinded to the assigned treatment.

Instructions for preparation of PTM by an unblinded pharmacist or designee are presented in the Investigational Medicinal Product Handling Instructions.

2.5.1.3 Packaging and Labeling

The investigational products will be packaged and labeled according to current ICH GMP and Good Clinical Practice (GCP) guidelines, and national legal requirements.

Investigational products will be packaged in an unblinded manner and prepared at the site by an unblinded pharmacist or designee.

2.5.1.4 Supply and Storage

Investigational products will be supplied to the study sites by CSLB or delegate.

AAT must be stored under temperature-controlled and monitored conditions at the predefined temperature range in a secure storage area as specified in the Investigational Medicinal Product Handling Instructions.

PTM must be stored under temperature-controlled conditions between 2-8° C.

2.5.1.5 Drug Accountability and Destruction

1. Investigational products must be used only as directed in the clinical study protocol.
2. The unblinded pharmacist or designee must provide reasons for any discrepancies in drug accountability.
3. All investigational products must be accounted for throughout the study.
4. Records for the delivery of investigational products to the study site, the inventory at the study site, the dispensing and the destruction of investigational product to CSLB / designee must be maintained by the unblinded pharmacist or designee as described in the IMP Handling Instructions. Administration of investigational products is recorded in the electronic case report forms (eCRF).
5. Any unused, partially used, or empty vials of investigational product should be retained, if possible. On-site destruction must be in compliance with applicable local & national regulations and per site's SOPs.
6. All drug accountability records must be stored in the Suvoda Interactive Response Technology system (IRT) and site file and must be readily available for inspection by the unblinded study monitor and / or auditor, and open to regulatory inspection at any time.
7. The Investigator (and/or hospital pharmacist) will ensure that all study drug is stored in a secured area under required storage conditions and is dispensed by qualified staff members. All study drug will be accounted for in the Suvoda IRT system.
8. The Investigator is responsible for maintaining drug accountability records. Drug accountability records will be reviewed during monitoring visits. Study drug must be administered only to patients enrolled in this study as per the protocol.
9. The unblinded study monitor will be allowed at intervals, and upon request during the study, to check unused supplies. Accounting for the use of supplies will be by reference

to each center's record of supplies received, the dispensing records for the total number of patients enrolled at each center and the unused and returned supplies.

10. Further details regarding accountability and destruction of investigational product are provided in the IMP Handling Instructions and the Suvoda IRT Manual.

2.5.1.6 Reconstitution/Preparation of AAT (Zemaira®)/PTM

Details with regard to preparation and distribution of the study drug can be found within the IMP Handling Instructions. AAT/PTM should be administered within 24 hours of preparation.

2.5.1.7 Dose and Administration

The first dose of study drug (AAT/PTM) **must be initiated as soon as possible after enrollment and must be within 12 hours of enrollment**. Each dose of study drug will be 120 mg/kg (actual weight) based on the patient's weight recorded at enrollment. Subsequent doses should be given on Days 4, 8, 12, 16, 20, 24, 28 (Table 2-1). **The patient's weight recorded at enrollment will be used to determine the dose for all infusions** (i.e., dose adjustments for changes in weight during the treatment period will not be made).

Patients that are non-responders or progress will be considered to be steroid-refractory (SR) and may start next-line therapy as described in section 2.5.3.3. Ruxolitinib is recommended for next-line therapy as it is an FDA-approved treatment for SR-GVHD.

Responding patients (CR/PR) at Day 28, compared to maximum GVHD organ staging within 72 hours prior to enrollment, that are receiving study drug alone, or study drug plus ruxolitinib, should continue to receive treatment at a dose of 120 mg/kg (actual weight) on Days 35, 42, 49, and 56 (Table 2-1). Patients who are not in CR/PR at Day 28 will not continue to receive study drug. Responding patients at Day 28 who progress after Day 28 may continue to receive weekly doses through Day 56.

Patients that receive next-line therapy other than ruxolitinib may not continue receiving study drug unless approval is granted by the BMT CTN 1705 Protocol Chairs.

Table 2-1 Study Drug Schedule

	Day											
	0[1]	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**35**	**42**	**49**	**56**
All patients	X	X	X	X	X	X	X	X				
CR/PR (VGPR) patients only									X	X	X	X

[1] Enrollment is Day 0, First dose of study drug must occur within 12 hours after enrollment and may fall on Day 0 or Day 1.

Patients can receive study drug (AAT/PTM) as either an inpatient or outpatient. All subsequent treatments will have a window of ± 3 days to allow for efficient scheduling of patients for holidays/weekends/etc. Following administration of the first or second dose, adjusting future infusions to accommodate a Monday/Thursday or Tuesday/Friday schedule is encouraged.

Infusion Rate: AAT/ PTM should be administered at a rate not exceeding 0.08 mL/kg body weight/minute. If adverse events occur, the rate should be reduced, or the infusion interrupted until the symptoms subside. The infusion may then be resumed at a rate tolerated by the subject.

Monitoring: Vital signs (temperature, heart rate, blood pressure, respiratory rate) should be recorded at a minimum: pre-infusion, approximately 10 minutes into the infusion and approximately 20 minutes after completion of the infusion. It should be noted that infusion reactions during and following AAT administration have been reported but are rare. These include:

- Uncommon (≥ 1/1,000 to < 1/100): hypersensitivity reactions (including tachycardia, hypotension, confusion, syncope, oxygen consumption decreased and pharyngeal oedema), flushing, infusion-site reactions (including infusion site hematoma)
- Very rare (< 1/10,000): anaphylactic reactions

If anaphylactic or severe anaphylactoid reactions occur, the infusion should be discontinued immediately. Epinephrine and other supportive therapy should be available for the treatment of any acute anaphylactic or anaphylactoid reaction.

2.5.1.8 Dose Interruptions

Study drug (AAT or PTM) may be held at the discretion of the treating physician and resumed at their discretion. Rationale for missed doses should be recorded. Patients that do not receive study medication for 3 consecutive doses or miss 4 doses in total will be removed from further study treatment.

Infections

Patients with severe infections resulting in hemodynamic instability requiring use of vasopressor medication may have study drug held at the discretion of the treating physician.

2.5.1.9 Adverse Events Related to Study Drug (AAT or PTM)

Adverse Events of Study Drug (AAT or PTM)

Patients with a non-hematologic grade 3 or higher adverse event which is not attributable to an anticipated post-transplant event may have their study drug held at the attending physician's discretion. Anticipated post-transplant events include GVHD and chemotherapy toxicities (see Appendix E and Section 2.5.2.2 respectively.). Study drug should be restarted after recovery of related toxicities to grade 2 or lower or identification of an alternative cause for these toxicities.

Observed adverse reactions as identified from clinical studies in AAT-deficient subjects and during post marketing use of AAT can be found in the Investigator Brochure AAT CSL964.

Adverse Events related to PTM (Alburex)

Adverse reactions to albumin 5% are rare, although nausea, fever, chills or urticaria may occasionally occur.

2.5.1.10 Study Drug Discontinuation Criteria

Patients that progress or are non-responders and receive ruxolitinib as next-line therapy are encouraged to continue study drug (AAT/PTM).

Study drug shall be discontinued and not re-instituted if any one of the following criteria is met:

- Patient does not receive study medication for 3 consecutive doses or 4 doses in total for any reason, including toxicity.
- Patients that receive next-line therapy other than ruxolitinib not approved by the BMT CTN 1705 Protocol Chairs.
- Relapse or progression of underlying malignancy.
- Pregnancy or initiation of breastfeeding during the study; refer to Section 4.4.5.
- Withdrawal of consent from study and/or study drug

The reason for discontinuation of study drug must be documented by the study team. All randomized patients, including those that did not receive any study drug, discontinued study drug and/or failed treatment, are evaluable for all endpoints of the study and should continue with protocol-specific follow-up and data collection unless the patient has specifically withdrawn consent to provide data.

2.5.2 Corticosteroids (CS)

2.5.2.1 CS Dosing and Taper

All patients enrolled on this trial must receive CS at a minimum dose of prednisone 2 mg/kg/day PO (or methylprednisolone 1.6 mg/kg/day IV) divided into 1-2 daily doses as therapy for acute GVHD for at least 72 hours post enrollment. For patients that weigh over 100 kg, maximal starting dose of prednisone will be 200 mg (or methylprednisolone-equivalent). For calculation of subsequent CS doses/kg on subsequent measures, the modified starting weight of 100 kg will be used. For patients less than 100 kg, dosing of steroids may follow institutional practices (with respect to actual versus adjusted weight). Daily dose per kg is based on the weight of the patient at time of enrollment.

For responding patients with evidence of improvement in signs and symptoms of acute GVHD after treatment initiation, a suggested CS taper is provided below or may follow institutional practice***. However, taper must not start sooner than 72 hours after enrollment and the CS dose must not be tapered to less than 0.25 mg/kg/day prednisone-equivalent (or 0.2 mg/kg/day methylprednisolone) before Day 29.*** A suggested taper schedule is provided below.

Table 2-2 Suggested Steroid Taper for Responding Patients

Suggested taper for <u>responders</u> (Prednisone/Methylprednisolone)	
Days 1-5	2 mg/kg/day **(please note that taper <u>cannot</u> start until 3 days after enrollment)**
Days 6-10	1.5 mg/kg/day
Day 11-15	1.0 mg/kg/day
Days 16-20	0.5 mg/kg/day
Days 21-28*	0.25 mg/kg/day
Days 29-56	Gradual further taper with a goal of reaching < 0.2 mg/kg/day of prednisone (or < 0.16 mg/kg/day of methylprednisolone) by Day 56.
***Do NOT taper below 0.25mg/kg/day prednisone (or 0.2 mg/kg/day methylprednisolone) before Day 29.**	

2.5.2.2 Adverse Events Related to CS

The side effects of prednisone are broad and well recognized. They include:

- Cardiac and vascular: hypertension
- Cutaneous: impaired wound healing, skin fragility, erythema, acne
- Endocrine: cushingoid state, growth suppression (pediatric), secondary adrenocortical and HPA (hypothalamic pituitary adrenal)-axis suppression, diabetes mellitus, hypokalemia, body fluid retention, weight gain
- Gastrointestinal: peptic ulcers, esophagitis, pancreatitis, gastrointestinal perforation gastrointestinal hemorrhage
- General: edema, increased appetite
- Hematologic: leukocytosis (transient), lymphopenia
- Musculoskeletal: myopathy, osteoporosis, tendon rupture, bone fractures, avascular necrosis of bones
- Neurologic: Increased intracranial pressure, convulsions, headache, increased intraocular pressure, glaucoma, mood swings, restlessness, insomnia, personality changes, seizure, severe depression, or frank psychotic manifestations
- Miscellaneous: increased risk of infection

2.5.3 Supportive Care

2.5.3.1 GVHD Prophylaxis Medications

Non-investigational medications such as cyclosporine, tacrolimus, mycophenolate mofetil (MMF), sirolimus (if used as GVHD prophylaxis prior to the development of acute GVHD) may be continued, resumed, or increased to therapeutic doses per provider discretion, and adjusted/discontinued as necessary for renal, central nervous system (CNS) or other toxicity using institutional management guidelines. Resumption of a GVHD prophylaxis agent is not considered the addition of a next-line agent. Changing prophylactic agents within a drug class or to similar agents due to toxicity would not represent new therapy or treatment failure.

2.5.3.2 Topical and Ancillary GVHD Therapies

Topical therapy for acute GVHD is allowed and should be used according to institutional practices. Topical therapy including corticosteroid creams, topical tacrolimus, oral beclomethasone or budesonide, topical azathioprine, and ophthalmic glucocorticoids, is not considered as secondary systemic therapy.

Ancillary/supportive care measures for acute GVHD such as the use of anti-motility agents for diarrhea, including octreotide, is allowed at the discretion of the treating physician. Use of ursodiol to prevent/reduce gall bladder sludging or prevent hepatic transplant complications is also allowed according to institutional guidelines.

2.5.3.3 Acute GVHD Progression or Non-response

Acute GVHD **Progression** (see Section 3.1.1 Response Definition for **Progression**):

A patient cannot be declared as having **progression** in acute GVHD before 72 hours from the time of study randomization. Patients should receive at least 2 doses of AAT/PTM before being declared as having acute GVHD progression (and starting next-line therapy). Patients receiving

ruxolitinib are encouraged to continue study drug (AAT/PTM), however, study treatment may be discontinued at the discretion of the treating physician.

Acute GVHD **No Response** (see Section 3.1.1 Response Definitions for **No Response**):

An additional line of therapy (for non-response) must not be started before Day 7. A patient should receive at least 3 doses of study drug (AAT/PTM) before starting next line therapy for **non-response**.

Ruxolitinib is recommended for next-line therapy as it is an FDA-approved treatment for SR-GVHD. Patients receiving ruxolitinib are encouraged to continue study drug (AAT/PTM) to determine if the continuation of AAT can improve outcomes. Patients that are non-responders and receive next-line therapy other than ruxolitinib may not continue study drug (AAT or PTM) unless approval is granted by the BMT CTN 1705 Protocol Chairs.

Patients who receive a non-protocol approved therapy for treatment of acute GVHD will be considered as treatment failure and study drug must be discontinued.

Table 2-3 Timeline for Declaring Patient as having Acute GVHD Progression or No Response Categories

Response Definition	Minimum Number of Doses of AAT/ PTM Before Starting Next Line Therapy	Mandated Minimum Time from Enrollment Before Starting Next Line Therapy
Progression	2 doses	72 hours
No Response	3 doses	7 days

2.5.3.4 Acute GVHD Flare

A flare is defined as any progression of acute GVHD after an initial response (i.e., earlier CR or PR) that requires re-escalation of steroid dosing, or initiation of additional topical or systemic therapy.

If acute GVHD flares during taper of CS, the dose of steroids may be increased at the discretion of the treating physician as long as this increase is to less than 2.5 mg/kg/day of prednisone (or methylprednisolone equivalent of 2 mg/kg/day). Doses otherwise above these parameters will be considered as a "new agent" and the patient will be scored as a failure. Other Supportive Care Guidelines

In addition to prescribed study drug plus CS, all patients should receive the following per institutional practice:

- Transfusion support
- Anti-infective prophylaxis against herpes viruses, *Pneumocystis jiroveci,* bacterial and fungal infections.
- Routine CMV antigenemia/viral load testing by hybrid capture or PCR-based methods (with preemptive therapy in patients who develop a positive assay). CMV testing is recommended weekly through at least Day 100 post-transplant. Prophylaxis against CMV is allowed.

- Co-enrollment onto supportive care and infectious disease protocols will be allowed on case-by-case basis and requires approval by study chairs and sponsor.

2.6 Study Unblinding

Subjects and all personnel involved with the conduct and interpretation of the study will be blinded to the study treatment except for the investigational pharmacy staff involved in study drug preparation. In addition, CSL's Clinical Trial Supply team, CSL's IRT team, and the Suvoda IRT team and system are unblinded. Unblinding of the study drug should only be considered for participant safety contingent upon knowing the blinded study drug assignment. In situations that satisfy these criteria, the Investigator may break the blind per the processes laid out in the BMT CTN 1705 Unblinding Procedures. The reason for unblinding must be fully documented. Any unblinding by the investigational staff must be reported immediately as outlined in the BMT CTN 1705 Unblinding Procedures and must include an explanation of why the study medication was unblinded. If possible, CSL Behring should be consulted prior to unblinding of the study drug. The Sponsor may break the treatment code for participants who experience a suspected unexpected serious adverse reaction (SUSAR), to determine if the individual case or a group of cases requires expedited regulatory reporting.

2.7 Study Conduct

This study will be conducted in accordance with the protocol, the IMP Manual, the Suvoda IRT system, the BMT CTN Administrative MOP, and the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The National Marrow Donor Program (NMDP) single Institutional Review Board (IRB) of Record will oversee this study and conduct the study-specific reviews as required by federal regulations and per the NMDP IRB Standard Operating Procedures (SOPs).

Site personnel will enter enrollment data and post randomization data in the electronic case report forms (eCRFs) in Advantage eClinical as described in the BMT CTN 1705 Forms Guide. Randomization will be conducted in the Suvoda IRT system after enrollment has been completed in Advantage eClinical. Source documentation should be made available for monitoring visits, audits, and regulatory inspections as described in the BMT CTN Administrative MOP.

Participating Principal Investigators bear ultimate responsibility for training of site staff as well as the scientific, technical, and administrative aspects of conduct of the protocol, even when certain tasks have been delegated to coinvestigators, sub-investigators, or staff. The PIs have a responsibility to protect the rights and welfare of participants and comply with all requirements regarding the clinical obligations and all other pertinent requirements in 21 CFR part 312. In addition to following applicable federal, state, and local regulations, investigators are expected to follow ethical principles and standards and receive training in GCP every three years and human subjects training within the past 3 years and thereafter as per institutional requirements.

2.8 Study Termination

Regulatory agencies have the right to terminate the study at any time in case of safety concerns or if special circumstances concerning the study drug or the company itself occur, making further treatment of patients impossible. The study sponsor also has the right to terminate the study. In this event, the Investigator(s) and relevant authorities will be informed of the reason for study termination.

CSLB reserves the right to prematurely discontinue or suspend the study either at a particular site or at all study sites at any time and for any reason. If such action is taken, the Study Monitor (or delegate) will discuss this with the Investigator at each study site at that time and notify the Investigators in writing. If the study is suspended or terminated for safety reasons, all Investigators and the relevant regulatory agencies will be immediately notified of the action as well as the reason for the suspension/termination. The Investigator at each study site will advise their local Institutional Review Board (IRB) / Institutional Ethics Committee (IEC). The BMT CTN DCC will notify the NMDP single IRB of Record overseeing the study of the suspension/ termination.

CHAPTER 3

3 STUDY ENDPOINTS

3.1 Primary Endpoint

The primary endpoint is the proportion of participants with an overall (complete or partial) response to acute GVHD treatment at Day 28 post-randomization, compared to maximum GVHD organ staging within 72 hours prior to enrollment. Patients without a Day 28 response assessment will be considered failures. Acute GVHD will be graded and assessed for response based on Harris (Mount Sinai Acute GVHD International Consortium [MAGIC]) Criteria (refer to APPENDIX C).

3.1.1 Response Definitions

Response definitions are listed below:

1. Complete Response (CR) is defined as a score of 0 for the GVHD staging in all evaluable organs.
2. Partial response (PR) is defined as improvement in one or more organs involved with GVHD symptoms without progression in others.
    a. Very good partial response (VGPR) is defined as a quantitative and functional response that closely approximates CR. Patients must have all of the following clinical features. i.e., (1) no rash or rash involving < 25% of body surface area (BSA) without bullae (residual faint erythema and hyperpigmentation do not count), (2) serum bilirubin < 2mg/dl or < 25% of baseline at enrollment and (3) ability to tolerate food or enteral feedings with predominantly formed stool (no overt GI bleeding or cramping and no more than occasional nausea or vomiting).
3. Mixed response (MR) is defined as improvement in one or more organs with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ.
4. No Response (NR) is defined as absence of any improvement or progression as defined.
5. Progression is defined as deterioration in at least one organ without any improvement in others.

Patients who receive next-line systemic GVHD therapy (or escalation of steroids to ≥ 2.5 mg/kg/day of prednisone [or methylprednisolone equivalent of 2 mg/kg/day]) before Day 28 will be classified as NR. **Responses categorized as NR, MR and progression will be treated as a failure for the primary endpoint.**

3.2 Secondary Endpoints

3.2.1 Duration of Response (DOR)

The duration of response (DOR) is defined as time from the Day 28 response (CR or PR) to any of the following events: recurrence/progression of acute GVHD, new systemic salvage therapy for

acute GVHD, re-escalation of steroids to greater or equal to 2.5 mg/kg prednisone or equivalent, or death from any cause. Patients without any of these events will censored at last follow up. DOR will be evaluated at 6- and 12-months post-randomization.

3.2.2 Non-Relapse Mortality (NRM)

An event for non-relapse mortality (NRM) is death without prior evidence of relapse/progression of the primary disease, where relapse/progression is treated as a competing risk. The cumulative incidence of NRM at 6 months and 12 months post-randomization will be evaluated.

3.2.3 Overall Survival (OS) and Progression-Free Survival (PFS)

Overall survival (OS) and progression-free survival (PFS) will be evaluated at 6 months and 12 months post-randomization. An event for OS is death from any cause, while an event for PFS is death from any cause or relapse/progression of the primary disease.

3.2.4 GVHD-Free Survival

Patients alive, free of active acute or chronic GVHD, and without other systemic agents (or escalation of steroids to ≥ 2.5 mg/kg/day of prednisone [or methylprednisolone equivalent of 2 mg/kg/day]) added for treatment of GVHD will be considered successes for this endpoint. The proportion of participants with GVHD-free survival at Day 56 post-randomization will be evaluated.

3.2.5 Proportion of Response

The proportion of patients with CR, PR (including subset with VGPR), and treatment failure (TF) will be described at Days 7, 14, 21, 28, 56 and 86 post-randomization. The Day 86 response will be assessed in patients remaining on maintenance treatment after Day 28. The designation of TF will consist of patients with NR, MR, progression or initiation of additional systemic (next-line) GVHD therapies (or escalation of steroids to ≥ 2.5 mg/kg/day of prednisone [or methylprednisolone equivalent of 2 mg/kg/day]). Death from any cause will also be considered a TF.

3.2.6 Proportion of Response (Next-Line Therapy)

The proportions of patients with CR, PR (including subset with VGPR), and treatment failure (TF) at Days 7, 14, 21, 28, 56, and 86 post-randomization for patients who receive ruxolitinib or other next-line therapies approved by the protocol Chairs as next-line therapy and remain on AAT/PTM.

3.2.7 Systemic Infections

The incidence of Grade 2 to 3 systemic infections, as defined in Appendix G, occurring from randomization until 30 days after last dose of study drug (AAT or PTM) will be described. Infections developing after randomization will be recorded by site of disease, date of onset, and severity.

3.2.8 Adverse Events

The incidence of Grade 3-5 treatment-emergent adverse events (per Common Terminology Criteria for Adverse Events [CTCAE] Version 5.0) that occur from randomization until 30 days after last dose of study drug (AAT or PTM) will be described.

3.2.9 Chronic GVHD

The cumulative incidence of chronic GVHD will be evaluated at 6- and 12-months post-randomization, where death is treated as a competing risk. Chronic GVHD is defined per National Institutes of Health (NIH) Consensus Criteria (see APPENDIX C). Diagnosis of chronic GVHD of any severity (mild, moderate, or severe) is considered an event for this endpoint. Organ involvement and maximum severity will also be described at 6 and 12 months.

3.2.10 Disease Relapse

The cumulative incidence of relapse/progression of the primary disease will be evaluated at 6- and 12-months post-randomization, with death prior to relapse/progression treated as a competing risk.

Malignancy relapse is defined as follows:
Relapse is defined by either morphological or cytogenetic evidence of acute leukemia or myelodysplastic syndrome (MDS) consistent with pre-transplant features, or radiologic evidence of lymphoma, documented or not by biopsy. Progression of disease applies to patients with lymphoproliferative diseases (lymphoma or chronic lymphocytic leukemia) not in remission prior to transplantation. The event is defined as increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy.

Acute leukemia– Relapse will be diagnosed when there is:

- Reappearance of leukemia blast cells in the peripheral blood; or,
- > 5% blasts in the bone marrow, not attributable to another cause (e.g., bone marrow regeneration)
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid

Myelodysplastic Syndrome (MDS) – Relapse will be diagnosed when there is:

- Satisfying criteria for evolution into acute myeloid leukemia (blasts greater than 20%), OR
- Reappearance of pre-transplant morphological abnormalities detected in bone marrow specimens combined with a decrease in PB chimerism; OR
- Reappearance of pre-transplant cytogenetic abnormality in at least one metaphase by routine metaphase cytogenetics or a positive test by FISH on each of two separate consecutive examinations at least one month apart, regardless of the number of metaphases analyzed
- For patients with:
    - Less than 5% blasts: greater or equal to 50% increase in blasts to greater or equal to 5% blasts confirmed by flow cytometry
    - 5%-10% blasts: greater or equal to 50% increase to greater or equal to 10% blasts confirmed by flow cytometry

Lymphoproliferative Diseases – Relapse or progression will be diagnosed when there is:

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased fluorodeoxyglucose (FDG) uptake in a previously unaffected site will only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.
- At least a 50% increase from nadir in the sum of the product diameters of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by $\geq$ 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.
- Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (< 1.5 cm in its long axis by CT).
- In addition to the criteria above, patients with chronic lymphocytic leukemia (CLL) who present in complete remission prior to transplantation may fulfill the relapse definition if there is reappearance of circulating malignant cells that are phenotypically characteristic of CLL.

For other diseases not listed probable or definitive evidence of progression must be documented by biopsy, imaging, or peripheral blood findings that are diagnostic or otherwise consistent with that disease state.

***Institution of any therapy to treat persistent, progressive or relapsed malignancy, including the withdrawal of immunosuppressive therapy, or donor lymphocyte infusion, will be considered evidence of relapse/progression regardless of whether the criteria described above were met.**

Non-malignant Diseases:
Non-malignant diseases will be considered to have a transplant status of persistent/active disease. Graft failures will be considered recurrence.

3.3 Exploratory Endpoints

3.3.1 AAT Levels

AAT levels will be assessed using measurements of serum antigen concentration and functional activity just prior to infusion and 30 min (+/- 15 min) post-end of infusion on Day 0, and on Days 16, 28, and 56 post-initiation of treatment. Post-infusion measurements are limited to subjects receiving a dose of AAT/PTM. AAT levels will be measured only prior to infusion on Days 8 and 24 post-initiation of treatment.

3.3.2 Stool AAT, Microbiome, and Metabolome

Stool concentrations of AAT at baseline and at Days 8 and 28 post-initiation of treatment (or at treatment discontinuation (TD) if prior to Day 28) at a subset of centers for a limited cohort of

patients. Samples will be stored for future analysis of the stool microbiome, metabolome, and biomarkers.

3.3.3 Immune Subsets

The changes in peripheral blood ratios of T regulatory to T effector (Treg/Teff) cells and Natural Killer (NK) cells and other relevant immune subsets at baseline and at Days 16, 28, and 56 post-initiation of treatment (or at treatment discontinuation (TD) if prior to Day 56).

3.3.4 Inflammatory Cytokines and GVHD Biomarkers

Serum levels of IL-1β, IL-6, IL-10, TNFα, amphiregulin (AREG), HS, ST2, and REG3α will be evaluated at baseline and at Days 8, 28, and 56 post-initiation of treatment (or at treatment discontinuation (TD) if prior to Day 56). Remaining samples will be retained for analysis of additional relevant cytokines, DAMPs and GVHD biomarkers.

3.3.5 Overall and Organ-Specific Response Rates by Refined Minnesota Risk and Biomarker-Based Risk Classifications

The proportions of patients with an overall response (complete or partial) at Day 28 post-randomization will be described by Refined Minnesota risk category (high or standard risk) and by Ann Arbor biomarker risk score (1, 2, or 3). Additionally, the response rate of each GVHD target organ will be described at Day 28 by Refined Minnesota risk and Ann Arbor biomarker risk. An organ response is defined as a decrease in staging of that organ from baseline. The organ-specific response rate will be evaluated only using patients with a staging greater than 0 in the corresponding organ at baseline.

3.3.6 Systemic Corticosteroid Dose

The systemic corticosteroid dose will be evaluated at baseline and on Days 7, 14, 21, 28, 56, 86, 6 months and 12 months post-randomization.

3.3.7 CMV-Reactivation

The proportion of patients requiring new systemic treatment for an increasing CMV PCR level per institutional practice for CMV-reactivation will be evaluated at Day 56 post-randomization. Patients receiving only standard of care viral prophylaxis will not be included in this assessment

3.3.8 Patient-Reported Outcomes

Patient-reported outcomes for both adolescents and adults will be assessed at baseline, Days 28, 56 and 180 post-randomization by the MD Anderson Symptom Inventory[85] (MDASI), and selected Patient-Reported Outcome Measurements Information System (PROMIS) subscales for gastrointestinal symptoms, physical function and satisfaction with participation in social roles. Adolescent versions should be completed by patients who are less than 18 years of age at the time of enrollment. The instruments will be scored according to the recommendations of the developers. Patient-reported outcomes (PRO) data will be collected electronically. The MDASI instrument will only be offered to English- and Spanish-speaking patients. The PROMIS instrument will only be offered to English-speaking patients. The instruments cannot be completed by surrogates.

CHAPTER 4

4 PATIENT ENROLLMENT AND EVALUATION

4.1 Approaching Patients, Eligibility Screening and Obtaining Consent

Patients with previously untreated, acute GVHD meeting eligibility criteria will be approached as soon as possible after diagnosis. Patients willing to participate in the trial will sign a NMDP IRB-approved consent or assent form, and eligibility for enrollment will be further evaluated.

4.2 Enrollment and Randomization

Patients will be (1) enrolled using the BMT CTN Advantage eClinical system and then (2) randomized in the Suvoda Interactive Response Technology (IRT) system. The IRT system will also provide the study drug supply management. Patients will be randomized to AAT versus PTM in a 1:1 ratio. The following procedures shall be followed:

Patients must be enrolled in Advantage eClinical prior to randomization in the Suvoda IRT System.

1. An authorized user at the clinical center completes the initial screening by entering patient demographics, eligibility information (consent date, inclusion/exclusion criteria) and patient's weight on the Enrollment Form in Advantage eClinical.
2. If the patient is eligible, "successful enrollment' is displayed along with a patient study ID# number and patient's weight. Information regarding participant's log-in information for the ePRO system is also included. The coordinator should print two copies of the screen: one for the patient's chart and one for the study participant.
3. **Immediately after the patient is successfully enrolled/ deemed eligible in Advantage eClinical, the authorized user must register the patient in the Suvoda IRT system for randomization to occur.** The user enters the Patient ID# assigned in eClinical, demographics (year of birth, age, and sex) and weight at time of enrollment.
4. The Suvoda IRT system will determine the patient's randomization assignment and automatically email the site's investigational pharmacist on record with the following information: Patient ID #, Visit # and the specific kits to be used to prepare the patient's assigned treatment.
5. A visit schedule based on enrollment date is displayed in Advantage eClinical for printing.

If a connection is interrupted during an enrollment session in Advantage eClinical, the process is completely canceled and logged. A backup manual enrollment system will also be available to provide for short-term system failure or unavailability. If the Suvoda IRT system is interrupted or down, the Suvoda Help Desk must be notified.

A patient is considered on the study once randomization in the IRT system is complete.

Patient must begin study drug (AAT or PTM) within 12 hours of enrollment in Advantage eClinical.

4.3 Study Monitoring

4.3.1 Follow-Up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4-1. The timing of follow-up visits is based on the date of enrollment. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the BMT CTN 1705 Forms Guide. Forms that are not entered in Advantage eClinical within the specified time will be considered delinquent. Transplant Centers can view past due forms via Advantage eClinical. A missing form will continue to appear until the form is entered into Advantage eClinical, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Forms Guide.

Reporting Patient Deaths: The Recipient Death Information must be entered into the web-based data entry system within 24 hours of knowledge of a patient's death even if the cause of death is unknown at that time. Once the cause of death is determined, the form must be updated.

Center for International Blood and Marrow Transplant Research (CIBMTR) Data Reporting: Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the CIBMTR. Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) CIBMTR post- transplant Report Forms must continue to be submitted for all patients enrolled on this trial. Additionally, CIBMTR pre- and post- transplant Report Forms must also be submitted for all patients enrolled on this trial according to the randomization assigned to the patient at the time of initial registration with the CIBMTR. Long-term follow-up of patients on this study will continue through routine CIBMTR mechanisms.

4.3.2 Assessments

Assessment and/or reporting of the following is required for patients enrolled on this study. All assessments are considered standard of care unless identified below by "*".

Pre-Enrollment

1. Performance status (Karnofsky for patients age 16 and older; Lansky for patients age 12-15.99 at the time of enrollment), history and physical exam including height and weight within 7 days prior to enrollment.
2. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and liver abnormalities prior to enrollment.
3. Corticosteroid start date and dose (including administered dose and patient weight) prior to enrollment.
4. Liver function tests (bilirubin, alkaline phosphatase, AST, ALT) within 48 hours prior to enrollment.

5. Complete blood count (CBC) with differential and platelet count within 48 hours prior to enrollment.
6. Pregnancy test (by blood) for females of childbearing potential within 30 days prior to enrollment.

Post- Randomization (Prior to initiation of Study Drug (AAT/PTM))

1. Baseline patient-reported outcome measures prior to initiation of AAT/PTM. Only English- and Spanish-speaking patients may participate in the MDASI Health Quality of Life (HQL) component of this trial; only English-speaking patients may participate in the PROMIS HQL component. Adolescent versions should be completed by patients who are less than 18 years of age at the time of enrollment. Surveys may not be completed by surrogates.*
2. GVHD prophylaxis medication and complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, and liver abnormalities prior to initiation of AAT/PTM.
3. Blood research samples for serum AAT levels in peripheral blood collected pre-treatment (just prior to first dose of study drug) Note: samples will also be collected 15-45 minutes post-end of infusion.*
4. Blood research samples collected pre-treatment for serum inflammatory cytokines, GVHD biomarkers and flow cytometry.*
5. Optional stool research sample pre-treatment from consenting patients (*select centers only*).*

Post- Randomization (Treatment and Post-Treatment Follow-Up)

1. Performance status (Karnofsky for patients age 16 and older; Lansky for patients age 12-15.99) at Days 28 and 56, and at 3 months, 6 months, and 12 months.
2. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests weekly until Day 56, and 3, 6, and 12 months. Days 28 and 56 scoring must be performed by direct observation at the Transplant Center. Evaluations at other time points may be performed by clinicians familiar with assessing GVHD other than at the Transplant Center but the Transplant Center is responsible for collecting all required data. All GVHD treatment decisions must be made by the physician at the Transplant Center.
3. Liver function tests (bilirubin, alkaline phosphatase, AST, ALT) weekly until Day 56, and at 3 months, 6 months, and 12 months.
4. Chronic GVHD assessment at Days 28 and 56, and months 3, 6 and 12.
5. GVHD prophylaxis medication (if available) weekly through Day 28.
6. CBC with differential, platelet count on Days 14, 28, 56, and 86.
7. Toxicities at Day 120, 6 months and end of treatment (EOT).
8. Infections up to 3 months are reported on the event driven infection forms. Per protocol section 4.4.1 infections are anticipated events and do not require reporting as an AE/SAE.
9. Corticosteroid dose (including administered dose and patient weight) weekly until Day 56, and at months 3, 6 and 12 and/or EOT.

10. Second-line immune suppressive therapy weekly until Day 56 and at months 3, 6 and 12 and/or EOT.
11. Patient-reported outcome measures at Day 28, Day 56, and 6 months. Only English- and Spanish-speaking patients may participate in the MDASI HQL component of this trial; only English-speaking patients may participate in the PROMIS HQL component of the trial. Adolescent versions should be completed by patients who are less than 18 years of age at the time of enrollment Surveys may not be completed by surrogates.*
12. Blood research samples for serum AAT levels in peripheral blood in peripheral blood at Days 0, 8, 16, 24, 28, and 56. Samples should be drawn <u>just prior</u> to each study drug infusion (and within 15-45 minutes post-end of infusion for the Day 16, 28, and 56 samples). See APPENDIX B for further details.*
13. Blood research samples at Days 8, 16, 28, and 56 (or treatment discontinuation [TD] if prior to Day 56) for serum inflammatory cytokines, GVHD biomarkers and flow cytometry. The collection of required research blood samples should be coordinated with the collection of the pre-infusion blood samples for AAT level measurements. If study drug is administered before or after the indicated day, but within the treatment window (Section 2.5.1.7), the timing of the research sample can be adjusted accordingly. If it is not possible to coordinate the research sample collection, the window of collection for research blood samples is ± 3 days.*
14. Optional stool research samples at Days 8 and 28 (or within +/- 3 days of treatment discontinuation [TD]). Stool samples from consenting patients should be collected if possible, on the target visit day indicated or within +/- 3 days of the scheduled visit. (*Select centers only*)*

Table 4-1 Required Assessments Post- Enrollment

	Days														
	Pre-Enrollment	**Pre-Therapy[1]**	**7***	**14***	**21***	**28***	**35***	**42***	**49***	**56 ***	**86 (3 mos)****	**120****	**180 (6 mos)*****	**EOT[2] (+ 30 days)**	**365 (12 mos)******
History, physical exam, weight and height	X														
Karnofsky/Lansky Performance Status	X					X				X	X		X		X
Pregnancy test if applicable[3]	X					X				X					
Acute GVHD evaluation	X	X	X	X	X	X	X	X	X	X	X		X		X
Liver Function Tests (alkaline phosphatase, bilirubin, AST, ALT)	X		X	X	X	X	X	X	X	X	X		X		X
CBC with differential, platelet count	X			X		X				X	X				
Immunosuppressive drug monitoring (eg, sirolimus, tacrolimus, cyclosporine)		X	X	X	X	X	X	X	X	X	X		X	X	X
Determination of corticosteroid dose, including administered dose and patient weight	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Chronic GVHD evaluation[7]						X				X	X		X		X
Toxicity assessment												X	X		
Infection monitoring[4]		Reporting of infections at time of occurrence from time of enrollment through 3 months													
AE/SAE assessments[5]		X	X	X	X	X	X	X	X	X	X		X[5]	X[5]	X[5]
Patient-reported outcomes[6]: MDASI, PROMIS		X				X				X			X		

* ± 3 days from the target day to allow for scheduling flexibility, holidays, etc.
** ± 14 days from the target day to allow for scheduling flexibility
*** ± 28 days to allow for scheduling flexibility
**** 60 days to allow for scheduling flexibility

1 Pre-therapy assessments preferably should be performed prior to enrollment, however, up to 36 hours post- enrollment is allowed.
2 End of Treatment assessments (except specimen collection) for patients that terminate treatment early will be done 30 days after the last dose of study drug. A ± 3-day window is allowed.
3 Pregnancy test (by blood) for females of childbearing potential within 30 days prior to enrollment. Serum or urine test on Day 28 and Day 56.
4 Includes anti-viral prophylaxis
5 AEs and SAEs will be reported from randomization until 30 days post last dose. Beginning at 31 days from the last dose of study drug through the end of study follow-up, SAEs deemed to be related to the study drug require reporting, and any unanticipated SAE regardless of relationship to study drug require reporting.
6 Only English- and Spanish-speaking patients are eligible to participate in the Health Quality of Life (HQL) component of this trial. MDASI surveys to be completed by both English- and Spanish-speaking patients. PROMIS surveys to be completed by English speaking patients only. Surveys may not be completed by surrogates.
7 The Chronic GVHD Provider Survey is due at each Chronic GVHD evaluation and will record GVHD symptoms present in the last week and must be completed by a clinician on the day of the assessment.

Table 4-2 Required and Optional Research Assessments Post-Initiation of Treatment (see APPENDIX B for further details)

Type	Purpose	Day							
		Pre-Therapy[2]	**0**	**8**	**16**	**24**	**28**	**56**	**TD[3]**
Required	Study drug levels[1]	X	X	X	X	X	X	X	
	Serum Inflammatory Cytokines and GVHD Biomarkers[4]	X		X			X	X	X
	Flow Cytometry[4]	X			X		X	X	X
Optional *(at select centers)*	Stool research samples[5]	X		X			X		X

NOTE: Enrollment is Day 0. First dose of study drug must occur within 12 hours after enrollment and may fall on Day 0 or Day 1.

1 Samples should be drawn just prior to each study drug infusion (and also within 15 to 45 minutes post-end of infusion for the Day 0, 16, 28, and 56 samples).
2 Collect sample prior to administering study drug.
3 Sample should be collected at treatment discontinuation (TD) if that occurs prior to Day 28 (stool)/56 (blood). (Note: This is at the time that patient discontinues the study drug.)
4 The collection of required research blood samples for serum biomarkers and flow cytometry studies should be coordinated with the collection of the pre-drug infusion blood samples for AAT level measurements. If study drug is administered before or after the indicated day but within the treatment window (Section 2.5.1.7), the timing of the research sample collection can be adjusted accordingly. If not possible for some reason to not coordinate the research sample collection, the window of collection for research blood samples are ± 3 day.
5 Stool samples being collected from consenting patients at selected centers with active Microbiome laboratories should be collected if possible on the target visit day indicated or within ± 3 days of the scheduled visit.

4.4 Adverse Event Reporting

Adverse event (AE) reporting is summarized below with the specific requirements described under Section 4.4.4.

4.4.1 Definitions

Adverse Event: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) therefore is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether related or not related to the IMP/PTM medicinal (investigational) product.

An adverse event can be Anticipated or Unanticipated

- **Anticipated adverse events** are those that have been previously identified as resulting from the underlying disease, the HCT, or acute GVHD and not related to study drug. Investigator should refer to the anticipated event list (see Appendix E) to assess whether or not an event is anticipated. Anticipated events will be collected in the data capture system through calendar-driven forms (Toxicity, GVHD, etc.) and event-driven forms (Death, Infection, etc.). Events that meet serious criteria should be reported as detailed in Section 4.4.4.2.

- **Unanticipated adverse events** are those that vary in nature, intensity, or frequency from information in the current anticipated event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk. Unanticipated events would also include those that have not been previously described as a result of the underlying disease requiring HCT, the HCT or acute GVHD.

The following are anticipated adverse events and do NOT require reporting as an AE/SAE:

- Disease progression of primary malignancy
- GVHD and symptoms of GVHD (these are recorded in acute and chronic GVHD eCRFs)
- Infections
- Graft failure
- Medical or surgical procedures (the condition that leads to the procedure is the AE)
- Situations where an untoward medical occurrence has not taken place. For example:
  - Planned hospitalizations due to pre-existing conditions, which have not worsened (e.g., HCT)
  - Hospitalizations that occur for procedures not due to an AE (e.g., cosmetic surgery)
  - Hospitalizations for a diagnostic procedure where the hospital stay is less than 24 hours in duration or for normal management procedures (e.g., chemotherapy)
  - Overdose of AAT or any concomitant therapy that does not result in any adverse signs or symptoms. (An overdose is a dose higher than the dose established in the protocol. For this study, the protocol dose is 120 mg/kg. There is a 5% variance that is allowed due to the rounding permitted in the preparation of the IMP. Therefore, an

AAT dose ≥1.06% of 120 mg/kg (i.e., 126 mg/kg) that does not result in any adverse signs or symptoms would not require reporting as an AE/SAE.)

Laboratory findings do NOT need to be reported as AEs in the following cases:

- Laboratory parameters already beyond the reference range at screening, unless a further increase / decrease can be considered an exacerbation of a pre-existing condition.
- Abnormal hematological laboratory parameters considered anticipated due to myeloablative conditioning regimen or other permitted chemotherapy treatments are not considered AEs.
- An abnormal laboratory value that cannot be confirmed after repeat analysis, preferably in the same laboratory (i.e., the previous result could be marked as not valid and should not necessarily be reported as an AE).

Serious Adverse Event: A serious adverse event (SAE), as defined by per 21 CFR 312.32, is any adverse event that results in one of the following outcomes, regardless of causality, anticipation, and/or expectedness:

- **Results in death**
- **Is life-threatening.** Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- **Requires or prolongs inpatient hospitalization** (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- **Results in persistent or significant disability/incapacity.** Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- **Is a congenital anomaly or birth defect;** or
- **Is an important medical event** when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed in the definition above (e.g., suspected transmission of an infectious agent by a medicinal product is considered a

Serious Adverse Event). Any event is considered a Serious Adverse Event if it is associated with clinical signs or symptoms judged by the Investigator to have a significant clinical impact.

4.4.2 Classification of Adverse Events by Severity

The severity refers to the intensity of the reported event. The Investigator must categorize the severity of each SAE according to the National Cancer Institute (NCI) CTCAE Version 5.0. CTCAE guidelines can be referenced at the following website: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. For any term that is not specifically listed in the CTCAE scale, intensity will be assigned a grade of one through five using the following CTCAE guidelines:

- Grade 1: Mild; asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

4.4.3 Classification of Adverse Events by Relationship to Investigational Product

The relationship of each reported event to the study therapy will be assessed by the Investigator; after careful consideration of all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the SAE, temporal relationship to any study therapy interventions and dechallenge or rechallenge according to the following guidelines:

- **Possibly, Probably, or Definitely Related:** there is a reasonable possibility that the study therapy caused the event. A relationship of possibly, probably, or definitely related to the investigational product is considered **related** for the purposes of regulatory authority reporting.
- **Unlikely, or Not Related:** There is no reasonable possibility that the investigational product caused the event. An unlikely or not related relationship to the investigational product is considered **not related** for the purposes of regulatory authority reporting.

4.4.4 Required Adverse Event Reporting

4.4.4.1 Non-Serious Adverse Events

All non-serious adverse events will be reported from randomization until 30 days after the last dose of study drug (AAT/PTM). Some anticipated non-serious events will be collected on calendar-driven forms or event-driven forms (Infection, GVHD, Graft Failure, Readmission/ Hospitalization, and Progression/Relapse).

4.4.4.2 Serious Adverse Events (SAE)

All SAEs will be reported from randomization until 30 days after the last dose of study drug (AAT/PTM). In addition, any SAE assessed as related to the investigational product that occurs after the 30-day follow-up period must be reported. Any unanticipated SAE from 31 days after the last dose of study drug through the study defined follow up (1-year post enrollment) are required to be reported through the expedited AE reporting system in Advantage eClinical. **Additionally, any grade 4 anticipated event not collected on the calendar-driven toxicity or specified event-driven form must also be reported through the expedited AE reporting system in Advantage eClinical. For this study, the reporting will be based on unanticipated in place of the MOP definition of unexpected.**

All reported SAEs are to be followed up until resolved, the Investigator judges an event as no longer clinically significant, or until the event stabilizes/becomes chronic to the extent that the event can be fully characterized.

Serious adverse events (SAEs) will be reported through an expedited AE reporting system via electronic data capture. Life-threatening and fatal SAEs must be reported within 24 hours of knowledge of the event. All other SAEs must be reported within 1 business day of knowledge of the event. Events entered in the EDC will be reported using NCI's CTCAE Version 5.0.

The Data and Safety Monitoring Board will receive reports of all unanticipated/unexpected SAEs upon review by the BMT CTN Medical Monitor and CSLB. Summary reports for all reported SAEs will be reviewed by the DSMB on at least an annual basis.

4.4.4.3 Adverse Events of Special Interest (AESI)

The Sponsor has a list of events classified as Adverse Events of Special Interest (AESI). These include 'anaphylactic infusional reaction' and 'suspected transmission of a viral agent via a medicinal product.' Both events must be reported through the expedited AE reporting system via Advantage eClinical and must be reported within 24 hours of knowledge of the event even if it does not meet serious criteria.

4.4.4.4 Toxicity Reporting

Toxicities are a subset of anticipated adverse events determined to be from the underlying disease process, HCT, GVHD prophylaxis, or corticosteroid use, and generally are not considered to be related to the study product. The toxicities listed in APPENDIX E were pre-determined by the Protocol Team.

Once the participant has reached 31 days from last dose of study drug, toxicities listed in APPENDIX E should be reported at the specified time points on the Toxicity CRF. **If an event listed is determined to be serious and related to AAT, it should be reported via the expedited reporting process on the AE/SAE case report form (CRF).**

4.4.5 Pregnancy

A female subject or female partner of a male subject who becomes pregnant from randomization up to and including 30 days after the last dose of study drug, must notify the Investigator immediately. CSLB must be notified within 5 days of the Investigator becoming aware of the pregnancy.

If a female subject becomes pregnant, she must discontinue treatment with the study drug but may continue other study procedures at the discretion of the Investigator.

Whenever possible, a pregnancy in a subject or in a female partner of a male subject exposed to study drug should be followed to term to assess any potential occurrence of congenital anomalies or birth defects. Any follow-up information, including premature termination and the status of the mother and child after delivery, should be reported by the Investigator through the expedited AE reporting system in Advantage eClinical. All abnormal pregnancies and neonatal outcomes (e.g., spontaneous abortion, stillbirth, neonatal death, or congenital anomaly) will meet the criteria for SAE classification. The Investigator should follow the procedure for reporting these events as SAEs.

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 Study Design

This study is a phase III, multicenter, double-blinded, randomized, placebo-controlled trial designed to compare AAT and corticosteroids (CS) to placebo-to-match (PTM) and CS as first-line therapy for patients with high-risk acute GVHD.

The study consists of an interim analysis for futility and a 12-month analysis at the end of the study. An additional analysis will be conducted after all randomized patients in the study complete 6 months of follow-up. Multiplicity adjustment is not needed for this analysis as all the patients would have completed the Day 28 primary endpoint visit by the time of analysis.

The target enrollment is 136 patients from approximately 25 centers with 68 patients assigned to each arm.

5.1.1 Accrual

It is estimated that an accrual period of 36 months (assuming a steady state of 4 patients per month after ramp up) will be necessary to enroll the target sample size. Accrual will be reported by race, ethnicity, gender, and age.

5.1.2 Randomization

Following enrollment, patients will be stratified by centers and randomized within each center in a 1:1 ratio using permuted blocks of random sizes for the two treatment arms.

Treatment practices differ considerably by centers. This includes the time to declare a patient refractory to steroids and potentially initiate next-line therapy. The variability by center may produce greater differences in treatment groups compared with age (adolescent vs adult), GHVD risk or disease. Additionally, the pathobiology of GVHD in both adolescent and adult and in malignant and non-malignant disease patient populations are similar. Therefore, since the presentation of GVHD and the anticipated response to treatment are expected to be similar, center was prioritized as the primary stratification variable.

5.1.3 Primary Endpoint

The primary endpoint is the proportion of participants with an overall (complete or partial) response to acute GVHD treatment at Day 28 post-randomization. Patients without a Day 28 response assessment will be considered failures. Acute GVHD will be graded and assessed for response based on Harris Criteria (refer to APPENDIX C). Patients who receive next-line systemic GVHD therapy (or escalation of steroids to ≥ 2.5 mg/kg/day of prednisone [or methylprednisolone equivalent of 2 mg/kg/day]) before Day 28 will be classified as NR. Responses categorized as NR, MR and progression will be treated as a failure for the primary endpoint. The primary analysis will be performed using the intent-to-treat principle. Therefore, all randomized patients will be included in the primary analyses.

5.1.4 Primary Hypothesis

The primary null hypothesis is that the odds ratio for response (CR/PR) at Day 28 between the treatment groups, adjusted for center, is equal to 1 versus the alternative hypothesis that the odds ratio is greater than 1 (AAT+CS versus PTM+CS). Therefore, an odds ratio greater than one means the response rate is higher in the AAT+CS group compared with the PTM+CS group. This null hypothesis will be tested using a one-sided significance level of 2.5%.

5.2 Sample Size and Power Calculations

The primary analysis will be based on a Wald test from a mixed effects logistic regression to test the odds ratio for response between the groups, adjusted for center and GVHD risk (Group A vs Group B; see Section 2.4.1, #3) as described in Section 5.6. We approximate the sample size and power requirements for this study based on a two-sample Z test of response rates. Historical control data indicates that the overall response rate (CR/PR) at Day 28 for high risk acute GVHD is approximately 50%. We further anticipate that the treatment will increase response rate to 75%. We planned a single boundary (nonbinding futility) design for testing a one-sided hypothesis with one interim analysis for futility, when 56% of the information is evaluable (76 patients or 38 per arm), and a 12-month analysis using a design based on the Gamma family spending function with parameter $\gamma = -8$. Therefore, a sample size of 136 patients (68 per arm) is required to sufficiently maintain a one-sided type I error of 2.5% while providing 86% statistical power for a one-sided test to detect an effect size of 25%. All statistical analyses will be done using SAS Version 9.4 or higher or R Version 3.4.4 or higher.

5.3 Study Analysis Schedule and Safety Stopping Guidelines

The study will consist of an interim analysis for futility after 56% of the patients (n=76) are evaluable, at a time coincident with a regularly scheduled meeting of the NHLBI-appointed DSMB, and a 12-month analysis after accrual and follow up for the primary endpoint is complete.

An additional analysis will be conducted after all the randomized patients in the study complete 6 months of follow-up post-randomization. This analysis is conducted to expedite the primary endpoint evaluation for potential regulatory submission. Multiplicity adjustment is not needed for this analysis as all the patients would have completed the Day 28 primary endpoint visit by the time of analysis. The details for this 6-month analysis, including the data to be included, will be specified in the statistical analysis plan.

Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review and are not formal "stopping rules" that would mandate automatic closure of study enrollment. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each meeting.

5.3.1 Interim Analysis for Futility

Analyses will be performed as described below in Section 5.6 for the primary endpoint. At the interim analysis time point, a Wald test for testing whether the odds ratio is equal to 1 in a mixed effects logistic regression model will be compared to the critical values shown in Table 5-1. All patients with at least 28 days of follow-up post randomization prior to the time of the interim analyses will be used to compute this statistic. Therefore, if the response rate in the placebo group

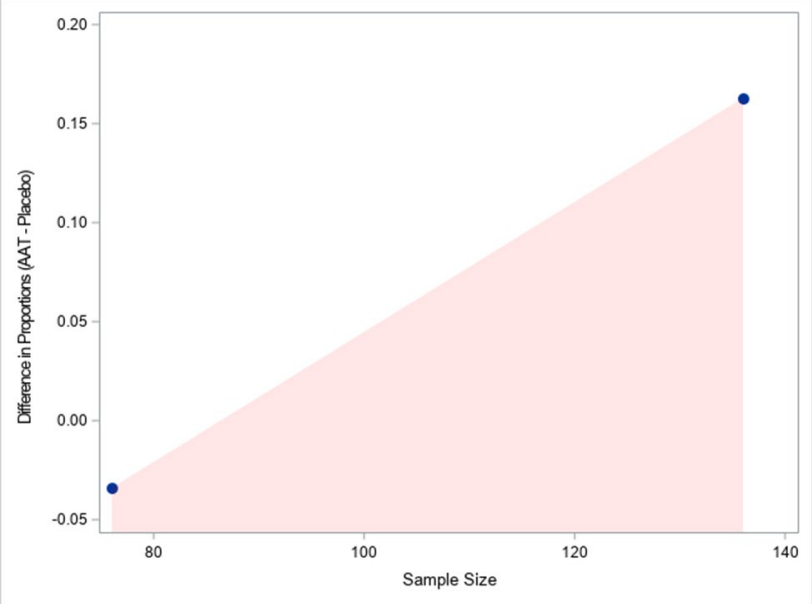
is at least 0.034 or higher than the AAT group, the DSMB will make a recommendation regarding termination or continuation of the trial.

Non-binding futility stopping rules—maintaining type I error control even if not followed exactly—were obtained from the Gamma family spending function with shape parameters $\gamma = -8$. This boundary corresponds to a conditional power of 8.9% under the alternative. This would also correspond approximately to an effect size (on a difference in response rates scale) less than -0.034 (Figure 5-1) or a Z statistic less than -0.31. In other words, if we were to observe a test statistic of -0.31 or smaller at the interim analysis, there is only an 8.9% chance that the study would be statistically significant at the 12-month analysis, under the alternative hypothesis.

Table 5-1 Single Boundary Non-Binding Futility Stopping Thresholds with One-Sided Type I Error 2.5%, Power 86% and an Effect Size of 25%

Info. Fraction	Sample Size	Nominal Cumulative Type II Error Rate	Futility Boundary Scales				Probability of Stopping Early for Futility
			Difference in Proportions	Z-Statistic	Conditional Power Under Alternative	Under Null	Under Alternative
0.56	76	0.004	-0.034	-0.31	0.089	0.378	0.004
1.0	136	0.139	0.163	1.96			

Figure 5-1 Stopping Boundary on Difference in Proportions Scale



5.3.2 Operating Characteristics of the Design

The statistical power to reject the null hypothesis under various treatment effect sizes using the Z test for comparing binomial proportions is shown in Table 5-2 as an approximation to the power using the primary analysis of mixed effects logistic regression. Kahan and Morris (2012) showed that a stratified analysis improves statistical power. Therefore, the unadjusted power analysis is a conservative estimate of the actual power. Also shown are the stopping probabilities for futility

at each effect size for the interim and 12-month analysis. This table shows that the target sample size of 136 patients has 86% power to detect a 25% difference in the response rate and is based on a simulation study with 10,000 simulated datasets.

Table 5-2 Summary of Operating Characteristics

Effect Size	Expected Sample Size	Overall Cumulative Power	Probability of Stopping Early
0.00	114	2.3%	0.360
0.10	129	21.24%	0.110
0.15	133	41.9%	0.046
0.20	135	65.2%	0.015
0.25	136	85.9%	0.003

5.3.2.1 Stratified Mixed Effects Logistic Simulation

We simulated a stratified randomized block design using blocks of size 6, based on the distribution of patients observed in each center of the BMT CTN 1501 study. Centers with fewer than 10 patients were combined resulting in a total of 6 centers of size 23, 19, 17, 15, 14 and 48. We assumed these centers are a random sample from a larger population with standard deviations 0.05, 0.6 and 1.0. Additionally, we estimated that 35% of the patients will be classified as "high risk"; a covariate that we adjust for in all the simulations done

Simulation results indicate that in the absence of a random effect (i.e., when the standard deviation of the random effect is close to zero) our statistical power is at least 94.2% to detect an odds ratio of 3. Further, when the random effect is present (std. dev. of 0.6 and 1.0) statistical power is at least 92.9% and 90.1% to detect an odds ratio of 3 as shown in Table 5-3. We also obtain statistical power ranging from 20.8% to 25.5% when the odds ratio is 1.5 regardless of the variability between centers.

Table 5-3 Stratified Mixed Effects Logistic Model with 1000 Simulations of Size 136

Effect Size			Power (%)		
			Variance of Random Effects		
AAT - PTM	Odds Ratio	Coef.	0.0025	0.36	1.0
10%	1.500	0.406	25.5	24.2	20.8
15%	1.857	0.619	53.8	51.3	48.4
20%	2.500	0.916	85.0	83.4	79.6
25%	3.000	1.099	94.2	92.9	90.1

5.3.3 6 Month Analysis for Clinical Study Report

A clinical study report for potential regulatory submission will be prepared after all randomized subjects have completed at least 6 months of follow-up post-randomization. The database will be locked to support this clinical study report. Data transfer and unblinding will follow the study's Blinding Management Plan. This report will include descriptive statistics based on available data at time of the analysis as well as formal hypothesis testing / treatment comparisons for the primary Day 28 overall response rate (CR/PR) endpoint and secondary endpoints including GVHD-free

survival, proportion of response, proportion of response allowing for next-line therapy, and systemic infections.

The BMT CTN 1705 Statistical Analysis Plan (SAP) will elaborate further on the contents of the 6-month report.

5.3.4 Guidelines for Safety Monitoring

Monitoring of a key safety endpoint will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guideline serves as a trigger for consultation with the DSMB for additional review.

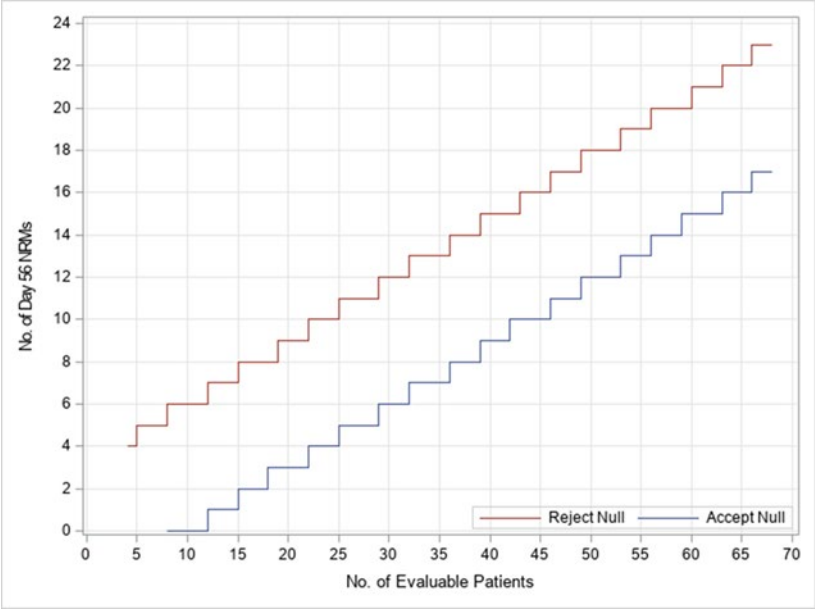
Safety monitoring will be based on Day 56 NRM. The proportion of NRM based on historical controls is estimated to be 20% versus an alternative hypothesis of 40%. This outcome will be monitored up to 56 days post-randomization, separately in each treatment arm, using a truncated Sequential Probability Ratio Test (SPRT) for binary data resulting in decision boundaries with a common slope of 0.293 and an upper intercept of 2.679 with nominal type I and type II error rates of, 0.065 and 0.10, respectively.

This sequential testing procedure conserves type I error at 5% across all the monthly examinations. The SPRT can be represented graphically. At each monthly examination, the number of evaluable patients is plotted against the cumulative sum of the endpoint of interest. The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring to protect against excessive Day 56 NRM. If the cumulative sum of the endpoint falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed number of patients evaluable. Otherwise, the SPRT continues until enrollment reaches the target sample size of 68 patients per arm. Table 5-4 and Figure 5-2 gives the rejection boundaries for the number of events for Day 56 NRM corresponding to the number of evaluable patients. As an example, if there are 15 evaluable patients with the number of Day 56 NRM being 8 or more, then this would trigger a review by the DSMB. Note that, at least four events must be observed to trigger review.

Table 5-4 Sequential Monitoring Plan for 68 Subjects per Arm ($\alpha = 0.065$, $\beta = 0.10$, $p_0 \leq 0.20$, $p_1 \geq 0.40$)

Number of Evaluable Patients	Rejection Boundary for the Null for the No. of Day 56 NRM	Number of Evaluable Patients	Rejection Boundary for the Null for the No. of Day 56 NRM	Number of Evaluable Patients	Rejection Boundary for the Null for the No. of Day 56 NRM
1-3		22-24	10	46-48	17
4	4	25-27	11	49-52	18
5-7	5	29-31	12	53-55	19
8-11	6	32-35	13	56-59	20
12-14	7	36-38	14	60-62	21
15-18	8	39-42	15	63-65	22
19-21	9	43-45	16	66-68	23

Figure 5-2 Acceptance and Rejection Boundaries for Safety Monitoring



The actual operating characteristics of the SPRT, shown in Table 5-5, were determined in a simulation study that assumed a uniform accrual of 68 patients to each arm over a 36-month accrual period.

Table 5-5 Operating Characteristics of the Sequential Probability Ratio Test for Day 56 NRM with 100,000 Simulation Replicates

True Day 56 NRM Rate	20%	25%	30%	35%	40%
Probability Reject Null	0.051	0.200	0.481	0.768	0.935
Mean No. of Day 56 NRMs	13.1	15.0	14.9	12.9	10.5
Mean No. of Patients Enrolled	66	60	50	37	26
Mean No. of Months	36	33	27	20	15

For example, the testing procedure rejects the null hypothesis in favor of the alternative 5.1%% of the time when the true Day 56 rate is 20% and 93.5% of the time when the rate is 40%. When the true Day 56 NRM rate is 40%, a review will be triggered when 10.5 events have been observed in 26 patients over a 15-month period.

5.3.5 Safety Analysis

The reporting of serious adverse events is described in detail in Section 4.4.4.2. All reported serious adverse events potentially associated with study drug will be carefully examined with respect to the severity and relationship to study drug. The type and severity of adverse events will be described.

5.4 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease, time from steroid initiation to randomization, acute GVHD grade at enrollment, MN classification, graft

source, GVHD prophylaxis, conditioning regimen, time from disease diagnosis to transplant, time from GVHD onset to randomization.

5.5 Analysis Populations

5.5.1 Primary Analysis Population

The intention-to-treat population will serve as the population for the primary analysis. All randomized subjects will be included in this population. Subjects will be included in the treatment group to which they are randomized. Intention-to-treat population consists of all randomized subjects whether or not treatment was administered. Any subject who receives a treatment randomization assignment will be considered to have been randomized. Analyses for the primary and secondary endpoints will use the primary analysis population.

5.5.2 Safety Analysis Populations

The safety analysis population in this study will comprise of all patients "as treated" in the study. This population will be used for the analysis of safety data. The "as treated" population consists of all randomized subjects who received at least one dose of study treatment. Subjects will be included in the treatment group corresponding to the study treatment they received for the analysis of safety data using the "as treated" population. For most subjects this will be the treatment group to which they are randomized. Subjects who take incorrect study treatment for the entire treatment period will be included in the treatment group corresponding to the study treatment received. Any subject who receives the incorrect study medication for one cycle but receives the correct treatment for all other cycles will be analyzed according to the correct treatment group and a narrative will be provided for any events that occur during the cycle for which the subject is incorrectly dosed.

5.5.3 General Analysis Guidelines

Primary analyses for the primary, secondary, and exploratory endpoints will use the primary analysis population except when otherwise specified. Analyses of each endpoint in each population will follow the analysis plans as described below in Sections 5.6, 5.7, and 5.8. We expect minimal missing data (< 5%) for the primary endpoint and secondary endpoints based on the past experience with a large phase III aGVHD treatment trial (BMT CTN protocol #0802. All secondary and exploratory analyses will use a two-sided significance level of 5%.

5.6 Analysis of Primary Endpoint

The response rate (CR/PR) at Day 28, compared to maximum GVHD organ staging within 72 hours prior to enrollment, is expected to be different by centers and GVHD risk (Group A vs Group B; see Section 2.4.1, #3). Therefore, we will fit a generalized linear mixed model with the response rate (CR/PR) at Day 28 as the outcome, treatment group as the main effect, and adjusted for GVHD risk fixed effect and center-specific random effects. A one-sided significance level of 2.5% will be used. The response rate (CR/PR) at Day 28 will be estimated using frequencies in each group along with corresponding 95% confidence intervals.

We plan on conducting two sensitivity analyses to assess the robustness of our findings. First, using the above generalized linear mixed model we will adjust for GVHD risk, age (adolescent vs adults) and disease (malignant vs non-malignant) all as main effects, in addition to

center specific random effects. The second analysis will include all the covariates from the first analysis in addition to the Mount Sinai Acute GVHD International Consortium (MAGIC) biomarker score. These two models will be compared to the primary analysis model in order to assess the impact on the main effect estimate.

5.7 Analysis of Secondary Endpoints

5.7.1 Duration of Response

Duration of response will be evaluated at 6- and 12-months post-randomization in the cohort of patients who achieve a Day 28 response. There will be a primary and secondary analyses of DOR. In the primary analysis, DOR is defined as the time from Day 28 response to progression, new salvage therapy for acute GVHD, death from any cause, or re-escalation of steroids to ≥ 2.5 mg/kg prednisone or equivalent. Patients without any of these events will censored at last follow up. For the secondary analysis, DOR will be defined as time from Day 28 response to death from any cause or new salvage therapy. Otherwise, patients are censored. For each definition, the probabilities of DOR will be estimated using the Kaplan-Meier method and compared between treatment arms using the log-rank test.

5.7.2 Non-relapse Mortality

Non-relapse mortality at 6 months and 12 months post-randomization will be estimated using the cumulative incidence function, treating relapse as a competing risk. The cumulative incidence curves for non-relapse mortality will be compared between the treatment groups using Gray's test. A Cox proportional hazards regression model with mixed effects will be used to compare the cause specific hazards of NRM between treatment groups adjusting for GVHD risk with center-specific log-normal frailties.

5.7.3 Overall Survival and Progression-Free Survival

Progression-free survival (PFS) at 6- and 12-months post-randomization will be estimated using the Kaplan-Meier method, treating death or relapse of malignancy as events in patients with malignancies. Overall Survival (OS) at 6 and 12 months will also be estimated using the Kaplan-Meier method. These endpoints will be compared between the treatment groups using the log-rank test. A Cox proportional hazards regression model with mixed effects will be used to compare PFS and OS between treatment groups adjusting for GVHD risk with center-specific log-normal frailties.

5.7.4 GVHD-Free Survival

The proportion of patients surviving at Day 56 after randomization without acute or chronic GVHD and without other systemic agents (or escalation of steroids to ≥ 2.5 mg/kg/day of prednisone [or methylprednisolone equivalent of 2 mg/kg/day]) added for treatment of GVHD will be evaluated. Provided there is no censoring prior to 56 days, this endpoint we will be computed as a binary outcome and summarized using frequencies/proportions. GVHD-Free survival will be compared by treatment adjusting for GVHD risk and center-specific random effects using a generalized linear mixed model. Additionally, treatment groups will be compared using difference in proportions, relative risk and an odds ratio estimate along with corresponding

confidence intervals. Censoring before 56 days is highly unlikely; and should this occur we anticipate this will be minimal.

5.7.5 Proportion of Response

The proportions of patients with Complete Response (CR), Partial Response (PR) (including subset with "Very Good Partial Response" [VGPR]), and Treatment Failure (TF) at Days 7, 14, 21, 28, 56 and 86 post-randomization will be described and compared between the treatment groups using the chi-square test or Fisher's exact test as appropriate.

5.7.6 Proportion of Response (Next-Line Therapy)

The proportions of patients with CR, PR (including subset with VGPR), and treatment failure (TF) at Days 7, 14, 21, 28, 56, and 86 post-randomization who receive ruxolitinib or other next-line therapies approved by the protocol Chairs as next-line therapy and remain on AAT/PTM will be described and compared between the treatment groups, to evaluate any differences in safety and efficacy, using the chi-square test or Fisher's exact test as appropriate. The proportion of AE/SAE will be described in these patient populations.

5.7.7 Systemic Infections

Frequencies of grade 2 or 3 infections occurring from randomization until 30 days after last study drug or placebo treatment will be tabulated by site of disease, date of onset, and severity. The time to first serious (grade 2 or 3) infection will be described using the cumulative incidence function and compared between treatment groups using Gray's test, with death treated as a competing risk.

5.7.8 Adverse Events

Frequencies of grade 3-5 adverse events (per CTCAE Version 5.0) that occur from randomization through 30 days after completing study drug or placebo treatment will be tabulated using the Safety Population. The proportion of patients experiencing grade 3-5 adverse events will be compared between the treatment groups using an odds ratio estimate with confidence interval.

5.7.9 Chronic GVHD

The incidence of chronic GVHD will be computed at 6- and 12-months post- randomization using the cumulative incidence method, treating death prior to chronic GVHD as a competing risk. Diagnosis of chronic GVHD of any severity (mild, moderate, or severe) per NIH Consensus Criteria (see APPENDIX C) is considered an event for this endpoint. Cumulative incidence will be compared between treatment groups using Gray's test. Cause specific hazard for chronic GVHD will be compared by treatment adjusting for GVHD risk and center-specific random effects using a generalized linear mixed model.

5.7.10 Disease Relapse

Disease relapse will be summarized at 6- and 12-months post-randomization using the cumulative incidence function with death as a competing risk. Cumulative incidence will be compared between treatment groups using Gray's test. Cause specific hazards for disease relapse will be compared by treatment adjusting for GVHD risk and center-specific random effects using a Cox proportional hazards model with center specific log-normal random effects.

5.8 Analysis of Exploratory Endpoints

5.8.1 AAT Levels

The pharmacokinetics parameters maximum (Cmax) and trough (Ctrough) serum antigen concentration of AAT will be measured and summarized using descriptive statistics at Days 0, 8, 16, 24, 28 and 56 post-treatment initiation.

5.8.2 Stool AAT, Microbiome and Metabolome

Stool concentrations of AAT will be measured and summarized using descriptive statistics at baseline and Days 8 and 28 post-treatment initiation.

5.8.3 Immune Subsets

The changes in peripheral blood ratios of T regulatory to T effector (Treg/Teff) cells, Natural Killer (NK) cells and other cellular immune subsets from baseline to Days 16, 28, and 56 post-treatment initiation will be summarized using descriptive statistics at each time point and compared between treatment groups using Mann-Whitney nonparametric tests. Select immune subsets may also be analyzed using a linear mixed model adjusting for baseline value, time and treatment, as well as a treatment by time interaction term.

5.8.4 Inflammatory Cytokines and GVHD Biomarkers

Serum levels of inflammatory cytokines (e.g., IL-1β, IL-6, IL-10, TNFα), damage associated molecular patterns (e.g., heparan sulfate) and select GVHD biomarkers (eg, ST2, REG3α) at baseline and at Days 8, 28, and 56 post-treatment initiation will be summarized using descriptive statistics at each time point and compared between treatment groups using Mann-Whitney nonparametric tests. Select measurements may also be analyzed using a linear mixed model adjusting for baseline value, time, and treatment, as well as a treatment by time interaction term.

5.8.5 Overall and Organ-Specific Response Rates by Refined Minnesota Risk and Biomarker-Based Risk Classifications

The proportions of patients with an overall response (complete or partial) and response rate of each GVHD target organ at Day 28 post-randomization will be described by Refined Minnesota risk category (A vs B; see Section 2.4.1, #3) and by Ann Arbor biomarker risk score (1, 2, or 3) using sample proportions and confidence intervals and compared on each factor using the chi-square test or Fisher's exact test as appropriate.

5.8.6 Systemic Corticosteroid Dose

The systemic corticosteroid-dose (measured as prednisone-equivalent) will be summarized at baseline and at Days 7, 14, 21, 28, 56, 86, 6 months and 12 months post-randomization using descriptive statistics at each time point and compared between treatment groups using Mann-Whitney nonparametric tests.

5.8.7 CMV-Reactivation

The proportions of patients requiring new systemic treatment for CMV-reactivation by Day 56 post-randomization will be compared between the treatment groups using a chi-square test or Fisher's exact test as appropriate.

5.8.8 Patient-Reported Outcomes

Participant self-reported measures for both adolescents and adults will be assessed at baseline, Days 28, 56 and 180 post-randomization by MDASI, and selected PROMIS subscales for gastrointestinal symptoms, physical function and satisfaction with participation in social roles. These instruments will be scored according to the recommendations of the developers. The scores will be examined using the medians, means, ranges, and standard deviations. Linear regression models for the Day 28, Day 56 and 6-month score adjusting for baseline score and other covariates will be used to explore differences between treatment arms and changes in outcomes over time. Provided there is sufficient data, separate analyses will be conducted for adolescent and adult patients. Otherwise, their data will be combined/aggregated.

APPENDIX A
HUMAN SUBJECTS

1. Subject Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates, provide the patient with information about the purpose of the study, and obtain consent or assent. The BMT CTN DCC will provide a template of the consent and assent forms to each center. Each center will customize the templates according to their local requirements and submit for review by the DCC for adequacy prior to submitting to the NMDP IRB of Record. Each center must provide evidence of IRB approval to the DCC.

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 center. The ID code will be generated by and kept on file at the BMT CTN Data and Coordinating Center upon enrollment.

3. Participation of Adolescents

The protocol was amended (v4.0), after DSMB review and approval, to include adolescents ages 12 - 17.99 after 40 adult patients were enrolled onto the trial and completed the Day 56 follow-up assessment for safety.

4. Participation of Women and Minorities

Women, ethnic minorities, and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on incidence of high risk acute GVHD in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment reports.

5. 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
LABORATORY PROCEDURES

Research Samples for Protocol-Defined and Ancillary Correlative Laboratory Studies
This protocol involves collection of required and optional research samples for patients consenting to the BMT CTN 1705 study. The objectives are based on the current understanding of AAT biology informed by preclinical and clinical studies available at the time of protocol development. These studies will explore the impact of AAT administration on serum levels of AAT, key cellular immune subsets, inflammatory markers and GVHD biomarkers.

Required peripheral blood samples will be collected at calendar driven time points that are required to support study-specific secondary and exploratory endpoints that are summarized in the table below. Additionally, stool losses of AAT and changes in gut microbiome are known to occur during the development of GVHD. Stool samples, which are optional for patients at selected centers associated with active microbiome laboratories, will be collected for planned exploratory analysis of stool AAT levels and correlation with serum cytokines and/or validated proteomic GVHD related biomarkers. Remaining stool and serum biospecimens will be made available for future research of microbiome, microbial metabolome and correlative laboratory studies with potential to advance findings of the current trial. Sample collection, processing and shipping procedures are detailed in the BMT CTN 1705 Research Sample Information Guide.

Enrollment is Day 0 and the first dose of study drug must occur within 12 hours after enrollment. Therefore, the first dose may fall on Day 0 or Day 1 in the tables below.

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications	Shipping and Testing Location
All Patients (Required)	**Peripheral Blood** (SST Clot Tube) **5 mL** **Serum AAT Levels**	**Prior to study drug infusion *and* 15-45 min post-infusion** • Day 0 (baseline) • Day 16 • Day 28 • Day 56 **Prior to study drug infusion only** • Day 8 • Day 24 ***Note:*** Collection of blood samples for AAT levels are limited to subjects still receiving assigned study treatment.	Collect the blood sample and place 5 mL in SST Vacutainer tube containing no anticoagulant. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation and serum aliquot storage at -80ºC. Refer to the BMT CTN 1705 Research Specimen Information Guide for details related to sample processing, aliquot labeling, storing and shipping.	Frozen serum sample aliquots will be periodically batched-shipped to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository
All Patients (Required)	**Peripheral Blood** (SST Clot Tube) **6 mL** **Serum Inflammatory Cytokines and GVHD Biomarkers**	**Prior to study drug infusion on:** • Day 0 (baseline) **Prior to study drug infusion, on Days**: 8, 28 and 56 (or TD if prior to Day 56) Coordinate the collection with the pre-drug infusion blood samples for AAT level measurements. If not possible in rare circumstances, the window of collection for research blood samples is ± 3 days.	Collect the blood sample and place 5 mL in SST Vacutainer tube containing no anticoagulant. Allow blood samples to clot upright for 30-60 minutes in a tube rack prior to centrifugation and serum aliquot storage at -80ºC. Refer to the BMT CTN 1705 Research Specimen Information Guide for details related to sample processing, aliquot labeling, storing and shipping.	Frozen serum sample aliquots will be periodically batched-shipped to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications	Shipping and Testing Location
All Patients (Required)	**Peripheral Blood** (sodium heparin) **10 mL** **Flow Cytometry** (T regulatory, T effector and NK cell subsets)	**Prior to study drug infusion on:** • Day 0 (baseline) **Prior to study drug infusion, on Days**: 16, 28 and 56 (or TD if prior to Day 56) Coordinate the collection with the pre-drug infusion blood samples for AAT level measurements. If not possible in rare circumstances, the window of collection for research blood samples are ± 3 days.	Collect the blood sample and place 10 mL in Vacutainer tube containing sodium heparin anticoagulant. Gently mix sample by inversion 8-10 times to mix sample well with anticoagulant.	Blood sample tube will be shipped at ambient temperature, on the day of collection, to project laboratory by priority overnight FedEx® delivery.	RPCI Project Laboratory
Stool Samples for Patients Enrolled at Selected Centers with Participating Microbiome Labs					

Subjects	Sample Type	Sample Collection Time Points	Sample Collection and Sample Processing Summary	Shipping Specifications	Shipping and Testing Location
Patients at a Subset of Centers (Optional)	**Stool** **10-20 mL** **Stool AAT, Microbiome, and Metabolome Studies**	**Prior to study drug infusion on:** • Day 0 (baseline) **Prior to study drug infusion, on Days**: 8 and 28 (or TD if prior to Day 28) Stool samples should be collected if possible, on the target visit day indicated or within ± 3 days of the scheduled visit.	Collect stool samples according to applicable inpatient or outpatient procedures described in the Research Sample Information Guide. Transfer a 10-20 mL stool sample to a sterile specimen transport container. Also transfer a small amount of stool sample to the OMNIgene Gut stool stabilization tube provided by the BMT CTN. Refer to the BMT CTN 1705 Research Specimen Information Guide for details related to sample requirements, OMNIgene Gut tube sampling/mixing procedures, sample labeling and transport to center's Microbiome laboratory for further process and temporary frozen storage.	Frozen stool sample aliquots processed by the center's Microbiome Laboratory will be periodically batched-shipped to the BMT CTN Biorepository by priority overnight FedEx® delivery.	BMT CTN Biorepository

APPENDIX C
DIAGNOSIS AND SEVERITY SCORING FOR ACUTE AND CHRONIC GVHD

1. (2014 NIH Consensus Criteria[87])

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

Organ scoring of chronic GVHD. ECOG indicates Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; ULN, normal upper limit. *Weight loss within 3 months. Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring. To be completed by specialist or trained medical providers. **Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

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

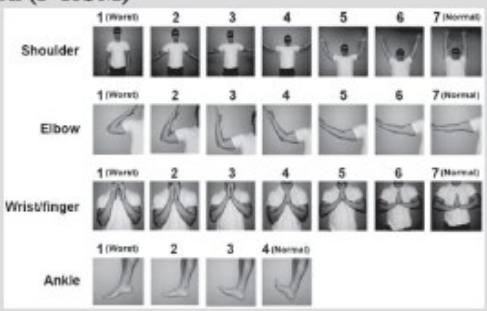
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs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

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

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

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

Photographic Range of Motion (P-ROM)



Chronic GVHD Grading according to the NIH Consensus Criteria chronic GVHD severity scale

Mild chronic GVHD

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

Moderate chronic GVHD

3 or more organs involved with no more than score 1

OR

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

OR

Lung score 1

Severe chronic GVHD

At least 1 organ with a score of 3

OR

Lung score of 2 or 3

Key points:

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

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

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

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

2. Harris (MAGIC) Criteria

Table 1
GVHD Target Organ Staging

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

Overall clinical grade (based on most severe target organ involvement):
Grade 0: No stage 1-4 of any organ.
Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement.
Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI.
Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI.
Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0-1 upper GI.

3. Categories of Acute and Chronic GVHD

Categories of Acute and Chronic GVHD			
Category	**Time of Symptoms after HCT**	**Presence of Acute GVHD Features**	**Presence of Chronic GVHD Features**
Acute GVHD			
Classic acute GVHD	≤ 100 d	Yes	No
Late-onset acute GVHD	> 100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

APPENDIX D
LIFESTYLE RESTRICTIONS

Female subjects of childbearing potential and all male subjects must use medically reliable methods of contraception from the first dose of investigational product until 1 month after the last dose of investigational product.

Childbearing potential is assumed in all female subjects except:

- Female subjects aged > 60 years.
- Female subjects aged 45 to 60 years (inclusive) with amenorrhea for ≥ 1 year.
- Female subjects who are surgically sterile for at least 3 months before providing informed consent.

Acceptable methods of contraception are:

- Abstinence, where abstinence is the preferred and usual lifestyle of the subject, including refraining from heterosexual intercourse during the entire period of risk associated with the investigational product. Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method are not acceptable definitions of abstinence.
- Hormonal methods: acceptable hormonal methods include oral contraceptives, contraceptive medication patch, contraceptive medication injection, estrogen/progestin vaginal ring, or contraceptive medication implant.
- 2 barrier methods; acceptable barrier methods include: female or male condoms, with spermicidal foam or spermicidal jelly, or diaphragm, with spermicidal foam or spermicidal jelly. The female condom and male condom should not be used together.
- Use of intrauterine device (placed more than 3 months before providing informed consent).
- Surgical sterilization (more than 3 months before providing informed consent) of subject or subject's partner.

APPENDIX E
ANTICIPATED TOXICITIES

The purpose of this appendix is to outline those AEs that commonly occur in the study population. The following is a list of events that the Sponsor considers to be common toxicities for agents used in HCT conditioning and GVHD prophylaxis regimens. The events listed below require reporting via the Toxicity CRF in Advantage eClinical and **do NOT change the reporting obligations, timelines or prevent the need to report an AE meeting the definition of an SAE.**

If aggregate analysis of these events indicates they occur more frequently with study drug, an expedited safety report may be submitted to regulatory agencies.

Lack of relationship to the investigational product is not automatically assumed; therefore, an assessment is required by the Investigator for any events specified below. The Sponsor will also perform the causality assessment on a case-by-case basis and expedite reporting to regulatory agencies when applicable.

General Disorders
Fever

Immune System Disorders
Allergic reaction

GI Disorders
Mucositis
Nausea
Vomiting
Gastric ulcer
Esophagitis
Pancreatitis
GI perforation
Increased appetite
Anorexia

Renal Disorders
Cystitis noninfective
Acute kidney injury
Chronic kidney injury

Hemorrhagic Disorders
Hemorrhage

Blood & Lymphatic Disorders
Leukocytosis
Anemia
Thrombotic thrombocytopenic purpura/Thrombotic microangiopathy
Hemolytic Uremic Syndrome

Vascular Disorders
Thromboembolic event

Respiratory Disorders
Hypoxia
Dyspnea

Investigations
Neutrophil count decreased
Platelet count decreased
ALT
AST
Bilirubin
Alkaline phosphatase
Hyperglycemia
Hypokalemia
Weight gain

Cardiac Disorders
Hypotension
Hypertension
Cardiac arrhythmia
Left ventricular systolic dysfunction
Pericardial effusion
Restrictive cardiomyopathy

Nervous System Disorders
Restlessness
Insomnia
Glaucoma
Headache
Somnolence
Seizure

Musculoskeletal Disorders
Myopathy
Tendon rupture
Bone fracture
Avascular necrosis
Osteoporosis
Arthralgia

Skin Disorders
Erythema
Rash acneiform

Endocrine/Metabolic Disorders
Cushingoid
Adrenal insufficiency
Edema

APPENDIX F
PATIENT REPORTED OUTCOMES MEASUREMENT INFORMATION SYSTEM (PROMIS)

1. In general, would you say your health is….

    a. Poor
    b. Fair
    c. Good
    d. Very good
    e. Excellent

2. In the past 7 days, how often did you have nausea—that is, a feeling like you could vomit?

    a. Never (skip next question)
    b. Rarely
    c. Sometimes
    d. Often
    e. Always

3. In the past 7 days, how often did you know that you would have nausea before it happened?

    a. Never
    b. Rarely
    c. Sometimes
    d. Often
    e. Always

4. In the past 7 days, how often did you have a poor appetite?

    a. Never
    b. Rarely
    c. Sometimes
    d. Often
    e. Always

5. In the past 7 days, how often did you throw up or vomit?

    a. Never
    b. One day
    c. 2-6 days
    d. Once a day
    e. More than once a day

6. In the past 7 days, how often did you have belly pain?
    - **a.** No days
    - **b.** One day
    - **c.** 2-6 days
    - **d.** Once a day
    - **e.** More than once a day

7. In the past 7 days, how many days did you have loose or watery stools?
    - **a.** No days
    - **b.** One day
    - **c.** Two days
    - **d.** 3-5 days
    - **e.** 6-7 days

8. In the past 7 days, how often did you feel like you needed to empty your bowels right away or else you would have an accident?
    - **a.** Never
    - **b.** One time during the past 7 days
    - **c.** 2-6 days during the past 7 days
    - **d.** Often once a day
    - **e.** More than once a day

9. In the past 7 days, how often did you have bowel incontinence—that is, have an accident because you could not make it to the bathroom in time?
    - **a.** No days
    - **b.** One day
    - **c.** 2-3 days
    - **d.** 4-5days
    - **e.** 6-7 days

10. Are you able to dress yourself, including tying shoelaces and buttoning your clothes?
    - **a.** Without any difficulty
    - **b.** With a little difficulty
    - **c.** With some difficulty
    - **d.** With much difficulty
    - **e.** Unable to do

11. Are you able to get out of bed into a chair?

a. Without any difficulty
b. With a little difficulty
c. With some difficulty
d. With much difficulty
e. Unable to do

12. Are you able to go for a walk of at least 15 minutes?

a. Without any difficulty
b. With a little difficulty
c. With some difficulty
d. With much difficulty
e. Unable to do

13. Are you able to go up and down stairs at a normal pace?

a. Without any difficulty
b. With a little difficulty
c. With some difficulty
d. With much difficulty
e. Unable to do

14. In the past 7 days, I feel fatigued

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

15. In the past 7 days, I have trouble starting things because I am tired

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

16. In the past 7 days, how run-down did you feel on average?

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

17. In the past 7 days, how fatigued were you on average?

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

18. In the past 7 days, how much did pain interfere with your day to day activities?

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

19. In the past 7 days, how much did pain interfere with your enjoyment of life?

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

20. In the past 7 days, how would you rate your pain on average?

a. No pain 0
b. 1
c. 2
d. 3
e. 4
f. 5
g. 6
h. 7
i. 8
j. 9
k. Worst imaginable pain, 10

21. In the past 7 days, how bothered were you by sores on your skin?

a. Not at all
b. Slightly
c. Moderately
d. Quite a bit
e. Extremely

22. In the past 7 days, how intense was your itch in general?

a. Had no itch
b. Mild
c. Moderate
d. Severe
e. Very severe

23. In the past 7 days, I felt worthless

a. Never
b. Rarely
c. Sometimes
d. Often
e. Always

24. In the past 7 days, I felt helpless

a. Never
b. Rarely
c. Sometimes
d. Often
e. Always

25. In the past 7 days, I felt depressed

a. Never
b. Rarely
c. Sometimes
d. Often
e. Always

26. In the past 7 days, I felt hopeless

a. Never
b. Rarely
c. Sometimes
d. Often
e. Always

27. In the past 7 days, my sleep quality was…

a. Very poor
b. Poor
c. Fair
d. Good
e. Very good

28. In the past 7 days, my sleep was refreshing

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

29. In the past 7 days, I had a problem with my sleep

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

30. In the past 7 days, I had difficulty falling asleep

a. Not at all
b. A little bit
c. Somewhat
d. Quite a bit
e. Very much

APPENDIX G
BMT CTN SEVERITY GRADING TABLE AND RECURRENCE INTERVAL DEFINITIONS

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
Bacterial infections	Bacterial focus NOS requiring no more than 14 days of therapy for treatment (e.g., urinary tract infection) Coag Neg Staph (S. epi), Corynebacterium, or Proprioniobacterium bacteremia Cellulitis responding to initial therapy within 14 days C. Difficile toxin positive stool with diarrhea < 1L without abdominal pain (child < 20 mL/kg)	Bacteremia (except CoNS) without severe sepsis *** Bacterial focus with persistent signs, symptoms or persistent positive cultures requiring greater than 14 days of therapy Cellulitis requiring a change in therapy d/t progression Localized or diffuse infections requiring incision with or without drain placement Any pneumonia documented or presumed to be bacterial C. Difficile toxin positive stool with diarrhea $\geq$ 1L (child $\geq$ 20 mL/kg) or with abdominal pain	Bacteremia with deep organ involvement (e.g., with new or worsening pulmonary infiltrates; endocarditis) Severe sepsis with bacteremia. Fasciitis requiring debridement Pneumonia requiring intubation Brain abscess or meningitis without bacteremia C. Difficile toxin positive stool with toxic dilatation or renal insufficiency with/without diarrhea

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
Fungal infections	Superficial candida infection (e.g., oral thrush, vaginal candidiasis)	Candida esophagitis (biopsy proven). Proven or probable fungal sinusistis confirmed radiologically without orbital, brain or bone involvement.	Fungemia including Candidemia Proven or probable invasive fungal infections (e.g., Aspergillus, Mucor, Fusarium, Scedosporium). Disseminated infections (defined as multifocal pneumonia, presence of urinary or blood antigen, and/or CNS involvement) with Histoplasmosis, Blastomycosis, Coccidiomycosis, or Cryptococcus. *Pneumocystis jiroveci* pneumonia (regardless of PaO2 level)
Fungal infections continued			
Viral infections	Mucous HSV infection Dermatomal Zoster Asymptomatic CMV viremia untreated or a CMV viremia with viral load decline by at least 2/3 of the baseline value after 2 weeks of therapy EBV reactivation not treated with rituximab Adenoviral conjunctivitis asymptomatic viruria, asymptomatic stool shedding and viremia not requiring treatment Asymptomatic HHV-6 viremia untreated or an HHV-6 viremia with a viral load decline by at least 0.5 log after 2 weeks of therapy	VZV infection with 3 or more dermatomes Clinically active CMV infection (e.g. symptoms, cytopenias) or CMV Viremia not decreasing by at least 2/3 of the baseline value after 2 weeks of therapy EBV reactivation requiring institution of therapy with rituximab Adenoviral upper respiratory infection, viremia, or symptomatic viruria requiring treatment Clinically active HHV-6 infection (e.g., symptoms, cytopenias) or HHV-6 viremia without viral load decline 0.5 log after 2 weeks of therapy	Severe VZV infection (coagulopathy or organ involvement) CMV end-organ involvement (pneumonitis, enteritis, retinitis) EBV PTLD Adenovirus with end-organ involvement (except conjunctivitis and upper respiratory tract)

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
Viral infections continued	BK viremia or viruria with cystitis not requiring intervention Viremia (virus not otherwise specified) not requiring therapy	BK viremia or viruria with clinical consequence requiring prolonged therapy and/or surgical intervention Enterocolitis with enteric viruses Symptomatic upper tract respiratory virus Any viremia (virus not otherwise specified) requiring therapy	Lower tract respiratory viruses Any viral encephalitis or meningitis
Parasitic infections			CNS or other organ toxoplasmosis Strongyloides hyperinfection
Nonmicrobiologically defined infections	Uncomplicated fever with negative cultures responding within 14 days Clinically documented infection not requiring inpatient management	Pneumonia or bronchopneumonia not requiring mechanical ventilation Typhlitis	Any acute pneumonia requiring mechanical ventilation Severe sepsis*** without an identified organism

*Concomitant or multimicrobial infections are graded according to the grade of the infection with the higher grade of severity.

**Therapy includes both PO and IV formulations

***Severe Sepsis:

Adults:

Hypotension

-A systolic blood pressure of <90 mm Hg or a reduction of >40 mm hg from baseline in the absence of other causes for hypotension

Multiple Organ Dysfunction Syndrome

-2 or more of the following: Renal failure requiring dialysis, respiratory failure requiring bipap or intubation, heart failure requiring pressors, liver failure

Pediatrics:

-Pediatric SIRS definition and suspected or proven infection and cardiovascular dysfunction or ARDS or TWO or MORE other organ dysfunctions

Pediatric SIRS definition:
Two or more of the following, *one of which must be abnormal temperature or leukocyte count*

1) Core temperature >38.5C ***or*** < 36C
2) Tachycardia, otherwise unexplained persistent in absence of external stimulus, chronic drugs or painful stimuli. ***or*** bradycardia, in < 1 year old, otherwise unexplained persistent.
3) Tachypnea or mechanical ventilation for an acute process not related to underlying neuromuscular disease or general anesthesia
4) Leukocytosis or leukopenia for age (not secondary to chemotherapy) or >10% bands

Pediatric organ dysfunction criteria:

Cardiovascular: despite administration of fluid bolus ≥40 ml/kg in 1 hour:

- Hypotension <5th percentile for age (***or*** per Table 1)
- Pressors at any dose
- Two of the following:
    - Capillary refill > 5 secs
    - Core to peripheral temperature gap > 3^{o}C
    - Urine output < 0.5 mL/kg/hr
    - Unexplained metabolic acidosis (Base deficit > 5.0 mEq/L)
    - Blood lactate > 2 x ULN

Respiratory:

- ARDS ***or***
- Intubated ***or***
- >50% FiO2 to maintain SaO2 > 92%

Neurological:

- Glasgow Coma Score ≤ 11 ***or***
- Acute change in mental status with a decrease in GSC ≥3 pts from abnormal baseline

Renal:

- Serum creatinine ≥ 2 x ULN for age ***or*** 2-fold increase in baseline creatinine

Hepatic:

- Total bilirubin ≥4 mg/dL ***or***
- ALT ≥2 x ULN for age

TABLE 1: FOUR AGE GROUPS RELEVANT TO HCT:

Age	Tachycardia (bpm)	Bradycardia (bpm)	Tachypnea (breaths/min)	Leukocytosis / Leukopenia (WBC)	Hypotension Systolic BP mmHg
1 mo to 1 yr	>180	<90	>34	>17.5 to <5.0	<100
2 yr to 5 yr	>140	NA	>22	>15.5 to <6.0	<94
6 yr to 12 yr	>130	NA	>18	>13.5 to <4.5	<105
13 yr to < 18 yr	>110	NA	>14	>11 to <4.5	<117

Disseminated Infections:

1. Two or more non-contiguous sites with the SAME organism
2. A disseminated infection can occur at any level of severity, but most will be grade 2 or 3.

Recurrence Intervals to Determine Whether an Infection is the Same or New:

1. CMV, HSV, EBV, HHV6: 2 months (< 60 days)
2. VZV, HZV: 2 weeks (< 14 days)
3. Bacterial, non-C. difficile: 1 week (< 7 days)
4. Bacterial, C. difficile: 1 month (< 30 days)
5. Yeast: 2 weeks (< 14 days)
6. Molds: 3 months (< 90 days)
7. Helicobacter: 1 year (< 365 days)
8. Adenovirus, Enterovirus, Influenza, RSV, Parainfluenza, Rhinovirus: 2 weeks (< 14 days)
9. Polyomavirus (BK virus): 2 months (< 60 days)

For infections coded as "Disseminated" per the *Infection Form*, any previous infection with the same organism but different site within the recurrence interval for that organism will be counted as part of the disseminated infection.

APPENDIX H
ABBREVIATIONS

A1-PI: Alpha1-Proteinase Inhibitor
AA1: Ann Arbor 1
AA2: Ann Arbor 2
AA3: Ann Arbor 3
AAT: Alpha 1 – Antitrypsin
ADL: Activities of Daily Living
AE: Adverse Event
ALT: Alanine Transaminase
AP: Alkaline Phosphatase
AREG: Amphiregulin
AST: Aspartate Transaminase
BLA: Biologics License Application
BMT CTN: Blood and Marrow Transplant Clinical Trials Network
BSA: Body Surface Area
CBC: Complete Blood Count
CD: Cluster of Differentiation 3
CI: Confidence Interval
CIBMTR: Center for International Blood and Marrow Transplant Research
CLL: Chronic Lymphocytic Leukemia
CMV: Cytomegalovirus
CNS: Central Nervous System
CR: Complete Response
CRF: Case Report Form
CRP: C-Reactive Protein
CS: Corticosteroids
CSLB: CSL Behring
CT: Computed Tomography
CTCAE: Common Terminology Criteria for Adverse Events
DAMP: Danger-Associated Molecular Pattern
DCC: Data Coordinating Center
DLI: Donor Lymphocyte Infusion
DOR: Duration of Response
DSMB: Data and Safety Monitoring Board
ECOG: Eastern Cooperative Oncology Group
eCRF: Electronic Case Report Form
EDC: Electronic Data Capture
EOT: End of Therapy
FCBP: Females of Childbearing Potential
FDG: Fluorodeoxyglucose
FEV1: Forced Expiratory Volume in 1 Second
FOXP3: Forkhead Box Protein P3
FVC: Forced Vital Capacity
GCP: Good Clinical Practice
GI: Gastrointestinal
GMP: Good Manufacturing Practice
IRT: Suvoda Interactive Response Technology
IV: Intravenous
KPS: Karnofsky Performance Status
LDH: Lactate Dehydrogenase
LFT: Liver Function Test
LPS: Lipopolysaccharide
MAGIC: Mount Sinai Acute GVHD International Consortium
MCP-1: Monocyte Chemotactic Protein-1
MDASI: MD Anderson Symptom Inventory
MDS: Myelodysplastic Syndrome
MHC: Major Histocompatibility Complex
MIN-6: Mouse Insulinoma 6
MMF: Mycophenolate Mofetil
MN: Minnesota
MOP: Manual of Procedures
MP: Methylprednisolone
MR: Mixed Response
MRD: Minimal Residual Disease
NCI: National Cancer Institute
NF-κβ: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NHLBI: National Heart, Lung, and Blood Institute
NIH: National Institutes of Health
NK: Natural Killer
NMDP: National Marrow Donor Program
NR: No Response
NRM: Non-Relapse Mortality
OS: Overall Survival
OT-II: Ovalbumin-Specific, MHC Class II-Restricted αβ T Cell Receptor
OVA: Ovalbumin
PBMC: Peripheral Blood Mononuclear Cells
PCR: Polymerase Chain Reaction
PET: Positron Emission Tomography
PFS: Progression-Free Survival
PK: Pharmacokinetic
PO: Per Os
PR: Partial Response
PRO: Patient-Reported Outcomes
PROMIS: Patient-Reported Outcome Measurements Information System
PRR: Pattern-Recognition Receptor
PTM: Placebo To Match
REG3α: Regenerating Islet-Derived 3-alpha
SAE: Serious Adverse Event
SCTOD: Stem Cell Transplant Outcomes Database
SOS: Sinusoidal Obstruction Syndrome

GVHD: Graft Versus Host Disease
HCT: Hematopoietic Cell Transplant
HL-1: Cardiac Muscle Cell Line Designated "HL-1"
HPA: Hypothalamic Pituitary Adrenal
HQL: Health Quality of Life
HR: High Risk
HS: Heparan Sulfate
HSCT: Hematopoietic Stem Cell Transplant
ICH: International Conference on Harmonisation
ID: Identification
IEC: Institutional Ethics Committee
IFNγ: Interferon Gamma
IgA: Immunoglobulin A
IL: Interleukin
IL-1RA: Interleukin 1 Receptor Antagonist
IMP: Investigational Medicinal Product
INS-1: Insulinoma 1
IRB: Institutional Review Board
SpO_2: Peripheral O_2 Saturation
SPRT: Sequential Probability Ratio Test
SR: Steroid Refractory
SST: Serum Separator Tube
ST2: Suppression of Tumorigenicity 2
SUSAR: Suspected Unexpected Serious Adverse Reaction
Tcon: Conventional T Cells
TD: Treatment Discontinuation
Teff: Donor Effector T Cells
TF: Treatment Failure
TNFR1: Tumor Necrosis Factor Receptor-1
TNF-α: Tumor Necrosis Factor Alpha
Tregs: Donor Regulatory T Cells
ULN: Upper Limit of Normal
VEGF: Vascular Endothelial Growth Factor
VGPR: Very Good Partial Response

APPENDIX I
REFERENCES

1. Jacobsohn, D.A. and G.B. Vogelsang, *Acute graft versus host disease.* Orphanet J Rare Dis, 2007. **2**: p. 35.
2. Jagasia, M., et al., *Risk factors for acute GVHD and survival after hematopoietic cell transplantation.* Blood, 2012. **119**(1): p. 296-307.
3. Lee, S.E., et al., *Risk and prognostic factors for acute GVHD based on NIH consensus criteria.* Bone Marrow Transplant, 2013. **48**(4): p. 587-92.
4. Martin, P.J., et al., *First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation.* Biol Blood Marrow Transplant, 2012. **18**(8): p. 1150-63.
5. Przepiorka, D., et al., *1994 Consensus Conference on Acute GVHD Grading.* Bone Marrow Transplant, 1995. **15**(6): p. 825-8.
6. MacMillan, M.L., et al., *Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems.* Biol Blood Marrow Transplant, 2002. **8**(7): p. 387-94.
7. Levine, J.E., et al., *A prognostic score for acute graft-versus-host disease based on biomarkers: a multicentre study.* Lancet Haematol, 2015. **2**(1): p. e21-9.
8. Alousi, A.M., et al., *Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network.* Blood, 2009. **114**(3): p. 511-7.
9. Couriel, D.R., et al., *A phase III study of infliximab and corticosteroids for the initial treatment of acute graft-versus-host disease.* Biol Blood Marrow Transplant, 2009. **15**(12): p. 1555-62.
10. Cragg, L., et al., *A randomized trial comparing prednisone with antithymocyte globulin/prednisone as an initial systemic therapy for moderately severe acute graft-versus-host disease.* Biol Blood Marrow Transplant, 2000. **6**(4a): p. 441-7.
11. Lee, S.J., et al., *Effect of up-front daclizumab when combined with steroids for the treatment of acute graft-versus-host disease: results of a randomized trial.* Blood, 2004. **104**(5): p. 1559-64.
12. Levine, J.E., et al., *Etanercept plus methylprednisolone as initial therapy for acute graft-versus-host disease.* Blood, 2008. **111**(4): p. 2470-5.
13. Bolanos-Meade, J., et al., *Phase 3 clinical trial of steroids/mycophenolate mofetil vs steroids/placebo as therapy for acute GVHD: BMT CTN 0802.* Blood, 2014. **124**(22): p. 3221-7; quiz 3335.
14. Saliba, R.M., et al., *Prognostic value of response after upfront therapy for acute GVHD.* Bone Marrow Transplant, 2012. **47**(1): p. 125-31.
15. MacMillan, M.L., T.E. DeFor, and D.J. Weisdorf, *The best endpoint for acute GVHD treatment trials.* Blood, 2010. **115**(26): p. 5412-7.
16. Levine, J.E., et al., *Graft-versus-host disease treatment: predictors of survival.* Biol Blood Marrow Transplant, 2010. **16**(12): p. 1693-9.
17. Weisdorf, D., et al., *Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome.* Blood, 1990. **75**(4): p. 1024-30.

18. MacMillan, M.L., T.E. DeFor, and D.J. Weisdorf, *What predicts high risk acute graft-versus-host disease (GVHD) at onset?: identification of those at highest risk by a novel acute GVHD risk score.* Br J Haematol, 2012. **157**(6): p. 732-41.
19. MacMillan, M.L., et al., *A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality.* Biol Blood Marrow Transplant, 2015. **21**(4): p. 761-7.
20. Srinivasan, R., et al., *Accurate diagnosis of acute graft-versus-host disease using serum proteomic pattern analysis.* Exp Hematol, 2006. **34**(6): p. 796-801.
21. Wang, H., et al., *Intact-protein-based high-resolution three-dimensional quantitative analysis system for proteome profiling of biological fluids.* Mol Cell Proteomics, 2005. **4**(5): p. 618-25.
22. Hartwell, M.J., et al., *An early-biomarker algorithm predicts lethal graft-versus-host disease and survival.* JCI Insight, 2017. **2**(3): p. e89798.
23. Leisenring, W.M., et al., *An acute graft-versus-host disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens.* Blood, 2006. **108**(2): p. 749-55.
24. Gooley, T.A., et al., *Reduced mortality after allogeneic hematopoietic-cell transplantation.* N Engl J Med, 2010. **363**(22): p. 2091-101.
25. Hockenbery, D.M., et al., *A randomized, placebo-controlled trial of oral beclomethasone dipropionate as a prednisone-sparing therapy for gastrointestinal graft-versus-host disease.* Blood, 2007. **109**(10): p. 4557-63.
26. Nikiforow, S., et al., *Upper gastrointestinal acute graft-versus-host disease adds minimal prognostic value in isolation or with other graft-versus-host disease symptoms as currently diagnosed and treated.* Haematologica, 2018. **103**(10): p. 1708-1719.
27. Schwartz, J.M., et al., *Severe gastrointestinal bleeding after hematopoietic cell transplantation, 1987-1997: incidence, causes, and outcome.* Am J Gastroenterol, 2001. **96**(2): p. 385-93.
28. Ross, W.A. and D. Couriel, *Colonic graft-versus-host disease.* Curr Opin Gastroenterol, 2005. **21**(1): p. 64-9.
29. Im, J.S., et al., *Histologic Grade 1 Is Associated With Increased Nonrelapsed Mortality in Lower Gastrointestinal Graft Versus Host Disease.* Am J Surg Pathol, 2017. **41**(11): p. 1483-1490.
30. Silverman, E.K. and R.A. Sandhaus, *Clinical practice. Alpha1-antitrypsin deficiency.* N Engl J Med, 2009. **360**(26): p. 2749-57.
31. Tuder, R.M., S.M. Janciauskiene, and I. Petrache, *Lung disease associated with alpha1-antitrypsin deficiency.* Proc Am Thorac Soc, 2010. **7**(6): p. 381-6.
32. Perlmutter, D.H., *Alpha-1-antitrypsin deficiency: importance of proteasomal and autophagic degradative pathways in disposal of liver disease-associated protein aggregates.* Annu Rev Med, 2011. **62**: p. 333-45.
33. Ekeowa, U.I., S.J. Marciniak, and D.A. Lomas, *alpha(1)-antitrypsin deficiency and inflammation.* Expert Rev Clin Immunol, 2011. **7**(2): p. 243-52.
34. Pott, G.B., et al., *Alpha-1-antitrypsin is an endogenous inhibitor of proinflammatory cytokine production in whole blood.* J Leukoc Biol, 2009. **85**(5): p. 886-95.
35. Tilg, H., et al., *Antiinflammatory properties of hepatic acute phase proteins: preferential induction of interleukin 1 (IL-1) receptor antagonist over IL-1 beta synthesis by human peripheral blood mononuclear cells.* J Exp Med, 1993. **178**(5): p. 1629-36.

36. Subramanian, S., et al., *Sustained expression of circulating human alpha-1 antitrypsin reduces inflammation, increases CD4+FoxP3+ Treg cell population and prevents signs of experimental autoimmune encephalomyelitis in mice.* Metab Brain Dis, 2011. **26**(2): p. 107-13.
37. Koulmanda, M., et al., *Curative and beta cell regenerative effects of alpha1-antitrypsin treatment in autoimmune diabetic NOD mice.* Proc Natl Acad Sci U S A, 2008. **105**(42): p. 16242-7.
38. Lewis, E.C., et al., *alpha1-Antitrypsin monotherapy induces immune tolerance during islet allograft transplantation in mice.* Proc Natl Acad Sci U S A, 2008. **105**(42): p. 16236-41.
39. Ozeri, E., et al., *alpha-1 antitrypsin promotes semimature, IL-10-producing and readily migrating tolerogenic dendritic cells.* J Immunol, 2012. **189**(1): p. 146-53.
40. Lewis, E.C., et al., *Alpha1-antitrypsin monotherapy prolongs islet allograft survival in mice.* Proc Natl Acad Sci U S A, 2005. **102**(34): p. 12153-8.
41. Marcondes, A.M., et al., *Inhibition of IL-32 activation by alpha-1 antitrypsin suppresses alloreactivity and increases survival in an allogeneic murine marrow transplantation model.* Blood, 2011. **118**(18): p. 5031-9.
42. Tawara, I., et al., *Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation.* Proc Natl Acad Sci U S A, 2012. **109**(2): p. 564-9.
43. Arora, P.K., H.C. Miller, and L.D. Aronson, *alpha1-Antitrypsin is an effector of immunological stasis.* Nature, 1978. **274**(5671): p. 589-90.
44. Lewis, E.C., *Expanding the clinical indications for alpha(1)-antitrypsin therapy.* Mol Med, 2012. **18**: p. 957-70.
45. Shahaf, G., et al., *alpha-1-antitrypsin gene delivery reduces inflammation, increases T-regulatory cell population size and prevents islet allograft rejection.* Mol Med, 2011. **17**(9-10): p. 1000-11.
46. Pileggi, A., et al., *Alpha-1 antitrypsin treatment of spontaneously diabetic nonobese diabetic mice receiving islet allografts.* Transplant Proc, 2008. **40**(2): p. 457-8.
47. Song, S., et al., *Recombinant adeno-associated virus-mediated alpha-1 antitrypsin gene therapy prevents type I diabetes in NOD mice.* Gene Ther, 2004. **11**(2): p. 181-6.
48. Grimstein, C., et al., *Alpha-1 antitrypsin protein and gene therapies decrease autoimmunity and delay arthritis development in mouse model.* J Transl Med, 2011. **9**: p. 21.
49. Grimstein, C., et al., *Combination of alpha-1 antitrypsin and doxycycline suppresses collagen-induced arthritis.* J Gene Med, 2010. **12**(1): p. 35-44.
50. Toldo, S., et al., *Alpha-1 antitrypsin inhibits caspase-1 and protects from acute myocardial ischemia-reperfusion injury.* J Mol Cell Cardiol, 2011. **51**(2): p. 244-51.
51. Jie, Z., et al., *Protective effects of alpha 1-antitrypsin on acute lung injury in rabbits induced by endotoxin.* Chin Med J (Engl), 2003. **116**(11): p. 1678-82.
52. Zhang, B., et al., *Alpha1-antitrypsin protects beta-cells from apoptosis.* Diabetes, 2007. **56**(5): p. 1316-23.
53. Loganathan, G., et al., *Culture of impure human islet fractions in the presence of alpha-1 antitrypsin prevents insulin cleavage and improves islet recovery.* Transplant Proc, 2010. **42**(6): p. 2055-7.

54. Kalis, M., et al., *alpha 1-antitrypsin enhances insulin secretion and prevents cytokine-mediated apoptosis in pancreatic beta-cells.* Islets, 2010. **2**(3): p. 185-9.
55. Congote, L.F., et al., *Comparison of the effects of serpin A1, a recombinant serpin A1-IGF chimera and serpin A1 C-terminal peptide on wound healing.* Peptides, 2008. **29**(1): p. 39-46.
56. Petrache, I., et al., *alpha-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis.* Am J Pathol, 2006. **169**(4): p. 1155-66.
57. Nita, I., et al., *Prolastin, a pharmaceutical preparation of purified human alpha1-antitrypsin, blocks endotoxin-mediated cytokine release.* Respir Res, 2005. **6**: p. 12.
58. Blanchard, V., et al., *N-glycosylation and biological activity of recombinant human alpha1-antitrypsin expressed in a novel human neuronal cell line.* Biotechnol Bioeng, 2011. **108**(9): p. 2118-28.
59. Guttman, O., et al., *Acute-phase protein alpha1-anti-trypsin: diverting injurious innate and adaptive immune responses from non-authentic threats.* Clin Exp Immunol, 2015. **179**(2): p. 161-72.
60. Tawara, I., et al., *Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation.* Clin Cancer Res, 2011. **17**(1): p. 77-88.
61. Teshima, T., et al., *Acute graft-versus-host disease does not require alloantigen expression on host epithelium.* Nat Med, 2002. **8**(6): p. 575-81.
62. Welniak, L.A., B.R. Blazar, and W.J. Murphy, *Immunobiology of allogeneic hematopoietic stem cell transplantation.* Annu Rev Immunol, 2007. **25**: p. 139-70.
63. Brennan, T.V., et al., *Heparan sulfate, an endogenous TLR4 agonist, promotes acute GVHD after allogeneic stem cell transplantation.* Blood, 2012. **120**(14): p. 2899-908.
64. Marcondes, A.M., et al., *alpha-1-Antitrypsin (AAT)-modified donor cells suppress GVHD but enhance the GVL effect: a role for mitochondrial bioenergetics.* Blood, 2014. **124**(18): p. 2881-91.
65. Reddy, P., *alpha-1 antitrysin DAMPens GVHD.* Blood, 2012. **120**(14): p. 2780-1.
66. Magenau, J.M., et al., *alpha1-Antitrypsin infusion for treatment of steroid-resistant acute graft-versus-host disease.* Blood, 2018. **131**(12): p. 1372-1379.
67. Marcondes, A.M., et al., *Response of Steroid-Refractory Acute GVHD to alpha1-Antitrypsin.* Biol Blood Marrow Transplant, 2016. **22**(9): p. 1596-1601.
68. Aldonyte, R., et al., *Polymerized alpha-antitrypsin is present on lung vascular endothelium. New insights into the biological significance of alpha-antitrypsin polymerization.* Histopathology, 2004. **45**(6): p. 587-92.
69. Carrell, R.W., *alpha 1-Antitrypsin: molecular pathology, leukocytes, and tissue damage.* J Clin Invest, 1986. **78**(6): p. 1427-31.
70. AmericanThoracicSociety and EuropeanRespiratorySociety, *American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency.* Am J Respir Crit Care Med, 2003. **168**(7): p. 818-855.
71. Kueppers, F., *Genetically determined differences in the response of alpha-antitrypsin levels in human serum to typhoid vaccine.* Humangenetik, 1968. **6**(3): p. 207-14.
72. Perez-Holanda, S., et al., *Serum concentration of alpha-1 antitrypsin is significantly higher in colorectal cancer patients than in healthy controls.* BMC Cancer, 2014. **14**: p. 355.

73. Lisowska-Myjak, B., et al., *Serum alpha-1-antitrypsin concentration during normal and diabetic pregnancy.* Eur J Obstet Gynecol Reprod Biol, 2001. **99**(1): p. 53-6.
74. Lisowska-Myjak, B. and J. Pachecka, *Antigenic and functional levels of alpha-1-antitrypsin in serum during normal and diabetic pregnancy.* European Journal of Obstetrics & Gynecology and Reproductive Biology, 2003. **106**(1): p. 31-35.
75. Gao, W., et al., *alpha1-Antitrypsin inhibits ischemia reperfusion-induced lung injury by reducing inflammatory response and cell death.* J Heart Lung Transplant, 2014. **33**(3): p. 309-15.
76. Weir, G.C., et al., *Alpha-1 antitrypsin treatment of new-onset type 1 diabetes: An open-label, phase I clinical trial (RETAIN) to assess safety and pharmacokinetics.* Pediatr Diabetes, 2018. **19**(5): p. 945-954.
77. Umar, S.B. and J.K. DiBaise, *Protein-losing enteropathy: case illustrations and clinical review.* Am J Gastroenterol, 2010. **105**(1): p. 43-9; quiz 50.
78. Weisdorf, S.A., et al., *Graft-versus-host disease of the intestine: a protein losing enteropathy characterized by fecal alpha 1-antitrypsin.* Gastroenterology, 1983. **85**(5): p. 1076-81.
79. O'Meara, A., et al., *Fecal calprotectin and alpha1-antitrypsin dynamics in gastrointestinal GvHD.* Bone Marrow Transplant, 2015. **50**(8): p. 1105-9.
80. Rodriguez-Otero, P., et al., *Fecal calprotectin and alpha-1 antitrypsin predict severity and response to corticosteroids in gastrointestinal graft-versus-host disease.* Blood, 2012. **119**(24): p. 5909-17.
81. Gottlieb, P.A.e.a., *alpha1-Antitrypsin therapy downregulates toll-like receptor-induced IL-1beta responses in monocytes and myeloid dendritic cells and may improve islet function in recently diagnosed patients with type 1 diabetes.* J Clin Endocrinol Metabolism, 2014. **99**: p. 418-26.
82. Rachmiel, M.e.a., *Alpha-1 antitrypsin therapy is safe and well tolerated in children and adolescents with recent onset type 1 diabetes mellitus.* Pediatr Diabetes, 2016. **17**: p. 351-9.
83. Brener, A.e.a., *Long-term safety of alpha-1 antitrypsin therapy in children and adolescents with Type 1 diabetes.* Immunotherapy, 2018. **10**: p. 1137-1148.
84. Weir, G.C.e.a., *Alpha-1 antitrypsin treatment of new-onset type 1 diabetes: An open-label, phase I clinical trial (RETAIN) to assess safety and pharmacokinetics.* Pediatr Diabetes, 2018. **19**: p. 945-954.
85. Sheikh, I.N.M., J.;, et al., *Using the MDASI-Adolescent for Early Symptom Identification and Mitigation of Symptom Impact on Daily Living in Adolescent and Young Adult Stem Cell Transplant Patients.* Children, 2022. **9**(19).
86. Kittelson, J.M. and S.S. Emerson, *A unifying family of group sequential test designs.* Biometrics, 1999. **55**(3): p. 874-82.
87. Jagasia, M.H., et al., *National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report.* Biol Blood Marrow Transplant, 2015. **21**(3): p. 389-401 e1.

Signature Page

CSL964_5001 - Protocol Amendment - v5

Signed By	Date (GMT)
PPD	22-Sep-2023 17:24:01
Approved-Biostatistician PPD	

--- Do Not Detach This Signature Page ---