MCC-17-13299
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Therapeutic Use of Intravenous Vitamin C in Allogeneic Stem Cell Transplant Recipients

12/06/2022

Virginia Commonwealth University Massey Cancer Center

Protocol MCC-17-13299 **IND # 138924**

Therapeutic Use of Intravenous Vitamin C Followed by Oral Vitamin C in Allogeneic Stem Cell Transplant Recipients

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Version #: 6 **Version Date:** 12/06/2022

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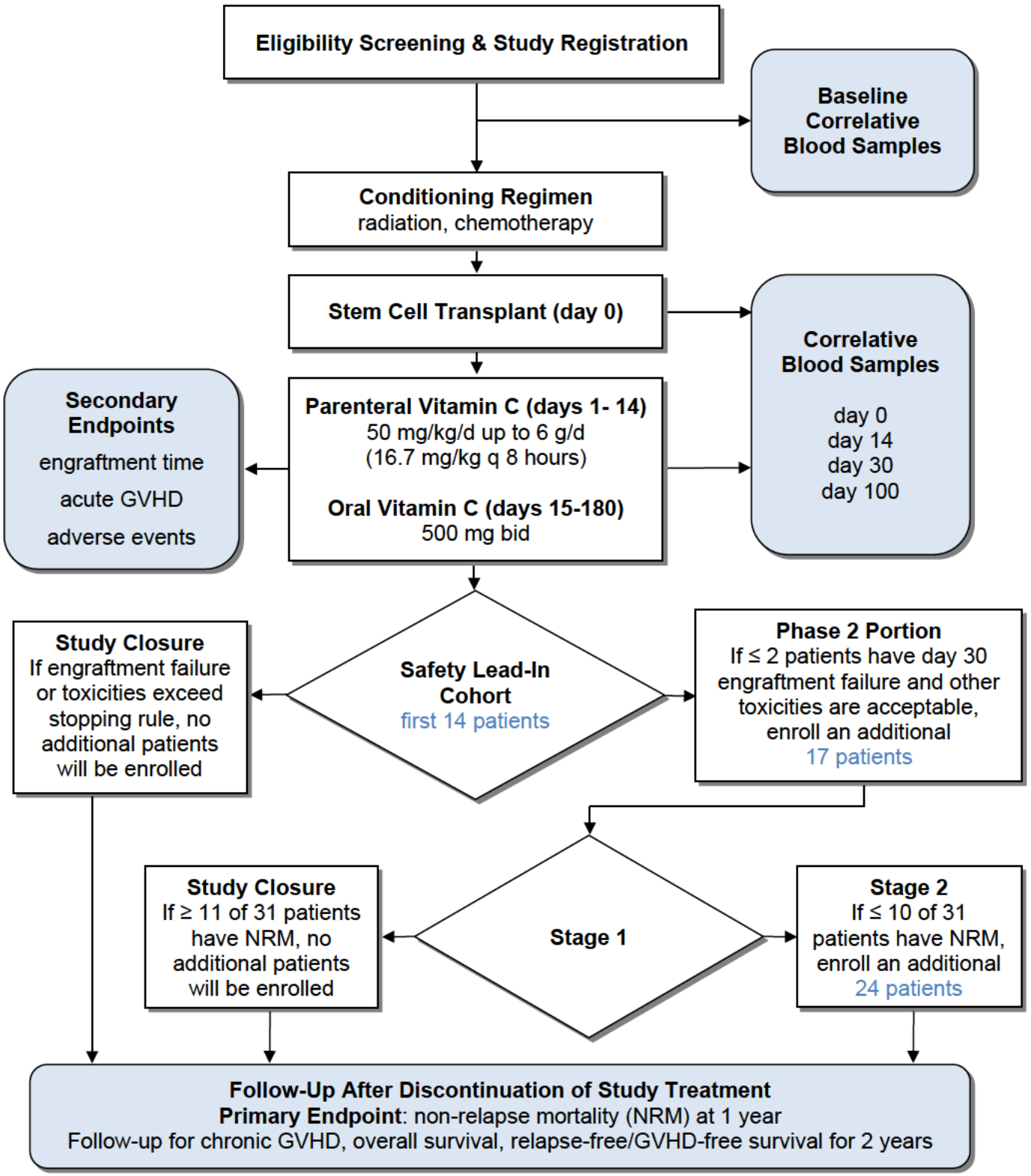
STUDY SCHEMA



REVISION HISTORY

Revision history is presented in reverse order so that the information pertaining to the most current version of the protocol is presented first in this section.

Version 6, Version Date 12/06/2022

- The version number and date have been updated throughout.
- William B. Clark, MD replaced Gary L. Simmons, DO as sponsor-investigator.
- Study coordination responsibilities were changed from Catherine H. Roberts, PhD to the VCU Massey Cancer Center Early Phase Development Department.
- The abbreviation for post office (PO) was removed from all VCU addresses.
- Investigators who have left the study were removed from the cover page.

Version 5, Version Date 10/20/2020

- The version number and date have been updated throughout.
- The name “Bone Marrow Transplant (BMT) Program” has been changed to “Cellular Immunotherapies and Transplant (CIT) Program” throughout.
- Infectious disease marker testing requirements have been updated to reflect current CIT Program practices (Section 12).

Version 4, Version Date 06/12/2019

- The version number and date have been updated throughout.
- On the cover page and throughout the protocol, the term “Principal Investigator” was changed to “Sponsor-Investigator.”
- Exploratory endpoints were modified to include newly funded biomarker analyses (Sections 3.4.5, 3.4.6, and 13.1.3).
- The window for IV vitamin C administration was defined as every 8 ± 3 hours (Section 6.1.2).
- The protocol now clearly states that missed doses of vitamin C will not be made up (Sections 6.1.3 and 7.1).
- Guidelines for holding vitamin C due to elevated serum creatinine were clarified, and the requirement to hold vitamin C for reduced calculated glomerular filtration rate was deleted (Section 7.1).
- FDA reporting requirements for adverse events were added to Section 8.
- AE collection instructions were clarified (Section 8.3).

- HepB sAB was removed from the list of infectious disease screening titers (Table 4).

Version 3, Version Date 06/14/2018

- The version number and date have been updated throughout.
- The normal range of vitamin C has been deleted from Section 1.2.1.
- The cutoff for pre-treatment levels of vitamin C in the safety lead-in cohort has been clarified as 0.5 mg/dL.

Version 2, Version Date 04/24/2018

- The version number and date have been updated throughout.
- Dr Ramesh Natarajan has been removed from the cover page as a co-investigator.
- A new schema has been created to reflect the safety lead-in cohort, two-stage design, and additional safety and tolerability objective.
- The Table of Contents and List of Tables have been updated.
- The study's FDA IND number has been added to the cover page and throughout.
- Additional information and references have been added to the rationale (Section 1.2) describing vitamin C's role in immune modulation with possible late effects of early treatment.
- A secondary objective (Section 2.2.3) and corresponding secondary endpoint (Section 3.3.3, Section 13.1.2) have been added to characterize the safety and tolerability of the vitamin C regimen by reporting adverse events.
- A safety lead-in cohort of 14 patients has been added to the study design and statistical analysis (Section 3.1, Section 13). The safety assessment for this cohort uses the existing stopping rules, which are based on severe toxicities and ANC engraftment failure. The tables describing the stopping rules have been divided to separate the safety lead-in cohort (Table 6) from the phase 2 portion of the trial (Table 7 and Table 8).
- A midpoint efficacy analysis has been added using a Simon's two-stage design. The first stage will consist of 31 patients. The total planned accrual remains 55 patients. The 14 patients in the safety lead-in cohort will be included in the 31 patients of stage 1 (Section 3.1, Section 13).
- The list of eligible hematological malignancies has been revised to reduce the heterogeneity of the study population (Section 4.1.1).
- Pre-treatment vitamin C testing has been added for the safety lead-in cohort of 14 patients to confirm deficiency prior to initiating the study regimen (Section 6.1.1, Section 12).
- The dose of IV vitamin C has been clarified as 16.7 mg/kg every 8 hours (Section 6.1.2).

- Standard urinalysis has been added at baseline and weekly through 1 week following the last dose of IV vitamin C (Section 6.3, Section 12).

- References to CTCAE version 4.0 have been changed to version 5.0 (Section 8.1.4, Section 13.3).

- The description of AE capture has been clarified to ensure that AEs of grade ≥3, including lab value AEs of grade ≥3 reflecting renal function, will be recorded as AEs (Section 8.4).

- Pharmaceutical information for vitamin C has been amended to clarify the risk of renal calculi (Section 9.1).

- The statistical analysis plan now specifies that primary and secondary outcomes will be evaluated by comparing to a matched historical cohort (Section 13.1.4).

Version 1, Version Date 02/20/2018

Initial submission of the protocol.

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LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
BID	twice per day
BMT	bone marrow transplant
BP	blood pressure
cfDNA	cell-free deoxyribonucleic acid
CIBMTR	Center for International Blood and Marrow Transplant Research
CIT	Cellular Immunotherapies and Transplant
CRF	case report form
CRP	C-reactive protein
CTCAE v5.0	Common Terminology Criteria for Adverse Events Version 5.0
cGVHD	chronic graft versus host disease
ddCD3	donor-derived $CD3^+$ (cell count)
DLCO	diffusing capacity of the lung for carbon monoxide
DSMC	Data Safety and Monitoring Committee
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
GVHD	graft versus host disease
HCT	hematopoietic cell transplant
HLA	human leukocyte antigen
HSC	hematopoietic stem cells
IBW	ideal body weight
LPS	lipopolysaccharide
MAC	myeloablative conditioning
MCC-VCUHS	Massey Cancer Center-Virginia Commonwealth University Health System
MRD	matched related donor
MUD	matched unrelated donor
NRM	non-relapse mortality
PBSC	peripheral blood stem cells
PO	by mouth
RIC	reduced intensity conditioning
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAE	serious adverse event
SCT	stem cell transplantation
TBI	total body irradiation
TRM	transplant-related mortality
ULN	upper limit of normal
UP	unanticipated problem
URD	unrelated donor
VCU	Virginia Commonwealth University
VOD	veno-occlusive disease
WBC	white blood cell
WCBP	woman of childbearing potential

1 BACKGROUND

1.1 Introduction

Approximately 20,000 hematopoietic cell transplants (HCT) are performed in the United States each year (Pasquini MC. 2014 CIBMTR Summary Slides). Myeloablative allogeneic HCT remains the only potential cure for leukemia, lymphoma, and hematopoietic disorders, however, it is associated with a high risk of morbidity and mortality. Myeloablative conditioning (MAC) refers to the necessary chemotherapy and/or radiation regimen used in stem cell transplant to eliminate residual leukemia/lymphoma and condition the host for the incoming graft of hematopoietic stem cells (HSC).

Non-relapse mortality (NRM), defined as mortality from complications of HCT but not tumor relapse, is usually from graft versus host disease (GVHD), infection, or organ failure. NRM after allogeneic HCT is influenced by type of donor (related vs. unrelated), conditioning chemotherapy doses, and co-morbidities of the recipient. In a study reported by Sorror et al, 2-year NRM in myeloablative allogeneic HCT was 14% to 41% based on patient comorbidities (1). In a recent clinical trial (BMTCTN 0901) comparing conditioning chemotherapy regimens of different intensities in acute myelogenous leukemia (AML), the overall NRM was 16% at 18 months (2).

HCT after myeloablative conditioning has several associated toxicities such as pancytopenia, mucositis, interstitial pneumonitis, hepatic sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD), and sepsis. These HCT toxicities are induced by MAC and are characterized by endothelial injury and a pro-inflammatory state with elevated inflammatory cytokines such as IL-1 and tumor necrosis factor-alpha (TNF-α) (3). The inflammatory response increases antigen presenting cell (APC) activity presenting minor histocompatibility antigens to T-cells resulting in acute GVHD in 35-50% of HCT recipients. Acute GVHD is a leading risk factor for chronic GVHD which is present in 40-70% of all HCT (3). GVHD, infection, and organ failure as a consequence of HCT contributes to NRM after allogeneic HCT. The one-year NRM at Virginia Commonwealth University (VCU) in 2014-2015 was 10-20% for patients receiving HLA-matched related donor grafts (MRD) and 20 - 40% in those with unrelated donor grafts (MUD) (4, 5).

The NRM risk, mediated by inflammation, endothelial injury, and GVHD, may be mitigated by an agent such as vitamin C which has several beneficial effects in patients with sepsis-associated acute inflammation including reduction in endothelial injury, hemodynamic instability, organ dysfunction and markers of inflammation (6). These effects are partly mediated by reduction in reactive oxygen species (ROS), an important consequence of both sepsis and high-dose chemoradiotherapy (7). We have demonstrated at our institution that patients' pre-HCT are deficient in vitamin C and remain deficient out to Day +60 following HCT (8).

This phase 2 trial will evaluate the efficacy of parenteral vitamin C in myeloablative allogeneic HCT patients. We hypothesize that mitigating the pro-inflammatory effects of HCT with early administration of vitamin C to restore normal blood levels will improve the outcomes of patients undergoing HCT by attenuating mucositis, endothelial and organ injury, as well as GVHD and will result in a reduction in the risk of transplant-related mortality.

1.2 Rationale and Previous Work

Myeloablative HCT utilizes a combination of chemotherapy dose intensity and donor T cell-mediated graft versus leukemia effect to achieve long-term freedom from disease progression in patients with recurrent hematological malignancies. This combination results in a pro-inflammatory state that resembles severe sepsis. The tissue damage results in secretion of pro-inflammatory cytokines IL-1 and tumor necrosis factor alpha (TNF-α), and the net effect is acute GVHD (9). Immunosuppression administered to prevent GVHD increases the risk of both relapse, as well as opportunistic infections, contributing to the observed high non-relapse mortality following allogeneic HCT. Evidence of the deleterious impact of the pro-inflammatory state comes from biomarker studies using ferritin and C-reactive protein (CRP) as well as cytokines IL-1 and TNF-α, which have been validated as predictors of outcomes after myeloablative allogeneic HCT. A review of the patients transplanted at VCU has found that elevated ferritin (>2020 G/dL) was associated with an increased risk for GVHD and inferior survival in patients transplanted for myeloid malignancies (4).

CRP produced by hepatocytes downstream of IL-6 is widely used as a reliable surrogate marker of inflammation (10). After allogeneic HCT, the elevation of CRP has been shown to be associated with transplant-related mortality (11). In a study by Fuji et al., the correlation between the pre-engraftment CRP value and subsequent clinical events was analyzed to test whether high CRP reflected the degree of tissue damage because of the conditioning regimen, infections, and allogeneic immune reactions and/or inflammation, all of which could contribute to subsequent GVHD and NRM. They found that CRP > 15 mg/dL was associated with a significant increase in GVHD and decreased overall survival post-allogeneic HCT (12). Another marker of tissue damage, cell-free DNA (cfDNA), is released as a result of cell necrosis or apoptosis and has been studied as a biomarker in severe sepsis (13). In a retrospective, observation study of 80 patients with severe sepsis in the intensive care unit (ICU), cfDNA had a sensitivity of 88% and specificity of 94% for predicting mortality in the ICU (13). Systemic release of TNF-α during pre-HCT conditioning is highly predictive for occurrence of acute GVHD and related complications and one-year transplant-related mortality in patients receiving allogeneic HCT (14, 15).

Vitamin C's pleotropic effect on the inflammatory process was demonstrated by Molina et al who stimulated lymphocytes with lipopolysaccharide (LPS) followed by treatment with vitamin C. Vitamin C reduced the reactive oxygen species and the production of pro-inflammatory cytokines TNF-α and IFN-γ, as well as increasing the anti-inflammatory cytokine IL-10. Also, glutathione (GSH) and G6PDH enzymes were increased in this study supporting the role of vitamin C as anti-oxidant (16).

The endothelial injury syndromes such as microangiopathy, VOD of the liver, diffuse alveolar hemorrhage, engraftment syndrome, and capillary leak syndrome are a major cause of NRM after allogeneic HCT. Further, biomarkers of endothelial injury such as thrombomodulin show a close relationship with GVHD (17). Tatekawa et al., generated a novel diagnostic system for transplant related complications from endothelial injury by analyzing 188 adult patients who received allogeneic HCT and found that the peripheral blood levels of biomarkers angiopoietin 2 (ANG2), CRP, D-dimer, and thrombomodulin at the onset were associated with transplant related complications with endothelial cell damage (18). These may then also serve as important biomarkers for endothelial injury and potentially its response to vitamin C therapy in the setting of HCT, as proposed in the MCC-17-132999 study.

High-dose vitamin C has been used in patients with septic shock to reduce inflammatory state. Nathens et al used parenteral vitamin C dosed at 1 gram every 8 hours combined with oral vitamin E for 28 days in 594 surgically critically ill patients and found a statistically significantly lower incidence of acute lung injury and multiple organ failure (19). In a phase 1 clinical trial performed at VCU, Fowler and colleagues found that high-dose parenteral vitamin C (200 mg/kg/24hours) was safe to administer with no adverse events and was effective at reducing inflammation in patients with severe sepsis. All patients with severe sepsis had below normal serum vitamin C levels. Patients treated with parenteral vitamin C had a significant rise in serum vitamin C compared to placebo (6). To assess the effects of parenteral vitamin C on inflammation they assessed organ function in severe sepsis by the sequential organ failure scores (SOFA), and inflammatory biomarkers, CRP, procalcitonin and thrombomodulin. CRP and SOFA levels decline with both low and high doses of parenteral vitamin C compared to placebo (Figure 1). Thrombomodulin levels remained stable compared to placebo, which trended upward (6). Given the significant immune modulatory and anti-inflammatory effects of parenteral vitamin C in patients with septic shock demonstrated in the above-referenced studies, we have hypothesized that IV ascorbic acid in BMT patients undergoing myleoablative allogeneic SCT will impact not only early conditioning-induced complications such as mucositis, sepsis, VOD, and acute lung injury, but also modulate immune reconstitution and ameliorate remote events such as acute and chronic GVHD. This will likely have a salutary effect on immune reconstitution as well as diminishing the likelihood of severe late opportunistic infections, resulting in improved survivability of myeloablative allogeneic SCT.

Patients undergoing myeloablative HCT are deficient in vitamin C, as reported by Nannya et al who found blood levels of vitamin C were normal before HCT, but deficient by Day 0 and remained low throughout the following 28 days with lowest levels at Day 14. In the same study, ferritin (a biomarker of inflammation) increases inversely as vitamin C declines (20). A similar state of vitamin C deficiency has been observed in transplant recipients conditioned with MAC at VCU. The pleiotropic anti-inflammatory effects of vitamin C make it an attractive intervention in HCT, which is a pro-inflammatory state that physiologically resembles severe sepsis.

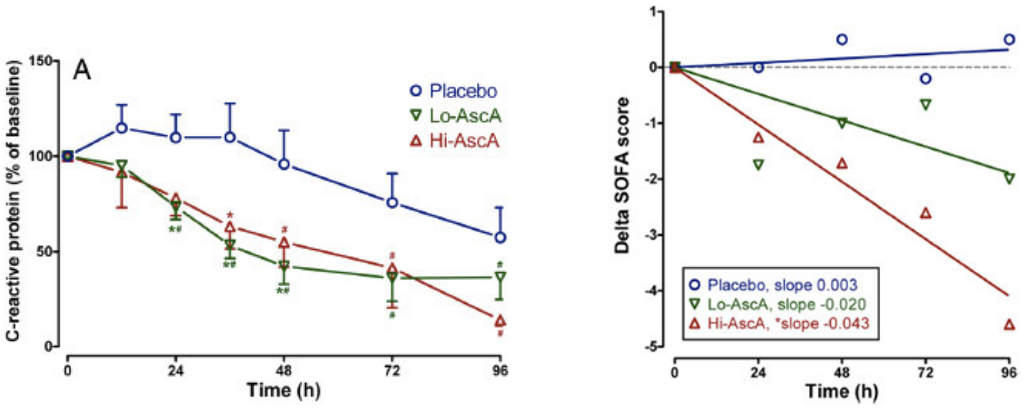

Figure 1. CRP Levels and SOFA Scores Lower with Parenteral Ascorbic Acid Compared to Placebo

There are multiple studies supporting the role of vitamin C augmenting hematopoiesis, which may reduce time to engraftment and minimize blood product transfusions in patients undergoing HCT. In preclinical trials, vitamin C has been shown to have an effect on

TET-2, T cell development, and responsiveness to growth factors. There is evidence that tissue injury and inflammation in the early weeks following SCT affects late endpoints such as GVHD and immune reconstitution (21), which in turn affect the likelihood of NRM. The profound impact of early milieu on late outcomes is also borne out by our group's work, which has demonstrated that the magnitude and rate of early lymphoid recovery following SCT modulates the risk of GVHD developing at remote times (22-24).

1.2.1 Preliminary Data

In a VCU prospective study, plasma vitamin C levels were measured at baseline and on Days 0, 14, 30, and 60 post-transplant in HCT recipients with MAC. To date, 15 patients have been studied: 4 underwent autologous and 11 allogeneic HCT. The results of the vitamin C levels are illustrated in Figure 2. The mean plasma vitamin C level was low at 40.8 µmol/L (±18.4) at baseline, falling to 27.3 µmol/L (±14.1) at Day 0 ($p < 0.05$, compared with baseline), and reaching nadir at 21.5 µmol/L (± 13.8) on Day 14 ($p < 0.05$ compared with baseline) post-HCT. Plasma vitamin C levels recovered to 34.2 µmol/L (± 20.5) at Day 30 and subsequently to 37.2 µmol/L (± 27.9) at Day 60 ($p < 0.05$ compared with Day 14). Of the patients studied, none maintained normal vitamin C levels throughout the study period and 13/15 developed significantly deficient vitamin C levels of < 28 µmol/L. Patients with severe mucositis tended to have lower vitamin C levels at Day 14. These patients also had cfDNA levels measured, and as can be seen in Figure 3, elevated cfDNA during the conditioning phase of HCT and throughout the 60 days after HCT (n=15). This is consistent with tissue injury sustained during conditioning prior to HCT.

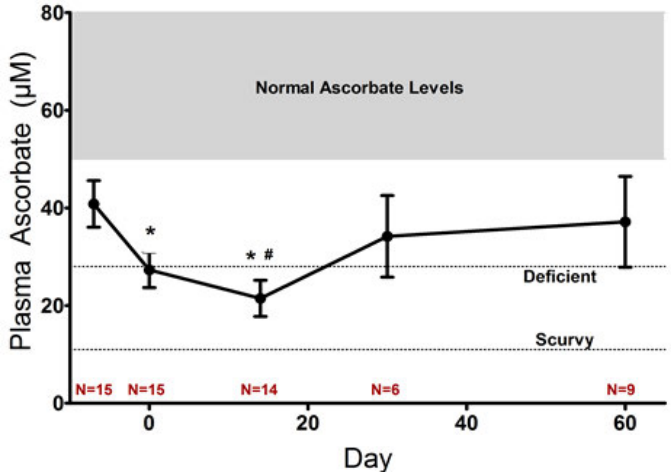

Figure 2. Vitamin C Deficient Levels at Baseline Up Until Day +60 from HCT

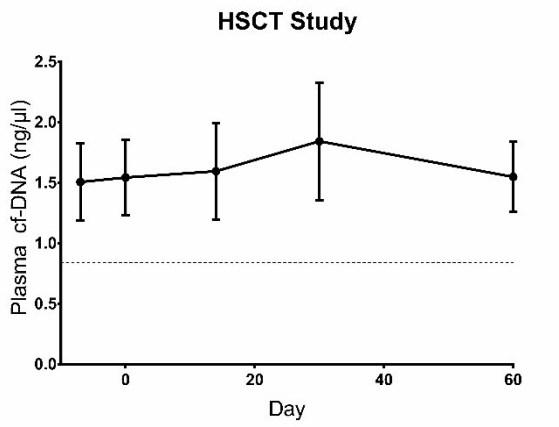

Figure 3. cfDNA Levels Elevated in Transplant Recipient

1.3 Proposed Study

In the MCC-17-13299 trial, we will attempt to determine the effect of parenteral vitamin C administered for 2 weeks beginning on Day +1 followed by oral vitamin C beginning on Day +15 and continuing through Day +180 on NRM at one year following myeloablative allogeneic HCT. Other transplant interventions and supportive care, transfusions, and infection prophylaxis will be applied per the VCU CIT Program's standard procedures.

1.4 Potential Risks and Benefits of the Investigational Regimen

1.4.1 Potential Benefits

Vitamin C is water-soluble and acts as an anti-oxidant and anti-inflammatory indicated for the prevention and treatment of scurvy. The anti-inflammatory effect of vitamin C and endothelial protection will potentially result in less GVHD, NRM, incidence and severity of mucositis, VOD, and infections. Its parenteral administration is desirable for patients with acute deficiency or those whose absorption of orally ingested vitamin C is uncertain.

1.4.2 Potential Risks

Too rapid infusion of intravenous vitamin C solution may cause temporary faintness or dizziness. Other side effects include nausea, vomiting, heartburn, and stomach cramps.

Previous clinical studies in patients with severe sepsis receiving parenteral vitamin C did not have any adverse events. During the 96-hour infusion period, no patients were withdrawn due to study-related adverse events (ie, hypotension, tachycardia, hypernatremia, or nausea/vomiting).

1.5 Correlative Studies

Blood samples will be collected for the following purposes:

- To determine the effect of parenteral vitamin C on levels of vitamin C in the blood

- To determine the effect of parenteral vitamin C on inflammatory markers C-reactive protein (CRP), cell free DNA (cfDNA), and tumor necrosis factor-α (TNF-α) after myeloablative allogeneic HCT

- To determine the effect of parenteral vitamin C on endothelial cell injury measured by thrombomodulin levels post myeloablative allogeneic HCT

Sample collection will be performed at baseline (before beginning conditioning), on transplant day 0, and on days 14, 30, and 100 (refer to Section 11 for additional information).

Samples collected on the aforementioned dates will be analyzed for vitamin C levels and by enzyme-linked immunosorbent assay (ELISA) method for CRP. Samples will also be stored for future TNF-α, cfDNA, and thrombomodulin assays.

2 OBJECTIVES

2.1 Primary Objective

To determine the effect of parenteral vitamin C on non-relapse mortality (NRM) at one year following myeloablative allogeneic HCT

2.2 Secondary Objectives

In patients who have had myeloablative allogeneic HCT:

2.2.1 To determine the effect of the vitamin C regimen on the time to hematopoietic engraftment

2.2.2 To determine the effect of the vitamin C regimen on the rate of acute GVHD

2.2.3 To characterize the safety and tolerability of the vitamin C regimen

2.3 Exploratory Objectives

2.3.1 [redacted]

3 STUDY DESIGN

3.1 General Description

This is a prospective phase 2 trial investigating the therapeutic effects of parenteral vitamin C followed by oral vitamin C for patients undergoing myeloablative HCT. All transplant-related treatment interventions are performed according to the VCU CIT Program's standard transplant procedures. Patients enrolled in the study will undergo myeloablative HCT with bone marrow or mobilized peripheral blood stem cells with matched related or unrelated or mismatched unrelated donor types. Blood samples will be collected for the correlative studies at baseline and 4 time points following the transplant.

A Simon's two-stage design will be utilized in this study. In the first stage, if there are ≥ 11 instances of NRM in the first 31 evaluable patients, then the trial will end for futility. If there are ≤ 10 instances of NRM, patient accrual will continue into the second stage. The total sample size for the Simon's two-stage design is 55 patients. Additionally, a safety lead-in phase will be conducted in the initial 14 patients enrolled in the study for identification of any clinically significant increase in toxic events or engraftment. The 14 safety lead-in patients will be counted toward the 31 patients included in the first stage of the Simon's two-stage design.

3.2 Primary Endpoint

NRM at one year following myeloablative allogeneic HCT

3.3 Secondary Endpoints

3.3.1 Number of days from transplant to hematopoietic engraftment

3.3.2 Proportion of patients with a diagnosis of acute GVHD

3.3.3 Adverse events (AEs) reported using criteria in the National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0)

3.4 Exploratory Endpoints

3.4.1 [redacted]

4 PATIENT SELECTION

4.1 Inclusion Criteria

A patient must meet all of the following inclusion criteria to be eligible to participate in the study.

4.1.1 Any of the following hematological malignancies:

- Acute lymphoblastic leukemia
- Acute myelogenous leukemia
- Chronic myelogenous leukemia
- Myelodysplasia

4.1.2 Candidate for HCT

Note: Patients with or without previous myeloablative autologous transplant are eligible.

4.1.3 HLA-matched stem cell donor, either related (6/6 or 5/6 loci matched) or unrelated (8/8 or 7/8 loci matched)

4.1.4 Stem cell graft from either bone marrow or peripheral blood

4.1.5 Negative serology for HIV

4.1.6 Age ≥ 18 to < 78 years of age

4.1.7 Karnofsky Performance Status of 70-100%

4.1.8 Women who are not postmenopausal or have not undergone hysterectomy must have a documented negative serum pregnancy test per standard MCC-VCUHS CIT Program guidelines

4.1.9 Ability to understand and the willingness to sign a written informed consent document

Note: The consent form must be signed and dated prior to initiation of SCT preparative treatments.

4.2 Exclusion Criteria

A patient who meets any of the following exclusion criteria is ineligible to participate in the study.

4.2.1 Known allergy to vitamin C

4.2.2 Inability to swallow oral medication

4.2.3 Known or suspected malabsorption condition or obstruction

4.2.4 G6PDH deficiency

4.2.5 Uncontrolled viral, fungal, or bacterial infection

4.2.6 Active meningeal or central nervous system disease

4.2.7 Alternative HCT including haplo-identical and umbilical cord transplants

4.2.8 Non-myeloablative conditioning defined as TBI < 2 cGy

4.2.9 Pregnancy or breastfeeding

4.2.10 Medical, psychological, or social condition that, in the opinion of the investigator, may increase the patient's risk or limit the patient's adherence with study requirements

5 STUDY ENTRY AND WITHDRAWAL PROCEDURES

5.1 Study Entry Procedures

5.1.1 Required Pre-Registration Screening Tests and Procedures

Refer to the study calendar in Section 12 for the screening tests and procedures that are required prior to registration.

5.1.2 Study Enrollment

The following are needed for patient registration:

- Completed, signed, and dated eligibility checklist
- Signed and dated consent form

The patient's initial enrollment data (eg, demographics, consent, eligibility, on study) will be entered into the OnCore database before treatment begins.

5.2 Study Withdrawal

A patient will be removed from the study for any of the following reasons:

- Consent withdrawal for study treatment and study procedures
- If, in the opinion of the investigator, it is in the best interest of the patient to do so
- The study has been closed by the Sponsor-Investigator

The reason for withdrawal from the study and the date the patient was removed from the study must be documented in the source documents and OnCore database.

6 TREATMENT PLAN

6.1 Study Treatment

6.1.1 Safety Lead-In Cohort

For the initial safety lead-in cohort of 14 patients, vitamin C deficiency will be confirmed by a standard clinical pathology laboratory blood test before the first dose of parenteral vitamin C (see Sections 12 and 13.3). Safety lead-in patients with confirmed vitamin C levels of 0.5 mg/dL or less (20) may proceed on study treatment.

6.1.2 Planned Vitamin C Regimen

All study patients will receive the following vitamin C regimen:

- Parenteral vitamin C 50 mg/kg/day divided in 3 doses beginning on post-transplant Day +1 and continuing through Day +14; each dose (16.7 mg/kg) given in 50 mL of 5% dextrose and water over 30 minutes every 8 ± 3 hours
- After completion of the parenteral vitamin C doses, oral vitamin C 500 mg twice each day beginning on Day +15 and continuing until Day +180

The vitamin C regimen will continue as described above even if the vitamin C levels are found to be within normal range at the time of testing (refer to Section 6.3 for testing time points).

6.1.3 Missed Doses

Missed doses of IV vitamin C will not be made up (see Section 7.1).

Patients will be instructed to take a missed dose of oral vitamin C on the same day, as soon as they remember, and to not take more than 2 doses of vitamin C on the same day to make up for doses that were missed on the previous day.

6.1.4 Monitoring Patient Adherence (Oral Vitamin C Regimen)

Following hospital discharge, patients will be instructed to bring any unused supply of vitamin C to visits with their study team.

Patient reports of self-administration and review of unused medication will be used to assess the patient's compliance with the oral vitamin C regimen.

6.2 Standard HCT Management

Other than the vitamin C regimen (Section 6.1) and the collection of blood samples for the correlative studies (Section 11), all pre- and post-transplant assessments, examinations, procedures, and treatment required for patients undergoing HCT will be carried out according to the standard approach of the MCC-VCUHS CIT Program.

6.3 Post-Treatment Evaluation

- All patients will undergo standard blood tests for vitamin C and CRP at baseline, on transplant day 0, and at post-transplant days +14, +30, and +100.
- All patients will undergo a standard urinalysis at baseline and weekly through 1 week following the last dose of IV vitamin C.
- All patients will have blood samples collected for thrombomodulin, TNF-α, and cfDNA assays at baseline, on transplant day 0, and at post-transplant days +14, +30, and +100.
- Relevant and disease-specific restaging studies will be performed per standard procedures of the MCC-VCUHS CIT Program following transplantation or earlier if clinically indicated.

6.4 General Concomitant Medication and Supportive Care Guidelines

Standard transplant-related supportive medications, other than vitamin C, may be administered at the investigator's discretion.

6.5 Follow-Up Period

The patient's follow-up status will be recorded in the source documents and the CRFs. Study follow-up will be conducted as follows:

6.5.1 Evaluation of Adverse Events (AEs)

Patients will continue to be evaluated for AEs until 6 months post-transplant.

6.5.2 Non-Relapse Mortality

Follow-up for NRM will continue for one year following transplantation per standard post-transplant procedures or until relapse or death, whichever occurs first.

6.5.3 Survival

Patients will be followed for survival for 2 years.

7 DOSING DELAYS/DOSE MODIFICATIONS

7.1 Vitamin C

During days 1-14, IV vitamin C will be held in patients who develop a serum creatinine of >2.0 mg/dL and resumed once serum creatinine is ≤ 2.0 mg/dL. Missed doses will not be made up.

During days 15-180, if a patient has a serum creatinine level of > 2.0 mg/dL definitely, probably, or possibly related to vitamin C (Section 8.1.8), the patient will be instructed not to take their oral vitamin C until the serum creatinine level is ≤ 2.0 mg/dL. Missed doses will not be made up.

7.2 Standard SCT Treatment

There are no study-required dosing delays or dose modifications for any of the agents included in the SCT preparatory, GVHD prophylaxis, infection prophylaxis, or transplantation regimens. Dose modifications and delays, if needed for patient support and safety, will be per investigator discretion.

8 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

8.1 Definitions

8.1.1 Adverse Event (AE)

AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

8.1.2 Suspected Adverse Reaction (SAR)

A SAR is any AE for which there is a reasonable possibility that the drug caused the AE. “Reasonable possibility” means that there is evidence to suggest a causal relationship between the drug and the AE.

An AE with an attribution of possible, probable, or definite (Section 8.1.8) is a SAR.

8.1.3 Serious AE (SAE) or Serious SAR (SSAR)

An AE or SAR is considered “serious” if, in the view of the investigator, it results in any of the following outcomes:

- death,
- a life-threatening AE or SAR (An AE or SAR is considered “life-threatening” if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.),
- inpatient hospitalization or prolongation of existing hospitalization,

  Planned inpatient hospitalizations are exempt from SAE reporting. Events that prolong hospitalization beyond the expected period of time and otherwise meet reporting criteria are, however, subject to SAE reporting requirements.
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical

judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.4 Unexpected SAR

A SAR is considered “unexpected” if:

- it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed;
- or, if an investigator brochure is not required, is not consistent with the risk information described in the prescribing information.

“Unexpected” as used in this definition, also refers to SARs that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the drug under investigation.

8.1.5 Unanticipated Problem (UP)

Unanticipated problems include any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the patient population being studied;
- related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.6 AE Description and Grade

The descriptions and grading scales found in the revised Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) will be utilized for AE reporting.

8.1.7 AE Expectedness

AEs can be ‘Unexpected’ or ‘Expected’. Refer to Section 8.2 regarding expected AEs. Unexpected AEs are those AEs occurring in one or more patients participating in the study, the nature, severity, or frequency of which is not consistent with either:

- The known or foreseeable risk of AEs associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or

- The expected natural progression of any underlying disease, disorder, or condition of the patient(s) experiencing the AE and the patient's predisposing risk factor profile for the AE.

8.1.8 AE Attribution

- Definite – The AE *is clearly related* to the study intervention.
- Probable – The AE *is likely related* to the study intervention.
- Possible – The AE *may be related* to the study intervention.
- Unlikely – The AE *is doubtfully related* to the study intervention.
- Unrelated – The AE *is clearly NOT related* to the study intervention.

8.2 Known AEs Related to Vitamin C

Too rapid infusion of intravenous vitamin C solution may cause temporary faintness or dizziness. Other side effects include nausea, vomiting, heartburn, diarrhea, and stomach cramps.

8.3 Recording AEs, SAEs, and UPs

All recordable AEs per protocol (defined in Section 8.4), all SAEs, and all UPs will be recorded in MCC's OnCore Clinical Trial Management System. In most cases, it is acceptable to record only the highest grade of a toxicity occurring during a particular study segment when an event has serial fluctuations in grade over time.

SAEs will be entered into the OnCore SAE domain. UPs will be entered into the OnCore Deviations domain. An SAE that is both an SAE and a UP will be entered in both domains. For all SAEs, a corresponding entry should be made in the routine AE record to match the event entries in the SAE domain. Additionally, events related to stopping criteria will be entered in OnCore as an event of special interest.

8.4 Time Period and Grade of AE Capture

AEs ≥ grade 3 regardless of expectedness or attribution will be recorded for study tabulation and analysis beginning on Day 0 and continuing until Day 180. Toxicity assessments will include a review of all toxicities experienced during each assessment period. The highest grade of each relevant toxicity during that period will be recorded.

Exception: Cytopenias and changes in electrolytes will **not** be recorded. Only clinical events and lab values reflecting renal function (eg, creatinine, urinalysis results) will be recorded as AEs.

8.5 AEs Requiring Expedited Reporting

All patients in this study will be receiving potentially toxic preparative therapy, therefore, significant regimen-related toxicity is anticipated, ie, expected AEs.

8.5.1 Expedited Reporting Requirements

All grade 3, 4, and 5 ***unexpected*** AEs regardless of attribution will be reported in an expedited manner from the first dose of vitamin C (on Day 1) until post-transplant Day 180. (Refer to Section 8.5.2 for AEs that should NOT be considered unexpected.)

8.5.2 Expedited Reporting Exceptions

The following ≥ grade 3 toxicities/events are **expected** AEs:

- All laboratory abnormalities regardless of grade
- Hospitalization including hospitalization for the transplant procedure
- Infection
- GVHD
- Graft failure
- Progression or relapse
- Death
- Adverse events that are commonly observed after hematopoietic cell transplantation including diarrhea, hematuria, hemorrhage, hypoxia, sepsis, mental status changes, pneumonitis, anemia, minor bleeding episodes (eg, epistaxis), hepatic VOD, renal insufficiency, blood clots, and thrombotic microangiopathy

8.6 Expedited Reporting Procedures for SAEs, and UPs

Refer to the table below for expedited reporting requirements.

Table 1. Expedited Reporting Requirements

SAEs	UPs	SSARs
Sponsor-Investigator[A] Gary Simmons, DO Phone: 804-827-7952 Email: gary.simmons@vcuhealth.org	**Sponsor-Investigator**[A] Gary Simmons, DO Phone: 804-827-7952 Email: gary.simmons@vcuhealth.org	**Sponsor-Investigator**[A] Gary Simmons, DO Phone: 804-827-7952 Email: gary.simmons@vcuhealth.org
Study Coordinator[A] Catherine H. Roberts, PhD Phone: 804-828-1292 Email: croberts2@vcu.edu	**Study Coordinator**[A] Catherine H. Roberts, PhD Phone: 804-828-1292 Email: croberts2@vcu.edu	**Study Coordinator**[A] Catherine H. Roberts, PhD Phone: 804-828-1292 Email: croberts2@vcu.edu
	DSMC[B] Email: masseydsmc@vcu.edu	**FDA**[D]
	IRB[C]	

A. Report event within 1 business day of becoming aware of the occurrence.
B. Report event within 2 business days of becoming aware of the occurrence.
C. Each UP must be reported to the VCU IRB within 5 business days of becoming aware of the occurrence.
D. Using MedWatch Form 3500A, the Sponsor-Investigator will report to the FDA any
- AE that is **all of the following**: 1) possibly, probably, or definitely related to study drug (ie, the event is a SAR); 2) serious (ie, a SSAR); and 3) unexpected. Events meeting these criteria will be reported to the FDA **within 15 calendar days of determining that the event is reportable.**
- clinically important increase in the rate of SSARs **within 15 calendar days** of determining that the event is reportable.
- unexpected fatal or life-threatening SSARs **within 7 calendar days of receipt of the information** regarding the event.

9 PHARMACEUTICAL INFORMATION

Refer to the current FDA-approved prescribing information for the pharmaceutical information for all of the agents used in the management of patients enrolled on the study. Information regarding vitamin C is outlined in this section.

9.1 Vitamin C General Information

9.1.1 Description

The chemical name of vitamin C is L-ascorbic acid. The molecular formula is $C_6H_8O_6$. It occurs as white or slightly yellow crystals or powder.

9.1.2 Availability and Ordering

All study vitamin C will be ordered and dispensed by MCC Investigational Drug Services.

9.1.3 Agent Accountability

MCC Investigational Drug Services will retain records of the inventory and disposition of study vitamin C.

9.1.4 Agent Destruction and Return

At the conclusion of the study, any unused study vitamin C will be destroyed according to institutional policies.

9.1.5 Contraindications

Administration of vitamin C is contraindicated in patients with a known allergy to vitamin C or any other ingredient used in the formulation.

9.1.6 Drug Interactions

Acidification of the urine by ascorbic acid may cause precipitation of cysteine, urate or oxalate stones (renal calculi) and will alter the excretion of certain other drugs administered concurrently.

Large doses interfere with the anticoagulant effect of warfarin.

Ascorbic acid has on occasion been used as a specific antidote for symptoms resulting from interaction between ethanol and disulfiram (Antabuse®). It may be expected that the concurrent administration of high doses of vitamin C could interfere with the effectiveness of disulfiram given to patients to encourage abstention from alcohol.

Because ascorbic acid is a strong reducing agent, it interferes with numerous laboratory tests based on oxidation-reduction reactions. Diabetics taking more than 500 mg of ascorbic acid daily may obtain false readings of their urinary glucose test. No exogenous ascorbic acid should be ingested for 48 to 72 hours before amine-

dependent stool occult blood tests are conducted because false negative results may occur.

9.1.7 Adverse Reactions

The most common adverse events associated with high intake of vitamin C are gastrointestinal events, including nausea, vomiting, heartburn, abdominal cramps, and diarrhea. As stated in Section 9.1.6, there is also a risk of renal calculi.

9.2 Parenteral Vitamin C (Ascor L 500)

9.2.1 How Supplied

Ascor L 500 (Ascorbic Acid Injection, USP) is a sterile, nonpyrogenic solution of ascorbic acid prepared with the aid of sodium bicarbonate in water for injection. Each mL contains 500 mg of ascorbic acid, and edetate disodium 0.025% (w/v). It also contains sodium bicarbonate and may contain sodium hydroxide to aid in preparation and pH adjustment. The pH is 5.5-7.0. It contains no bacteriostat, antimicrobial agent, or added buffer. Ascor L 500 (ascorbic acid for injection, USP), 500 mg/mL, is available in trays of twenty-five, 50 mL, sterile, pharmacy bulk bottles, containing no preservative.

9.2.2 Storage Requirements

Protect from light. Store in refrigerator at 2°– 8°C (36°– 46°F). Do not allow to stand at room temperature before use. Failure to follow this caution may lead to excessive pressure inside the vial.

9.2.3 Stability

All vials of Ascor L 500 are labeled with a “Best Use” date.

9.2.4 Route of Administration

Intravenous.

9.2.5 Warnings and Precautions

Pain and swelling at the site of injection have been reported in some patients. Excessively rapid intravenous injection may result in temporary faintness or dizziness. Since high internal pressure may develop on long storage, precautions should be taken when withdrawing the solution from the vial. Do not administer unless solution is clear and container is intact. Discard unused portion.

Usage in pregnancy: Pregnancy Category C (in doses greater than the RDA). Animal reproduction studies have not been conducted with ascorbic acid injection. It is not known whether ascorbic acid injection can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. High doses of vitamin C taken during pregnancy have been reported to cause scurvy in infants removed from this environment at birth.

Nursing mothers: Ascorbic acid is excreted in breast milk. Caution should be exercised when ascorbic acid injection is administered to a nursing woman, and excessive doses should be avoided.

9.3 Vitamin C Tablets

9.3.1 How Supplied

Vitamin C for oral consumption is available in tablets containing 500 mg vitamin C each.

9.3.2 Storage Requirements

Vitamin C tablets may be stored at room temperature.

9.3.3 Stability

Containers of vitamin C tablets are labeled with an expiration date.

9.3.4 Route of Administration

Oral.

10 MEASUREMENT OF EFFECT

10.1 Engraftment

Hematopoietic engraftment is defined as both of the following:

- Recovery from post-transplant cytopenia with an absolute neutrophil count of ≥ 0.5 x 10^9 /L for 3 consecutive measurements or ≥ 1.0 x 10^9 /L for 1 day; and
- Platelet count of ≥ 20 x 10^9 /L for 7 days without transfusion.

10.2 Length of Relapse-Free/GVHD-Free Survival and Survival

All study patients including those removed from the treatment protocol will be followed for survival every 3 months.

- Length of relapse-free/GVHD-free survival is measured from transplant to relapse, GHVD diagnosis, death, or time of last contact, censoring for patients alive and relapse-free/GVHD-free at the time of last contact.

- Length of survival is measured from transplant to death or time of last contact, censoring for patients alive at time of last contact.

10.3 Relapse from Remission

10.3.1 Lymphoma (Hodgkin Lymphoma, Non-Hodgkin Lymphoma, and T-Cell Lymphoma)

In patients with Hodgkin lymphoma, non-Hodgkin lymphoma, or T-cell lymphoma, 25% or greater increase in involved lymph nodes and histologically documented relapse at another site will be indicative of relapse or PET scanning with an SUV >4.

10.3.2 Leukemia (Acute and Chronic Myeloid and Lymphoid) and Myelodysplasia

Patients with acute leukemia will be considered as having relapsed if there is an increase in blast count to beyond 5% or recurrent cytogenetic abnormalities or molecular aberration defined by an abnormal FISH or PCR.

10.3.3 Myelofibrosis

Re-emergence of clonal hematopoiesis or increased blasts in circulation in the presence of recipient chimerism, with or without persistent marrow fibrosis.

10.3.4 Multiple Myeloma

In patients with multiple myeloma, disease relapse will be defined by increase in the plasmacytosis to greater than 5% plasma cells and/or 25% or greater increase in paraproteinemia, appearance of new extramedullary disease, or appearance of new skeletal disease.

10.4 GVHD Staging and Grading

Acute GVHD will be graded according to the CIBMTR acute GVHD system. The staging and grading criteria for GVHD are outlined on Table 2 and Table 3.

Table 2. GVHD Staging

Stage	Skin Rash (BSA %)	Gastrointestinal	Liver (Total Bilirubin)
1	< 25%	Diarrhea > 500 mL/day or persistent nausea	2-3 mg/dL
2	25-50%	Diarrhea > 1000 mL/day or persistent nausea	3.1-6 mg/dL
3	> 50%	Diarrhea > 1500 mL/day or persistent nausea	6.1-15 mg/dL
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain +/- ileus	> 15 mg/dL
From the Technical Manual of Procedures Version 3.0 of the Blood and Marrow Transplant Clinical Trials Network, 2013.			

Table 3. GVHD Grading

Overall Grade	Skin		Gastrointestinal		Liver
I	Stage 1-2	*and*	0	*and*	0
II	Stage 3	*or*	Stage 1	*or*	Stage 1
III	---	---	Stage 2-4	*or*	Stage 2-3
IV	Stage 4	---	---	*or*	Stage 4
From the Technical Manual of Procedures Version 3.0 of the Blood and Marrow Transplant Clinical Trials Network, 2013.					

11 CORRELATIVE STUDIES

11.1 Participation in Correlative Studies

Plans for the correlative studies are described in Section 1.5. Participation in the correlative studies using collected blood samples is mandatory.

11.2 Processing and Distribution of Blood Samples

Samples for vitamin C and CRP will be processed and analyzed in the VCUHS clinical pathology laboratory.

The laboratory of Ramesh Natarajan, PhD, will receive, process, and store blood samples for TNF-α, TM, and cfDNA studies.

11.3 Blood Samples for Correlative Studies

Blood samples will be collected for the planned correlative studies at the following time points:

- Baseline (before beginning conditioning)
- Day 0
- Day +14
- Day +30
- Day +100

Approximately 4 mL will be collected in each of three tubes at each time point: one tube for vitamin C, one tube for CRP, and one tube to be processed and stored for TNF-α, TM, and cfDNA studies.

11.4 Labeling for Blood Samples

Blood samples for vitamin C and CRP testing should be labeled in accordance with the standard practice of the VCUHS clinical pathology laboratory.

Each blood sample collected for storage and future analysis should be labeled as follows:

- Study number
- Patient study identification number
- Date of sample collection
- Time of sample collection
- Study time point

11.5 Processing and Analysis of Blood Samples

Testing and analysis will be as follows:

11.5.1 Vitamin C

Serum levels of vitamin C will be measured according to standard practices of the VCUHS clinical pathology laboratory.

11.5.2 C-Reactive Protein (CRP)

Plasma/serum levels will be measured using by high sensitivity C-reactive protein (hsCRP) assay. The assay will be performed by ELISA using the Human C-Reactive Protein/CRP Quantikine ELISA Kit (R&D Systems, catalog # DCRP00).

11.5.3 Tumor Necrosis Factor-Alpha (TNF-α)

TNF- α levels in plasma/serum will be quantified using a Human TNF-alpha Quantikine ELISA Kit according to manufacturer's instructions (R&D Systems, catalog # DTA00C).

11.5.4 Thrombomodulin (TM)

Plasma levels will be quantified using an ELISA kit (IMUBIND; American Diagnostica Inc., Stamford, Connecticut, USA).

11.5.5 Cell-Free DNA (cfDNA)

The levels of cf-DNA in human serum/plasma will be quantified using the Invitrogen Quant-iT PicoGreen dsDNA assay kit according to the manufacturer's instructions (Life Technologies, Grand Island, NY). Fluorescence intensity will be measured on a SpectraMax Gemini XPS microplate reader with excitation at 490 nm and emission at 525 nm, with 515 nm emission cutoff filter (Molecular Devices, Sunnyvale, CA).

12 STUDY CALENDAR

Required assessments, tests, examinations, administration of the vitamin C regimen, and collection of blood samples for correlative studies are outlined on Table 4 and Table 5.

Table 4. Study Calendar – Screening/Pretreatment Requirements

Tests, Exams, Procedures, and Other Requirements [A]	Timeline
Informed Consent	Prior to initiation of SCT preparative treatments
Demographics	Timing prior to study enrollment should be consistent with the usual MCC-VCUHS CIT Program practices to screen patients for SCT.
Medical History	
Physical Exam	
Performance Status	
Weight & Height	
HLA Typing [B]	
CBC, Differential, Platelets	
Blood Chemistries [C]	
Creatinine Clearance	
Infections Disease Titers [D]	
G6PDH Deficiency Testing	
Cardiac Assessment [E]	
Chest X-Ray or Chest CT	
Disease Staging [F]	
Baseline Symptoms	
Urinalysis	
Serum Pregnancy Test	As applicable, within 30 days prior to initiation of the SCT preparatory regimen
Vitamin C Test [G]	Within 2 to 5 days prior to transplant day 0
Correlative Blood Sample Collection	After study registration but before initiation of the SCT preparatory regimen

Table 4 Footnotes:

A. In addition to the assessments/tests listed, other tests and exams may be performed per standard guidelines for the MCC-VCUHS CIT program and per investigator discretion.
B. Donor and recipient
C. Blood chemistries include serum creatinine, total bilirubin, alkaline phosphatase, AST, ALT, and additional tests per investigator discretion.
D. Infectious disease markers per standard VCU CIT program practices.
E. Cardiac assessments include ECG and left ventricular ejection fraction or shortening fraction by echocardiogram or MUGA.
F. Relevant and disease-specific staging according to the MCC-VCUHS CIT Program usual practice.
G. For safety lead-in cohort patients only

Table 5. Study Calendar – Treatment and Follow-Up

Study Requirements [A]	Transplant Day 0	Post-Transplant Day												Year 2 [D]
		1	7	14	15	21	30 [B]	60 [B]	100 [B]	120 [B]	180 [C]	270 [C]	365 [C]	Every 3 months [C]
Physical Exam				X			X	X	X	X	X	X	X	
Toxicity Assessment [E]				X			X	X	X	X	X			
GVHD Assessment				X			X	X	X	X	X	X	X	X
Study Drug Review					X			X	X	X	X			
Urinalysis			X [F]	X [F]		X [F]								
Correlative Blood Samples	X			X			X		X					
Stem Cell Transplant (SCT)	X													
Parenteral Vitamin C		X (Days 1-14)												
Oral Vitamin C					X (Days 15-180)									
CBC, Differential, Platelets [G]	Frequency per standard practice of the MCC-VCUHS CIT Program													
Blood Chemistries [G,H]	Frequency per standard practice of the MCC-VCUHS CIT Program													
Survival														X

Table 5 Footnotes:

A. In addition to the assessments/tests listed on Table 5, other tests and exams may be performed per standard guidelines for the MCC-VCUHS CIT Program and per investigator discretion.

B. Within +/- 1 week.

C. Within +/- 2 weeks.

D. For patients who are not able to return for evaluation during year 2, follow-up may be performed by telephone contact with the patient or through the patient's referring physician.

E. Refer to Section 8 for AE reporting requirements and instructions.

F. Within +/- 2 days

G. Labs are done as part of routine care, and lab values will not be collected on eCRFs except as relevant to endpoints or stopping criteria or in support of clinical event AEs.

H. Blood chemistries include serum creatinine, total bilirubin, alkaline phosphatase, AST, ALT, and additional tests per investigator discretion.

13 STATISTICAL CONSIDERATIONS

13.1 Statistical Methods

13.1.1 Primary Endpoint

The primary outcome in this study is non-relapse mortality (NRM) at one year.

13.1.2 Secondary Endpoints (with measurement type)

- Time to engraftment (in days)
- Acute GVHD (days to positive diagnosis: time-to-event; positive diagnosis: categorical)
- AEs of grade ≥ 3 possibly, probably, or definitely related to the vitamin C regimen (counts by event type)

13.1.3 Exploratory Endpoints

- Chronic GVHD (days to positive diagnosis: time-to-event; positive diagnosis: categorical)
- Overall survival
- Relapse-free/GVHD-free survival
- Levels in blood over time of vitamin C and exploratory biomarkers

13.1.4 Statistical Analysis

The primary and secondary outcomes will be compared between the study cohort and a historical cohort matched for age, donor type, and diagnosis. The NRM rate will be compared to the historical 35% rate (see Section 13.2 below) using a one-sided exact binomial test. Time-to-event outcomes (time to engraftment, acute and chronic GVHD, and overall and relapse-free survival) will be graphically represented with a Kaplan-Meier step-function and will be assessed for associations with serum vitamin C levels and patient demographic measurements using a Cox Proportional Hazards model. Associations between blood levels of vitamin C and other exploratory endpoints will be estimated using Spearman rank correlation coefficients.

13.2 Sample Size and Power Determination

The sample size and power are calculated based on Simon's two-stage minimax design, which minimizes the expected maximum sample size under the alternative hypothesis. The previous 2 years at VCU have seen an ~15% NRM rate in MRD patients and an ~40% NRM rate in MUD patients. Given that MRDs comprise ~25% of total transplantations and MUDs comprise ~75%, we then assume an overall 35% NRM rate. The null hypothesis that the NRM rate is 35% will be tested against a one-sided alternative. In order to achieve 80% power (with 5% significance) to declare an NRM of 20% significantly smaller than the

hypothesized null NRM of 35%, we will accrue 31 patients in stage 1 and an additional 24 patients in stage 2 (provided the trial is not stopped early for futility). At the end of stage 1, if there are 11 or more NRMs in the 31 stage 1 patients, the study will be stopped for futility. Otherwise, 24 additional patients will be accrued for a total of 55. The null hypothesis will not be rejected if 14 or more NRMs are observed in the total 55 patients. We anticipate a 32% screen failure rate, leading to a recruitment target of 81 subjects (VCU sees ~5 eligible MRD/MUD patients per month). The number of MUD patients will be limited to 80%.

The population evaluable for the primary endpoint includes all patients who receive at least one dose of IV vitamin C and who have a known survival status at one year. Patients who are enrolled to the study but do not receive any IV vitamin C or who are lost to follow up at one year will be replaced.

13.3 Safety Criteria for Lead-In Phase and Phase 2 Portions of the Trial

Early stopping criteria based on severe toxicities and ANC engraftment failure will be used to ensure patient safety in both the safety lead-in cohort and the phase 2 portion of the trial.

The following toxic events as defined per CTCAE v5.0 will be counted separately toward the stopping rules in Table 6 and Table 7:

- Grade 4 mucositis (any site)
- Any grade 4 renal toxicity
- Any grade 4 hepatobiliary toxicity
- Grade 4 respiratory failure
- Grade 4 sepsis

13.3.1 Safety Lead-in Cohort

The stopping rules for the initial safety phase are based on both the five toxic events listed above and ANC engraftment failure occurring with 30 days following transplant. The safety phase and the overall study will be stopped if either of the conditions stated for toxic events or ANC engraftment failure in Table 6 below are met. Both stopping rules are based on a one-sided exact binomial test with right-tailed significance level of $\alpha=0.05$ for sample sizes 4 to 14. For the toxic events the assumed null proportion is 0.30 for each of the five toxicities listed above, while for ANC engraftment failure the assumed null proportion of 0.10.

After enrollment to the safety lead-in cohort has been completed, accrual to the study will be temporarily suspended until all patients have completed toxicity and engraftment assessments through 30 days post-transplant. These assessments will be reviewed by study monitors and the Data and Safety Monitoring Committee (DSMC, Section 14.2). If the DSMC concludes that the reported toxicities and engraftment meet safety criteria to continue, the study will reopen to accrual.

Table 6. Number of Observed ANC Engraftment Failures and Toxic Events (Measured Separately for Each Toxicity Outcome) Needed to Stop Trial During Safety Lead-In Given Accrued Sample Size

Accrued Sample Size	ANC Engraftment Failure Stopping Rule	Toxic Event Stopping Rule
4,5	2	3
6,7	2	4
8	2	5
9-10	3	5
11,12	3	6
13,14	3	7

13.3.2 Stopping Criteria for Phase 2 portion

If criteria are met to proceed to the phase 2 portion of the trial, the rates of engraftment failures and severe toxicities will continue to be monitored, and the early stopping rules outlined in Table 7 and Table 8 will be employed.

The stopping rules for the phase 2 portion of the study are based on an exact binomial test using the null proportion of 0.30 for each of the five toxicities listed above and a one-sided, right-tailed significance level of α=0.05 for sample sizes 2 to 55 (Table 7).

ANC engraftment failure will also be used for a stopping rule based on an exact binomial test using the null proportion of 0.10 for engraftment failure and a one-sided right-tailed significance level of α=0.05 for sample sizes 4 to 55 (Table 8). ANC engraftment failure is defined as failure to achieve hematopoietic engraftment (Section 10.1) within 30 days after transplant.

Table 7. Number of Observed Toxic Events (Measured Separately for Each Toxicity Outcome) Needed to Stop Phase 2 Portion of Trial Given Accrued Sample Size

Accrued Sample Size	Stopping Rule
15-17	8
18-20	9
21-22	10
23-25	11
26-28	12
29,30	13
31-33	14
34-36	15
37-39	16
40,41	17
42-44	18
45-47	19
48-50	20
51-53	21
54,55	22

Table 8. Number of Observed ANC Engraftment Failures Needed to Stop Phase 2 Portion of Trial Given Accrued Sample Size

Accrued Sample Size	Stopping Rule
15-20	4
21-27	5
28-34	6
35-41	7
42-48	8
49-55	9

14 DATA AND SAFETY MONITORING

14.1 Study Team

The study team minimally consists of the sponsor-investigator, the co-investigators, the study coordinator, the clinical research associate, and the study biostatistician. While patients are on treatment, the sponsor-investigator and the study coordinator will meet at least monthly and will meet at least quarterly with the study biostatistician to review study status. This review will include, but not be limited to, reportable AEs and UPs and an update of the ongoing study summary that describes study progress in terms of the study schema. All meetings including attendance are documented.

14.2 Monitoring and Auditing

14.2.1 MCC Compliance Office

Compliance specialists in the MCC Compliance Office will provide ongoing monitoring and auditing for this study.

14.2.2 Data and Safety Monitoring Committee (DSMC)

The study will be reviewed by the MCC DSMC initially according to the risk level specified by the MCC Protocol Review and Monitoring Committee (PRMC) and then according to a schedule based on study status and quality indicators. The DSMC will review reports provided by the sponsor-investigator/study team and the MCC Compliance Office focusing on data integrity and patient safety.

15 REGULATORY COMPLIANCE AND ETHICS

15.1 Ethical Standard

This study will be conducted in conformance with the principles set forth in *The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research* (US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979).

15.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards); 21 CFR 312 (IND Application); and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children)

15.3 Institutional Review Board

The VCU IRB, which is registered with the Office for Human Research Protections, will review and provide approval for the protocol, the associated informed consent document, material that will be provided to participating patients, and any recruitment material. Any amendments to the protocol, consent form, or other materials will also be approved by the IRB.

15.4 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Discussion of risks and possible benefits of this therapy will be provided to the patients and their families. IRB-approved consent forms describing the study interventions, study procedures, and risks are given to the patient and written documentation of informed consent is required prior to starting intervention/administering study product.

The patient will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the patient and answer any questions that may arise. The patient will sign the informed consent document prior to any procedures being done specifically for the study. The patients should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. Patients may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to patients for their records. The rights and welfare of the patients will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.5 Patient Confidentiality and Access to Source Documents/Data

Patient confidentiality is strictly held in trust by the participating investigators and their staff. This confidentiality includes the clinical information relating to participating patients, as well as any genetic or biological testing.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor-investigator.

The sponsor-investigator will allow access to all source data and documents for the purposes of monitoring, audits, IRB review, and regulatory inspections.

The study monitor or other authorized representatives of the sponsor-investigator may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The clinical study site will permit access to such records.

16 DATA HANDLING AND RECORD KEEPING

16.1 Data Management Responsibilities

The sponsor-investigator is responsible for: (i) the overall conduct of the investigation; (ii) ongoing review of trial data including all safety reports; and (iii) apprising participating investigators of any UPs. Participating investigators are responsible for reporting SAEs and UPs as required in Section 8.

Any laboratory conducting correlative studies must maintain the laboratory records and documentation (laboratory notebooks, laboratory protocols, print-outs, recordings, photographs, etc).

16.2 Source Documents

Source documents for clinical information (patient history, diagnosis, clinical and diagnostic test reports, etc) are maintained in the patient's clinical file. Source documents for the correlative studies are maintained in the laboratory conducting the study.

16.3 Case Report Forms and Data Collection

MCC OnCore data management will provide standard and study-specific electronic case report forms (eCRFs) to capture all the information required by the protocol. The eCRFs will be approved by the study team to ensure the most effective data acquisition.

The investigator(s) and study coordinator(s) must maintain source documents for each patient in the study. All information on the eCRFs will be traceable to the source documents, which are generally maintained in the patient's file.

All eCRFs should be completed and available for collection within a timely manner, preferably no more than 14 days after the patient's visit.

16.4 Study Record Retention

As applicable, study records will be maintained a minimum of 6 years beyond: (i) the publication of any abstract or manuscript reporting the results of the protocol; (2) the submission of any sponsored research final report; or (iii) submission of a final report to clinicaltrials.gov.

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