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SHORT REPORT

Feasibility of intravenous vitamin C supplementation in allogeneic hematopoietic cell transplant recipients

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Abstract

Introduction: Intravenous vitamin C was administered following hematopoietic stem cell transplant to mitigate nonrelapse mortality (NRM) in a Phase II clinical trial.

Methods: Patients with advanced hematologic malignancies received IV vitamin C, 50 mg/kg/day, in three divided doses on days 1–14 after HSCT, followed by 500 mg bid oral until 6 months.

Results: All patients enrolled (55) were deficient in vitamin C at day 0 and had restoration to normal levels. Vitamin C recipients had a trend for lower nonrelapse mortality (NRM, 11% vs. 25%, p-value = 0.07) compared with propensity score-matched historical controls. A similar trend toward improved survival was observed (82% vs. 62% $p = 0.06$), with no attributable grade 3 and 4 toxicities to vitamin C.

Conclusion: In patients undergoing allogeneic HSCT, repletion of vitamin C is feasible and may reduce NRM and improve overall survival. Randomized trials in large uniform cohorts of patients are needed to confirm the utility of this easily available and inexpensive therapy.

KEYWORDS
allogeneic stem cell transplantation, graft versus host disease, nonrelapse mortality, parenteral ascorbic acid

Manjari Sriparna and Cody McIntire contributed equally to this work.

Preparative myeloablative conditioning regimens for hematopoietic stem cell transplant (HCT) work in large part by widespread, indiscriminate oxidative damage to DNA [1]. The oxidative stress produced by these regimens induces variable degrees of enteral mucositis, loss of functioning epithelial cells and disrupts tight junctions increasing the translocation of bacterial products and inflammatory cytokines, an essential factor in the pathogenesis of acute graft versus host disease (GVHD) [2], which contributes to nonrelapse mortality (TRM) in myeloablative allogeneic HCT [3, 4].

Therapy to mitigate GVHD incidence contributing to NRM in HCT recipients without the reciprocal immune deficiency, that immune suppressants cause, is urgently needed. Ascorbic acid (vitamin C) is an antioxidant/anti-inflammatory agent with the ability to inhibit NF-κB-driven inflammatory cytokine (IL-6, IL-8, and TNF-α) expression and to attenuate endothelial permeability [5, 6, 7]. In previous reports, vitamin C deficiency was observed during the acute phase of HCT and this was significantly associated with elevated levels of inflammatory markers, CRP, and ferritin [8, 9, 10].

A Phase II study was developed to prospectively administer parenteral vitamin C following HCT, to study the hypothesis that mitigating the proinflammatory/oxidant effects of HCT with early administration of vitamin C will attenuate endothelial and organ injury from high-dose conditioning and ameliorate GVHD and NRM.

This was a prospective Phase II clinical trial with a safety lead-in cohort (FDA-IND 138924). This trial was approved by the Institutional Review Board (IRB) at Virginia Commonwealth University (NCT03613727). The study population included patients 18–78 years old with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) who underwent their first allogeneic HCT from HLA-matched sibling and unrelated donors, matched at either 7/8 or 8/8, HLA-A, -B, -C, -DRB1 loci using high-resolution DNA-based typing. Patients undergoing nonmyeloablative conditioning were not included.

An initial cohort of patients (N = 14) were enrolled if they had low vitamin C levels (< 0.5 mg/dL). Vitamin C levels were checked at baseline and on day −2 in these patients. Patients were treated with parenteral vitamin C 50 mg/kg/day (Ascorbic Acid; McGuff Pharmaceuticals, Santa Ana, CA) divided into three doses from day +1 through day +14 and transitioned to oral vitamin C 500 mg twice daily from day +15 through day +180 (Figure S1). Vitamin C was given in 50 mL of 5% dextrose and water over 30 min every 8 h in UV-light-protected bags and tubing. Subsequently, consecutive patients meeting inclusion criteria were enrolled in the trial till study completion (N = 55). Patients in the vitamin C and historical control cohorts received rabbit antithymocyte globulin (ATG) as a part of the GVHD prophylaxis regimen predominantly on days −3 to −1 in the historical control and days −9 to −7 in the vitamin C recipients

The primary endpoint was NRM at 1 year; Simon's two-stage Minimax design was utilized for this study. In the first stage, after 14 patients became evaluable, a threshold of 3 or more patients experiencing NRM was set to result in study termination for futility; otherwise, the study would continue. The total sample size calculated for Simon's two-stage design was 55 patients for a reduction in NRM from 35% to a null-hypothesized value 20% (which would achieve a desired 15% absolute reduction or a 42% relative reduction). We reject the null hypothesis if there are 13 or fewer NRMs in the total 55 patients.

For this analysis, comparing clinical outcomes between the study cohort and a set of historical control patients, propensity score matching was performed to help balance select covariates between groups. The study team conducted chart review audits to identify similarly treated patients for the historical control cohort. To accomplish the matching, a logistic regression model was fit with an intervention indicator as the binary outcome (intervention vs. historical control), against three matching variables important in contextualizing allogeneic SCT recipients, which include diagnosis (ALL, AML, and CML + MDS), conditioning regimen (busulfan + cyclophosphamide, fludarabine + melphalan, and total body irradiation + cyclophosphamide), and CIBMTR disease risk index (high and intermediate/low). The resulting probabilities of belonging to the intervention group were used as propensity scores, with nearest neighbor matching of those scores used to identify the matching set of historical control subjects in a 1:1 ratio with the trial enrollees.

A Kaplan–Meier step function was performed for mortality assessment, while cumulative incidence curves were constructed for relapse (accounting for competing risk of nonrelapse mortality), acute GVHD, chronic GVHD (accounting for competing risks of relapse and mortality), and NRM (accounting for relapse). Cox proportional hazard models were used to estimate adjusted hazard ratios between the time-to-event outcomes and group, adjusted for patient age, donor type, stem cell source, disease type, conditioning regimen, and disease risk. The R (4.1.2) and RStudio (version 2022.02.0) statistical software platforms were used for all data management and analysis.

Patients were prospectively enrolled in this study between October 2018 and October 2021 and propensity scores matched with historical controls transplanted between 2015 and 2018. Follow-up was updated as of February 2023. Patient characteristics are described in Table S1; 55 patients received IV vitamin C, including 10/10 HLA-MRD and MUD (n = 48) and 9/10 HLA MUD recipients (n = 7; 4 HLA-DQ, 2 HLA-B and 1 HLA-A mismatch).

A safety lead-in cohort of 14 patients was initially enrolled. All patients enrolled were deficient in vitamin C at day 0, with a median level of 0.3 mg/dL (range: 0.1–0.5). Safety endpoints of lack of myeloid engraftment, grade 3 acute GVHD and NRM were evaluated in these patients, without triggering any of the stopping thresholds.

In the entire cohort of patients treated with Vitamin C (N = 55), times to neutrophil and platelet engraftment were 11 days (range: 9–15 days) and 12 days respectively (8–21 days). T-cell chimerism at days 30, 60, and 90 following HSCT was > 95% in the vitamin C group.

Clinical outcomes were generally improved in vitamin C recipients. NRM (at any time) in the vitamin C-treated group (11%) was nominally lower compared to the historical control (25%) (HR = 0.4, 95% CI: 0.1, 1.0, p-value = 0.069) (Figure 1A and B), and while overall survival tended toward improvement (HR = 0.5, 95% CI: 0.2, 1.0,

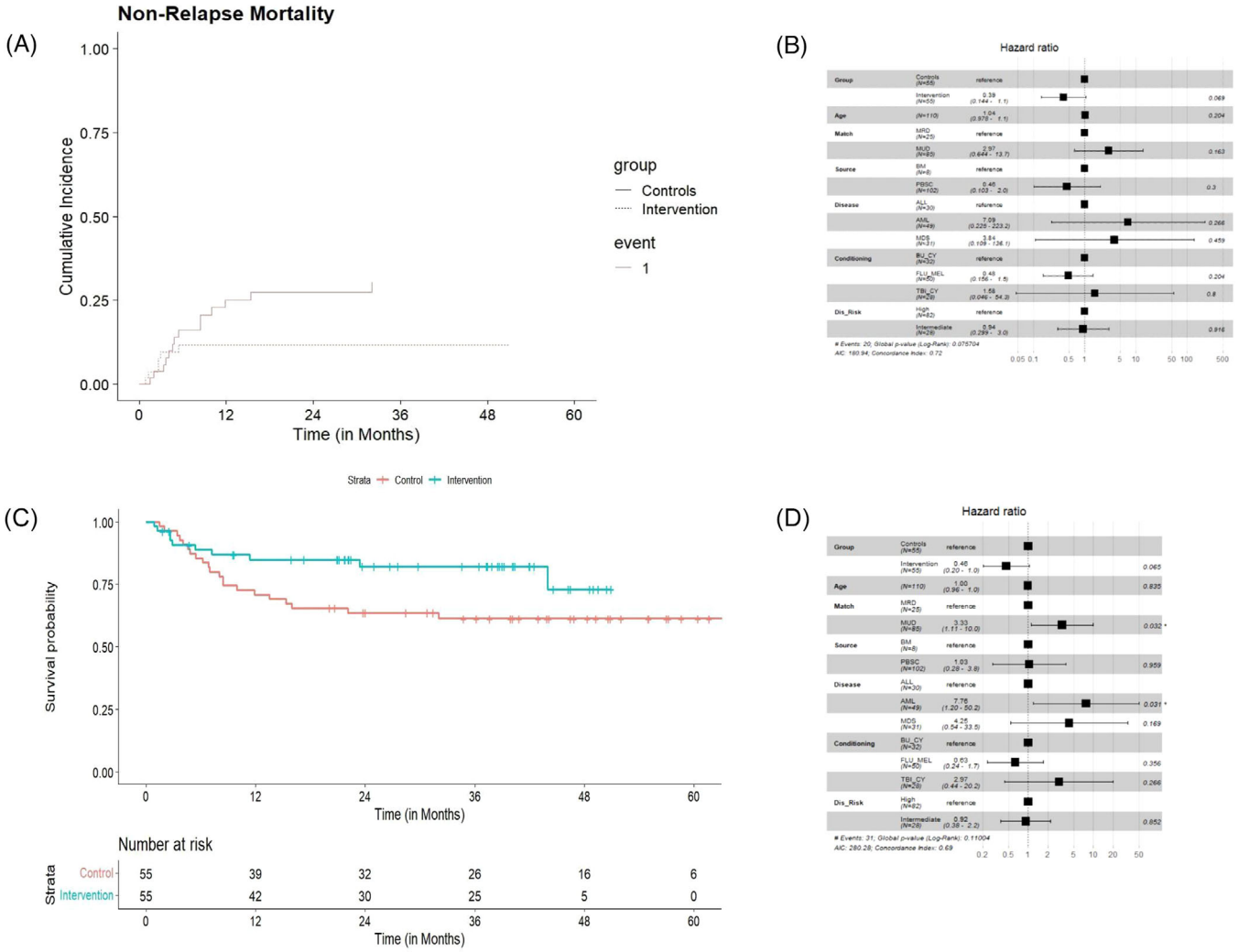

FIGURE 1 (A) Cumulative incidence curves depicting NRM in the VC-treated patient. (B) Multivariate model. (C) KM curves depicting improved survival in the VC-treated patients ($p = 0.057$). (D) Multivariate model, only donor type and diagnosis were significant.

p-value = 0.065) (Figure 1C and D), neither results achieved statistical significance in this small varied cohort of patients.

No difference was observed in the risk of acute GVHD in the control versus study cohorts (grade II–IV, (33% vs. 33%) *p*-value = 0.81 and grade III and IV, (24% vs. 17%) *p*-value = 0.35). Moderate to severe chronic GVHD rate when accounting for competing risks of relapse and mortality was lower in the Vitamin C group (11% vs. 24%) (adj HR = 0.60, 95% CI: 0.20, 1.76, *p*-value = 0.352) (Figure S2). Relapse incidence in the two cohorts was not different (24 vs. 22%, *p* value = 0.9).

CMV reactivation (> 200 IU/mL) was not different between historical controls (36%) and intervention cohort (24%; *p*-value = 0.35), nor EBV reactivation (> 500 IU/mL and requiring Rituximab) (33% vs. 35%; *p*-value = 0.88). There were no grade 3–5 adverse events attributable to vitamin C therapy in this trial, particularly no nephrotoxicity or renal calculi were observed (Table S2).

Vitamin C level (normal 0.4–2.0 mg/dL) was universally low prior to intervention and was restored to normal by day 14 (Figure 2), while CRP (normal 0–0.5 mg/dL) rose in the early aftermath of transplant following conditioning and engraftment, and came down later.

In this paper, the results of a prospective trial evaluating parenteral vitamin C in allogeneic HCT are presented, demonstrating the feasibility of its administration following allogeneic HCT. While there were nominal improvements in NRM and survival, there was no statistical evidence of differences between the study cohort and historical controls. These observations support the hypothesis of vitamin C associated benefit that merits being tested in a larger cohort of patients.

NRM diminishes the benefit that comes from the immune graft versus malignancy effect in HCT. The origin for NRM is in the initial mucosal and endothelial injury seen following high-dose therapy, which promotes alloantigen presentation to donor cells, setting up alloimmune response culminating in GVHD [11, 12]. Proinflammatory cytokines released following tissue injury from high-dose therapy is a major contributor to the pathophysiology in these instances [13]. Further, due to the oxidative stress of pretransplant conditioning with

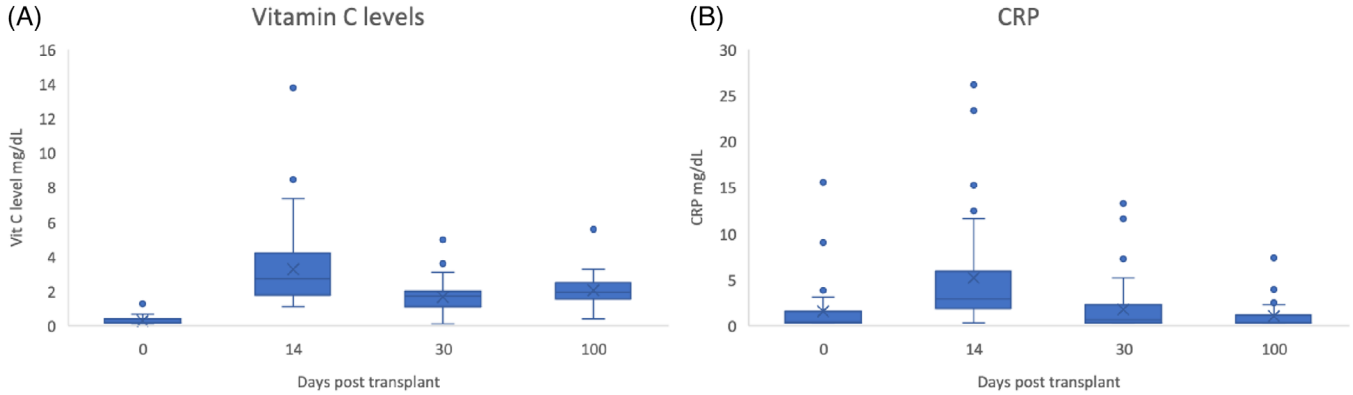

FIGURE 2 (A) Vitamin C levels in the entire cohort of patients. (B) CRP levels at the same time points.

radiation and alkylating agents [14, 15], followed by poor oral intake, nutrient levels, particularly vitamin C stores are rapidly depleted [16], leading to a severely deficient state [11, 17, 18]. Given this logic, the clinical trial reported here was designed to rapidly restore vitamin C following transplantation and utilize its endothelial stabilizing effect to reduce the impact of mucosal and endothelial injury post-transplant [19, 20]. The findings reported support the hypothesis that vitamin C administration may lower the risk for NRM with possible downstream benefit in survival, providing rationale for future studies of this inexpensive and readily available agent in HCT.

A reduction in inflammation may increase the risk of infections. Infections were seen in the vitamin C recipients, but not at a higher rate than the matched historical controls, nevertheless given a reduction in the inflammatory response, this bears close observation in future trials, as was suggested by a randomized trial of vitamin C administration in patients with early sepsis [21]. Such a trial, of necessity, must be randomized, and optimally conducted in patients who do not receive T-cell depletion (in vivo or ex vivo).

An inherent limitation of our approach is the use of a historical control group for comparison with our study cohort. The risks of using historical data are known, as such we contextualize our results as hypothesis formulating. To help overcome any structural differences between the two cohorts, we used propensity score matching to select a set of historical control patients whose aggregate covariate profile better matched the study cohort. While sample sizes were relatively small, the matching process did improve the comparability of the covariate distributions between groups.

If vitamin C repletion following high-dose therapy and HSCT is proven to be of benefit in patients undergoing T-cell replete allografts, it will be a significant advance on a global scale, particularly in economically challenged regions, given its low cost and wide availability. Therefore, our study demonstrating the absence of increased toxicity and the potential favorable impact on NRM represents a crucial first step in that direction.

AUTHOR CONTRIBUTIONS

Gary Simmons: Develop and conduct study; collect and analyze data and write paper. **Roy Sabo**: Develop and conduct study; analyze data and write paper. **Rehan Qayyum**: Evaluate and analyze data and write paper. **May Aziz**: Conduct study. **Erika Martin**: Conduct study. **Robyn Bernard**: Collect data. **Manjari Sriparna**: Collect data and write paper; **Cody McIntire**: Collect data and write paper. **Elizabeth Krieger**: Review and write paper. **Donald Brophy**: Conduct study and write paper. **Ramesh Natarajan**: Develop study and write paper. **Alpha Fowler III**: Conceptualize and develop study and write paper. **Catherine H. Roberts**: Develop and conduct study and collect and analyze data. **Amir Toor**: Conceptualize; develop; conduct study; collect; analyze data and write paper.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

Data will be made available for all reasonable requests.

ETHICS STATEMENT

The patients consented to participate in this prospective, IRB and US-FDA approved IND trial.

PATIENT CONSENT STATEMENT

All patients signed informed consent and were treated at VCU (NCT03613727).

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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REFERENCES

1. Ferry C, Gérard S. Busulfan-cyclophosphamide versus total body irradiation-cyclophosphamide as preparative regimen before

allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia: what have we learned? Exp Hematol. 2003;31:1182–86.
2. Lai HY, Chou TY, Cheng Hwai T, Oscar Kuang Sheng L. Cytokine profiles in various graft-versus-host disease target organs following hematopoietic stem cell transplantation. Cell Transplant. 2012;21:2033–45.
3. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. J Clin Oncol. 2017;35:1154–61.
4. Chae YS, Sohn S-K, Kim JG, Yy C, Moon JH, Hj S, et al. New myeloablative conditioning regimen with fludarabine and busulfan for allogeneic stem cell transplantation: comparison with BuCy2. Bone Marrow Transplant. 2007;40(6):541–47.
5. Molina N, Morandi AC, Bolin AP, Otton R. Comparative effect of fucoxanthin and vitamin C on oxidative and functional parameters of human lymphocytes. Int Immunopharmacol. 2014;22.
6. Kuck JL. Ascorbic acid attenuates endothelial permeability triggered by cell-free hemoglobin. Biochem Biophys Res Commun. 2018;495:433–37.
7. Fowler AA. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. J Am Med Assoc. 2019;322:1261–70.
8. Nannya Y, Shinohara A, Ichikawa M, Kurokawa M. Serial profile of vitamins and trace elements during the acute phase of allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20:430–34.
9. Dahl GB, Jeppsson RI, Tengborn HJ. Vitamin stability in a Tpn mixture stored in an eva plastic bag. J Clin Pharm Ther. 1986;11:271–79.
10. Rasheed M, Simmons GL, Fisher BJ, Leslie K, Reed J, Roberts C, et al. Reduced plasma ascorbic acid levels in recipients of myeloablative conditioning and hematopoietic cell transplantation. Eur J Haematol. 2019;103:329–34.
11. Ferrara JLM, Chaudhry MS. GVHD: biology matters. Blood Adv. 2018;2(22):3411–17.
12. Teshima T, Reddy P, Zeiser R. Acute graft-versus-host disease: novel biological insights. Biol Blood Marrow Transplant. 2016;22(1):11–16.
13. Piper C, Drobyski WR. Inflammatory cytokine networks in gastrointestinal tract graft vs. host disease. Front Immunol. 2019;10:163.
14. Wang W, Hu L, Chang S, Ma L, Li X, Yang Z, et al. Total body irradiation-induced colon damage is prevented by nitrate-mediated suppression of oxidative stress and homeostasis of the gut microbiome. Nitric Oxide. 2020;102:1–11.
15. Hu L, Yin X, Zhang Y, Pang A, Xie X, Yang S, et al. Radiation-induced bystander effects impair transplanted human hematopoietic stem cells via oxidative DNA damage. Blood. 2021;137(24):3339–50.
16. Chevion S, Or R, Berry EM. The antioxidant status of patients subjected to total body irradiation. Biochem Mol Biol Int. 1999;47(6):1019–27.
17. Umegaki K, Sugisawa A, Shin SJ, Yamada K, Sano M. Different onsets of oxidative damage to DNA and lipids in bone marrow and liver in rats given total body irradiation. Free Radic Biol Med. 2001;31(9):1066–74.
18. Saga R, Uchida T, Takino Y, Kondo Y, Kobayashi H, Kinoshita M, et al. Radiation-induced gastrointestinal syndrome is exacerbated in vitamin C-insufficient SMP30/GNL knockout mice. Nutrition. 2021;81:110931.
19. Fujii J, Osaki T, Bo T. Ascorbate is a primary antioxidant in mammals. Molecules. 2022;27(19):6187.
20. Kuck JL, Bastarache JA, Shaver CM, Fessel JP, Dikalov SI, May JM, et al. Ascorbic acid attenuates endothelial permeability triggered by cell-free hemoglobin. Biochem Biophys Res Commun. 2018;495(1):433–37.
21. Lamontagne F, Masse MH, Menard J, Sprague S, Pinto R, Heyland DK, et al. Intravenous vitamin C in adults with sepsis in the intensive care unit. N Engl J Med. 2022;386(25):2387–98.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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