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TRANSPLANTATION

Vorinostat plus tacrolimus/methotrexate to prevent GVHD after myeloablative conditioning, unrelated donor HCT

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Key Points

- Grade 2 to 4 acute GVHD in URD HCT patients who received vorinostat and tacrolimus/methotrexate after myeloablative conditioning was 22%.
- HDAC inhibition with vorinostat shows potential efficacy for GVHD prevention and should be investigated in a randomized phase 3 trial.

The oral histone deacetylase (HDAC) inhibitor (vorinostat) is safe and results in low incidence of acute graft-versus-host disease (GVHD) after reduced-intensity conditioning, related donor hematopoietic cell transplantation (HCT). However, its safety and efficacy in preventing acute GVHD in settings of heightened clinical risk that use myeloablative conditioning, unrelated donor (URD), and methotrexate are not known. We conducted a prospective, phase 2 study in this higher-risk setting. We enrolled 37 patients to provide 80% power to detect a significant difference in grade 2 to 4 acute GVHD of 50% compared with a reduction in target to 28%. Eligibility included adults with a hematological malignancy to receive myeloablative HCT from an available 8/8-HLA matched URD. Patients received GVHD prophylaxis with tacrolimus and methotrexate. Vorinostat (100 mg twice daily) was started on day −10 and continued through day +100 post-HCT. Median age was 56 years (range, 18-69 years), and 95% had acute myelogenous leukemia or high-risk myelodysplastic syndrome. Vorinostat was safe and tolerable. The cumulative incidence of grade 2 to 4 acute GVHD at day 100 was 22%, and for grade 3 to 4 it was 8%. The cumulative incidence of chronic GVHD was 29%; relapse, nonrelapse mortality, GVHD-free relapse-free survival, and overall survival at 1 year were 19%, 16%, 47%, and 76%, respectively. Correlative analyses showed enhanced histone (H3) acetylation in peripheral blood mononuclear cells and reduced interleukin 6 ($P = .028$) and GVHD biomarkers (Reg3, $P = .041$; ST2, $P = .002$) at day 30 post-HCT in vorinostat-treated subjects compared with similarly treated patients who did not receive vorinostat. Vorinostat for GVHD prevention is an effective strategy that should be confirmed in a randomized phase 3 study. This trial was registered at www.clinicaltrials.gov as #NCT01790568. (*Blood*. 2017;130(15):1760-1767)

Introduction

Allogeneic hematopoietic cell transplantation (HCT) outcomes have improved in recent years.[1-4] However, acute graft-versus-host disease (GVHD) remains a significant cause of morbidity and mortality.[2] Despite standard immunosuppressive prophylaxis, acute GVHD develops in 50% to 70% of the patients receiving allogeneic HCT from unrelated donors (URDs),[5-11] and GVHD accounts for 13% to 16% of the mortality in this type of HCT.[10,12] The incidence of severe (grade 3-4) acute GVHD after myeloablative, non–total-body irradiation (TBI)–based conditioning, URD HCT is as high as 32% at 100 days after transplant[13] and carries a 75% to 100% mortality rate.[5]

Vorinostat is a histone deacetylase (HDAC) inhibitor that is approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma.[14] At lower nontoxic concentrations, it has anti-inflammatory and immunoregulatory effects.[15,16] In preclinical models of acute GVHD, vorinostat reduced the GVHD rate, suppressed proinflammatory cytokines, regulated antigen-presenting cells, and enhanced regulatory T-cell function.[17,18] In a phase 1/2 clinical trial of vorinostat added to standard GVHD prophylaxis in patients receiving a reduced-intensity conditioning allogeneic HCT from a related donor, the rates of grade 2 to 4 and 3 to 4 acute GVHD at day 100 were 22% and 6%, respectively.[19] Given that most patients lack a suitable related donor and that the risks for GVHD and its related mortality are increased after URD HCT, we sought to expand the use of vorinostat in GVHD prevention to this higher-risk population and to determine whether a similar reduction in the incidence of acute GVHD can be achieved in the setting of URD HCT. Moreover, the effect of vorinostat combined with tacrolimus and methotrexate (standard GVHD prophylaxis) is not known. Therefore, we conducted a phase 2 clinical trial of oral vorinostat with standard GVHD prophylaxis in patients undergoing myeloablative conditioning URD HCT.

Methods

Study design

This was a single-center prospective phase 2 clinical trial to evaluate the feasibility of vorinostat combined with tacrolimus and methotrexate for GVHD

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prophylaxis after URD HCT. The protocol was approved by the Michigan Medicine Institutional Review Board (UMCC 2012.047; HUM00070080). All participants gave written informed consent. This trial was registered with ClinicalTrials.gov, number NCT01790568.

Inclusion criteria

Eligible patients were aged 18 to 75 years, diagnosed with hematologic malignancy, and had a Karnofsky performance score of ≥70% and life expectancy of >6 months and were candidates for myeloablative URD HCT. An 8/8 HLA allelic match between the URD and the recipient at HLA-A, HLA-B, HLA-C, and HLA-DRB1 by high-resolution typing was required. The graft source could be bone marrow or peripheral blood stem cells. Patients undergoing a TBI-based conditioning regimen (TBI 1200 cGy) with a history of prolonged QTc syndrome, or who had prior treatment with an HDAC inhibitor (ie, valproic acid) within 30 days, were excluded from this study.

Treatment

Patients received a myeloablative conditioning regimen consisting of intravenous fludarabine 160 mg/m^2 (40 mg/m^2/day on days −5 through −2) and intravenous busulfan 12.8 mg/kg (3.2 mg/kg/day on days −5 through −2), followed by an infusion of stem cells on day 0. GVHD prophylaxis consisted of tacrolimus (0.03 mg/kg/day intravenous or 0.045 mg/kg/day as an oral dose, to a goal level of 8 to 12 ng/mL, starting on day −3) and methotrexate (5 mg/m^2/dose once daily, intravenously, on days +1, +3, +6, and +11). In the absence of GVHD, tacrolimus tapering started on day +100 and discontinued on day +180 after transplant. Vorinostat was started on day −10 at a dose of 100 mg orally twice daily and continued until day 100 after transplant. Vorinostat dose delays or modification were approved according to the study protocol. Posttransplantation supportive care treatment was provided according to institutional guidelines. If clinically significant acute GVHD occurred, the patient was treated according to the standard of care under the institutional blood and marrow transplantation program clinical practice guidelines (eg, 2 mg/kg/day of methylprednisolone), and treatment with vorinostat was continued at the treating physician's discretion.

Outcomes assessment

The primary endpoint of this trial was the incidence of grades 2 to 4 acute GVHD by day 100 posttransplant. Acute GVHD grade was scored weekly according to the modified Glucksberg criteria.[20] Secondary outcomes included the safety and feasibility of vorinostat administration, incidence of infection (by day 30, 100, and 180), and relapse, nonrelapse mortality (NRM), overall survival, chronic GVHD requiring systemic therapy, and GVHD-free relapse-free survival (GRFS) at 1 year. Feasibility was defined as the successful administration of 60% or more of the planned vorinostat dose between days −10 to day 30. Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.01, and the likelihood of these events being related to vorinostat was determined. Dose modifications were designed with stringent criteria to avoid adverse toxicities.[18] Supportive care therapies, including anti-infectives and blood product transfusions, were administered according to institutional guidelines. Neutrophil engraftment was defined as the first of 3 consecutive days in which the absolute neutrophil count was ≥500 cells/μL, and for platelet engraftment, as the first of 3 consecutive days in which the platelet count was ≥ 20 000 cells/μL without transfusion of platelets in the preceding 3 days. Primary engraftment failure was defined as an inability to achieve absolute neutrophil count ≥500 cells/μL within 28 days posttransplant. GRFS is a new composite endpoint that represents ideal post-HCT recovery (ie, absence of grade 3 to 4 acute GVHD, systemic therapy-requiring chronic GVHD, relapse, or death in the first year post-HCT).[21] Participants who experienced any of these events in the first year post-HCT were censored for the GRFS endpoint on the date of diagnosis or occurrence of the first qualifying event.

Correlative studies

The effect of vorinostat in vivo was evaluated by correlative laboratory studies of patients' samples. Heparinized blood samples were collected coincident with routine blood draws on the participants. All samples were processed, frozen, and banked in the Core Immunology Laboratory at Michigan Medicine. Samples were batched and run simultaneously to maximize efficiency and enhance comparison of samples. For controls, cryopreserved blood samples were collected from 5 healthy donors and 36 historic patients who underwent URD HCT with the same myeloablative conditioning and who received tacrolimus and methotrexate for GVHD prophylaxis without vorinostat. Day 30 blood samples of study and control patients were analyzed.

Histone acetylation in peripheral blood mononuclear cells (PBMCs) was assessed by western blot. PBMCs were thawed, and whole-cell lysates were prepared. Protein concentrations were determined using the Pierce BCA Protein Assay (Thermo Scientific, Waltham, MA). Equal amounts of protein were separated by NuPAGE Novex 4% to 12% Bis-Tris Gel (Invitrogen, Carlsbad, CA) and subsequently transferred to nitrocellulose membrane. Primary antibodies to acetylated-histone 3 (Lys9/Lys14) and acetylated-histone 4 (Lys5) (both at 1:1000; Cell Signaling Technology, Danvers, MA) and β-actin (AC-15) (1:2500; Sigma-Aldrich, St. Louis, MO) were used to analyze the membranes. Horseradish peroxidase-conjugated anti-rabbit (1:3000; Santa Cruz Biotechnology, Dallas, TX) and anti-mouse (1:6000; Santa Cruz Biotechnology, Dallas, TX) antibodies were used as secondary antibodies and incubated for 1 hour at room temperature. Immunoreactive bands were developed with the Pierce ECL system (Thermo Fisher Scientific, Waltham, MA). Densitometry analysis was performed by ImageJ software (Ver 1.46R). Data for the acetylated-histones were adjusted for concentration relative to the β actin bands. Concentrations of interferon γ, interleukin 1β (IL-1β), IL-2, IL-6, IL-8, IL-10, tumor necrosis factor α, suppression of tumorigenicity-2 (ST2),[22] soluble IL-2 receptor α, soluble tumor necrosis factor receptor 1, chemokine (C-X-C motif) ligand 9, and regenerating islet-derived 3-α (Reg3α)[23] were measured in plasma, using Luminex multiplex assay kits (Invitrogen, Carlsbad, CA). Assays were performed according to the manufacturer's protocol. Samples were diluted to fall within the linear range of the standard concentration curve of the assay. Samples and standards were assayed in duplicate. Plates were read at 450 nm by using a microplate reader (Bio-Rad, Hercules, CA).

Statistical analysis

This study was designed to enroll 37 patients who would be assessable for the clinical outcomes. The incidence of grade 2 to 4 acute GVHD in 154 comparable patients treated at the University of Michigan between 2005 and 2011 who received tacrolimus and methotrexate GVHD prophylaxis after URD HCT with similar preparative regimen was 48% at day 100. A sample size of 37 total patients gave us 80% power to test the hypothesis that the addition of vorinostat would reduce the incidence of grade 2 to 4 acute GVHD at day 100 from 48% to 28% or lower, assuming a type I error of 5%. With 37 patients, a 95% confidence interval for the incidence of grade 2 to 4 GVHD by day 100 posttransplant will have a width of no more than ±16 points. Overall survival was estimated using the Kaplan-Meier method, which was determined from the date of transplantation to either death from any cause or last date/end of follow-up, whichever occurred first. We used the methods of Fine and Gray[24] to estimate the cumulative incidence of relapse, NRM, acute and chronic GVHD, and GRFS. Relapse and NRM were competing risks for each other, and relapse and death were competing risks for GVHD. Laboratory correlative samples from the study participants and control cohort were compared using the Mann-Whitney nonparametric test and GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA). A 2-sided *P*-value of <.05 indicated statistical significance.

Results

Between 1 June 2013 and 31 July 2016, 38 patients were enrolled into this single-center prospective phase 2 clinical trial. One patient experienced progression of his hematologic malignancy and did not proceed to transplantation, and therefore was removed from the study. Thirty-seven patients were included in the final analysis for the study endpoints. Table 1 depicts baseline patient demographics and clinical characteristics. The median age of study participants was 56 years (range, 18-69 years). Eighteen participants (49%) were male. The

Table 1. Patient demographics and clinical characteristics

Demographics	Participants, n = 37 (%)
Recipient age, years (range)	56 (18, 69)
Sex	
Male	18 (48.6)
Female	19 (51.4)
Race/ethnicity	
White non-Hispanic	33 (82.9)
White, Hispanic	2 (5.4)
Asian	1 (2.7)
Not reported	1 (2.7)
Clinical characteristics	
Diagnosis	
Acute myelogenous leukemia	27 (73.0)
Myelodysplastic syndrome	8 (21.6)
Chronic myelogenous leukemia	1 (2.7)
Acute undifferentiated leukemia	1 (2.7)
Disease status	
Low	21 (56.8)
Intermediate	9 (24.3)
High	7 (18.9)
Comorbidity index	
Low	10 (27.0)
Intermediate	12 (32.4)
High	15 (40.5)
Donor	
Matched-unrelated (10/10)	32 (86.4)
DQ-mismatched (9/10)	5 (13.5)
CMV status	
R^+, D^+	11 (29.7)
R^-, D^+	3 (8.1)
R^+, D^-	5 (13.5)
R^-, D^-	18 (48.6)
CD34 count (10^6 cells/kg)	5.0 (1.5, 10)
Engraftment day	
Neutrophil	12 (11, 23)
Platelet	12 (9, 35)

DQ, HLA-DQ.

majority of participants (83%) identified their race/ethnicity as white non-Hispanic, consistent with the demographic typically treated at our institution. Seventy-three percent of participants had a diagnosis of acute myelogenous leukemia (AML), and 22% were diagnosed with myelodysplastic syndrome (MDS). Nearly 20% of participants had high-risk disease, classified according to the American Society for Blood and Marrow Transplantation RFI 2016 Disease Classifications.[25] More than two-thirds of the participants had an intermediate or high comorbidity index.[26] A median of 5.0×10^6 cells/kg from donor bone marrow (n = 17, 46%) or peripheral blood (PBMC, n = 20, 54%) were infused into matched (8/8 HLA-A, HLA-B, HLA-C, and HLA-DRB1, n = 32, 86%) or DQ-mismatched (9/10, n = 5, 14%) URD HCT recipients.

Feasibility and safety

Thirty-six (97%) of the 37 patients met the feasibility criteria and received 60% or more (median, 98.8%; range, 51%-100%) of the planned vorinostat daily dose between days −10 and +30. The median duration on vorinostat for the entire study was 106 days (range, 39-111 days). Overall, the participants received a median of 73% (range, 37%-100%) of the twice-daily dose of vorinostat between day −10 and day 100. Dose modification was required (range, 1-67 days' duration) in 70% of the patients, and withholding vorinostat was required in 65% of the patients (range, 1-59 days). Per the a priori design of the study protocol, laboratory findings of a low platelet count, the presence of gastrointestinal (GI) symptoms (nausea, vomiting, and diarrhea), or elevated creatinine or bilirubin were the leading triggers of dose modifications. No deaths were related or attributable to vorinostat treatment. The safety profile was consistent with our previous study.[18] No drug-related toxic effects arose that warranted discontinuation of vorinostat.

Engraftment

Neutrophils and platelets engrafted a median of 12 days (range, 11-23 days) and 12 days (range, 9-35 days) after HCT, respectively. There were no cases of primary or secondary graft failures. Chimerism data at day 30 was available for 20 patients, with all of them demonstrating 100% donor CD33 cells. The median CD3 chimerism at day +30 was 69% (range, 22%-100%). The median donor CD3 chimerism increased to 79% (range, 29%-100%) at day 100 in the 25 patients' samples available. Donor CD33 chimerism at day 100 was 100% in 23 of the 25 available samples. Two patients demonstrated decreased donor CD33 chimerism at day 100 related (or associated with) relapse of primary malignancy.

Acute GVHD

The primary endpoint of this study was the incidence of acute GVHD grade 2 to 4 at day 100; this occurred in 8 of 37 patients (22%; 95% CI, 11%-39%), with a median time to onset of 32 days (range, 19-48 days) (Table 2 and Figure 1A). Five participants developed grade 2 acute GVHD, and 3 (8%; 95% CI, 3%-23%) developed grade 4 acute GVHD by day 100. The 3 patients who developed grade 4 acute GVHD died with death attributed to GVHD. Six of the 8 patients developed skin involvement, 4 of 8 had GI involvement, and 3 of 8 had liver involvement at day 100 (Table 3). The low incidence of acute GVHD persisted through day 180 (22%; 95% CI, 11%-39%) more than 2 months after cessation of vorinostat. At 1 year, the incidence of acute GVHD was 32% (95% CI, 19%-51%) because of the development of 3 cases of only GI acute GVHD after day 180. Among these, 1 participant developed late-onset grade 1 GI GVHD on day 184 and 2 participants developed grade 2 GI GVHD after discontinuation of immunosuppression.

Relapse and survival

The cumulative incidence of relapse at 1 year was 19% (95% CI, 9%-36%; Figure 1B). The median time to relapse was 94 days (range, 61-916 days) posttransplant. NRM incidence at 1 year was 16% (95% CI, 7%-34%; Figure 1C). The median time to death from any cause was 146 days (range, 51-939 days). Ten patients died during the study or follow-up period (Table 4). Four participants died after relapse, 3 as a result of GVHD-related complications, 1 as a result of failure to thrive, and 1 as a result of respiratory failure secondary to infection. One patient died because of previously known interstitial pulmonary fibrosis. Median follow-up was 368 days

Table 2. Incidence of GVHD

	Day 100	Day 180	1 y
Acute GVHD, % (95% CI)			
Grade 2-4	22 (11-39)	22 (11-39)	32 (19-51)
Grade 3-4	8 (3-23)	11 (4-27)	18 (8-36)
Skin*	16 (8-33)	16 (8-33)	20 (10-37)
Liver	8 (3-23)	8 (3-23)	8 (3-23)
Gut	11 (4-27)	14 (6-30)	24 (12-43)
Chronic GVHD, % (95% CI)†			29 (15-50)

*Skin GVHD includes grades 2 to 4.
†Chronic GVHD requiring systemic therapy.

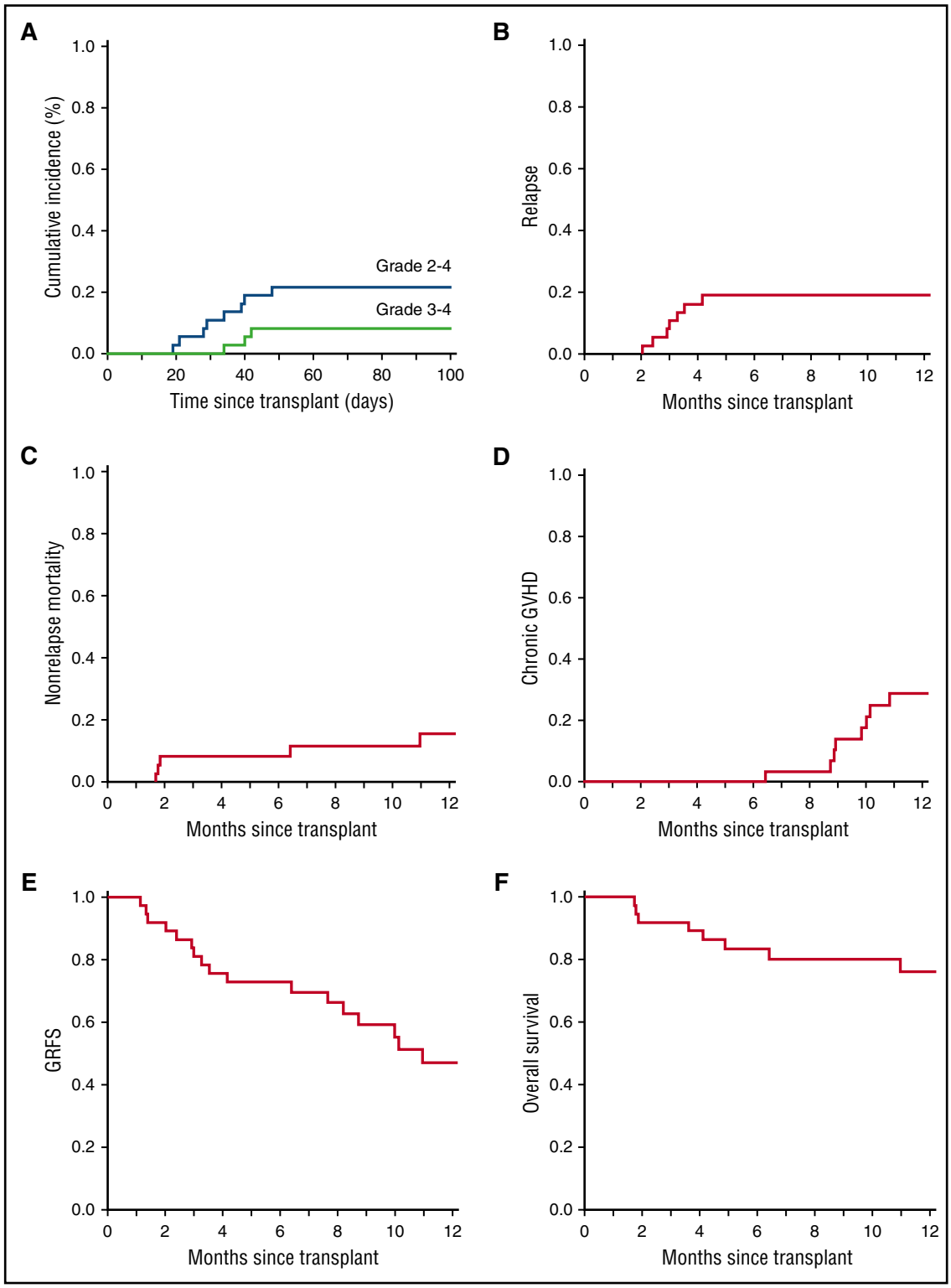

Figure 1. Acute GVHD, relapse, NRM, chronic GVHD, GRFS, and overall survival. Cumulative incidence (%) of (A) grade 2 to 4 and grade 3 to 4 acute GVHD at day 100, the primary endpoint of this study, (B) relapse, (C) NRM, (D) chronic GVHD, (E) GRFS, and (F) overall survival at 1 year.

(range, 51-1275 days) with an overall survival of 76% at 1 year posttransplant (95% CI, 63%-92%; Figure 1D).

Infections, chronic GVHD, and GRFS

Infection events were monitored for the first 180 days after HCT (Table 5). Eighteen infections were noted in 11 patients. Infections included 4 cases of gram-positive bacteremia, 8 cases of gram-negative bacteremia, 3 cases of cytomegalovirus (3 reactivation), and 3 fungal infections. The cumulative incidence of chronic GVHD requiring systemic treatment with corticosteroids at 1 year was 29% (95% CI, 15%-50%; Figure 1E), which resulted in a GRFS of 47% (95% CI, 32%-69%; Figure 1F).

Table 3. Maximum GVHD stage by organ involvement

	Day 100			Day 365		
	Skin	Liver	Gastrointestinal tract	Skin	Liver	Gastrointestinal tract
Maximum stage						
0	3	5	4	2	0	0
1	1	1	2	1	0	1
2	2	0	1	0	0	2
3	1	2	0	0	0	0
4	2	0	1	0	0	0

Correlative studies by assessment of histone acetylation

Acetylated histone 3 levels at day 30 post-HCT were significantly higher in PBMCs from study patients who received vorinostat compared with patients who were similarly transplanted but did not receive vorinostat. These data (Figure 2A) support in vivo HDAC inhibition by vorinostat.[27] Patients treated with vorinostat also demonstrated lower plasma levels of the cytokine IL-6 (median, 4.2 vs 7.6 pg/mL; $P = .028$) compared with patients who were not treated with vorinostat on day 30 (Figure 2B), which is consistent with attenuated systemic inflammation. Concentrations of plasma IL-1β, IL-2, IL-8, interferon γ, tumor necrosis factor α, soluble IL-2 receptor α, soluble tumor necrosis factor receptor 1, and chemokine (C-X-C motif) ligand 9 were not found to be significantly different between study patients who received vorinostat compared with patients who did not receive vorinostat 30 days after HCT (data not shown). Biomarkers of GVHD, Reg3α[23] (median, 4646 pg/mL vs 5554 pg/mL; $P = .041$; Figure 2C), and ST2[22] (6804 pg/mL vs 17 734 pg/mL; $P = .0017$; Figure 2D) were also significantly lower in the plasma of vorinostat-treated patients day 30 post-HCT compared with patients who were not treated with vorinostat.

Discussion

In this phase 2 study of oral vorinostat administered in combination with standard GVHD prophylaxis with tacrolimus and methotrexate in patients receiving myeloablative conditioning URD HCT, we show the strategy to be safe and feasible. These findings build on our previous report of the use of vorinostat in patients receiving matched related donor HCT.[19] All patients achieved sustained engraftment while taking oral vorinostat in combination with standard immunosuppression, and no cases of graft failure were reported. Inhibition of histone deacetylases also did not appear to augment the risk for infection after transplant. No unexpected adverse reactions were observed and were similar to those reported elsewhere,[14] as well as in our previous study.[27]

Although limited by the single-arm design, we are encouraged by the low incidence of acute GVHD observed in recipients of myeloablative URD HCT. Jagasia et al recently reported day 100 incidence of grade 2 to 4 acute GVHD of 50% to 60%, after myeloablative, non-TBI-based conditioning URD transplant with bone marrow cells or PBSC and prophylaxis with calcineurin inhibitor (tacrolimus or cyclosporine) plus methotrexate.[13] In a study of HCT for AML, in which 69% of 8/8 URD HCT recipients received tacrolimus and methotrexate-based GVHD prophylaxis, Saber et al reported a 51% incidence of grade 2 to 4, and a 25% incidence of grade 3 to 4, acute GVHD at day 100.[10] Our finding of 22% incidence of grade 2 to 4 and 8% grade 3 to 4 acute GVHD at day 100 compares favorably with these studies, as well as with seminal studies of GVHD prophylaxis with tacrolimus and methotrexate, which reported 42% to 49% incidence of grade 2 to 4, and 12% to 18% grade 3 to 4, acute GVHD at day 100 after URD HCT.[6,8] Furthermore, we observed chronic GVHD of only 29% of the patients who required systemic therapy at 1 year, which compares favorably with estimates of 45% to 48% reported in other studies.[6,10] It is possible that our low incidence of chronic GVHD could be influenced by the increased use of bone marrow cells as the donor source compared with historical use of peripheral blood stem cells.[11] We recognize, however, that randomized controlled trials are warranted to confirm the efficacy of vorinostat.

The therapeutic potential of allogeneic HCT relies on the graft-versus-leukemia effect to eradicate residual malignant disease. Although strategies that mitigate GVHD can result in increased incidence of relapse, we did not observe a rise in relapse, consistent with our previous observations in patients taking vorinostat after matched related donor HCT.[27] We recorded a relapse incidence of 19% (95% CI, 9%-36%) at 1 year in a cohort with predominantly AML/MDS diagnosis (95%), which is commensurate with the 20% to 40% reported in a recent registry study examining AML relapse after URD HCT.[28] Together, the low incidence of relapse and acute GVHD observed in this trial yielded an NRM of 16% (95% CI, 7%-34%) and an overall survival of 76% (95% CI, 63%-92%) at 1 year. These data compare favorably with the large study of 1328 patients with AML who received URD allografts in which 1-year OS ranged between 40% and 50%.[28] We also examined GRFS, which compared favorably with an incidence of 47% (95% CI, 32%-69%) at 1 year. Holtan et al recently reported a 1-year GRFS of 28% (95% CI, 17%-39%) after URD HCT.[21] Similarly, an adjusted GRFS of 27% (95% CI, 21%-33%) was observed at 1 year in a subgroup of 205 patients who received URD HCT.[29] In a retrospective review of 684 patients, the 1-year GRFS was 42% in 120 patients who received myeloablative URD HCT.[30] Although the outcomes here are encouraging and consistent with prior murine studies that showed preserved graft-versus-leukemia with HDAC inhibition,[17] we recognize the importance of confirming our observations in a larger, randomized setting.

In this trial, we conducted correlative laboratory tests on blood samples from study patients and from control patients. Our aim was to try and characterize vorinostat on target effect and its in vivo attenuation of inflammation.[18,31] Acetylated-H3 levels were significantly higher in vorinostat-treated patients in this trial compared with control patients. This is consistent with our previous data in the related-donor, reduced-intensity setting[32] and supports HDAC inhibition in the treated patients. Furthermore, the levels of IL-6 and biomarkers of GVHD soluble ST2 and Reg3α,[21,22] were significantly reduced in day 30 plasma samples of study patients compared with control patients. Pro-inflammatory cytokine IL-6 levels are elevated in acute GVHD[33] and have been a therapeutic target for GVHD prevention in a heterogeneous group of patients.[34] Reduction of IL-6 is consistent with preclinical and clinical data demonstrating that HDAC inhibition can ameliorate inflammation.[15,16,18,27,31,35] Although not measured at a time to allow prediction of acute GVHD onset, the lower soluble ST2 and Reg3α levels in our study cohort likely reflect an attenuated inflammatory response resulting

Table 4. Causes of death

Cause	No.
Relapse	4
Acute GVHD–related complication	3
Failure to thrive	1
Interstitial pulmonary fibrosis	1
Chronic GVHD–related complication	1

Table 5. Infections

Category	Study participants					Organisms
	Total	Before neutrophil engraftment	Days 0-29	Days 30-99	Days 100-180	
Gram-positive bacteria events	5	1	2	1	1	Coagulase-negative *Staphylococcus*, *Enterococcus faecium*, *Staphylococcus epidermis*
Gram-negative bacteria events	7	1	3	5		*Escherichia coli*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae**
Viral events	3			3		Cytomegalovirus
Fungal events	3			1	2	*Candida albicans* and *glabrata*, *Aspergillus*, *Pneumocystis carinii*
Total events	18	2	5	10	3	
Alive at interval start	N = 37		N = 37	N = 34	N = 32	

Listed are the counts of new infection events during the first 180 d after HCT and the count by interval within these 180 days. The table also shows the count of patients alive (N) at the start of each interval. Organisms not captured include enterococci from stool, rectum, skin; *Clostridium difficile* from stool; polyomavirus virus from urine; and oral thrush.

**M pneumoniae* not laboratory proven.

from vorinostat treatment 30 days after HCT.[22,23,36-38] These preliminary findings warrant further study in a larger, randomized controlled sample to allow robust mechanistic determinations on vorinostat treatment including whether modulation of cytokines and biomarkers correlates with clinical responses and outcomes of individual patients.

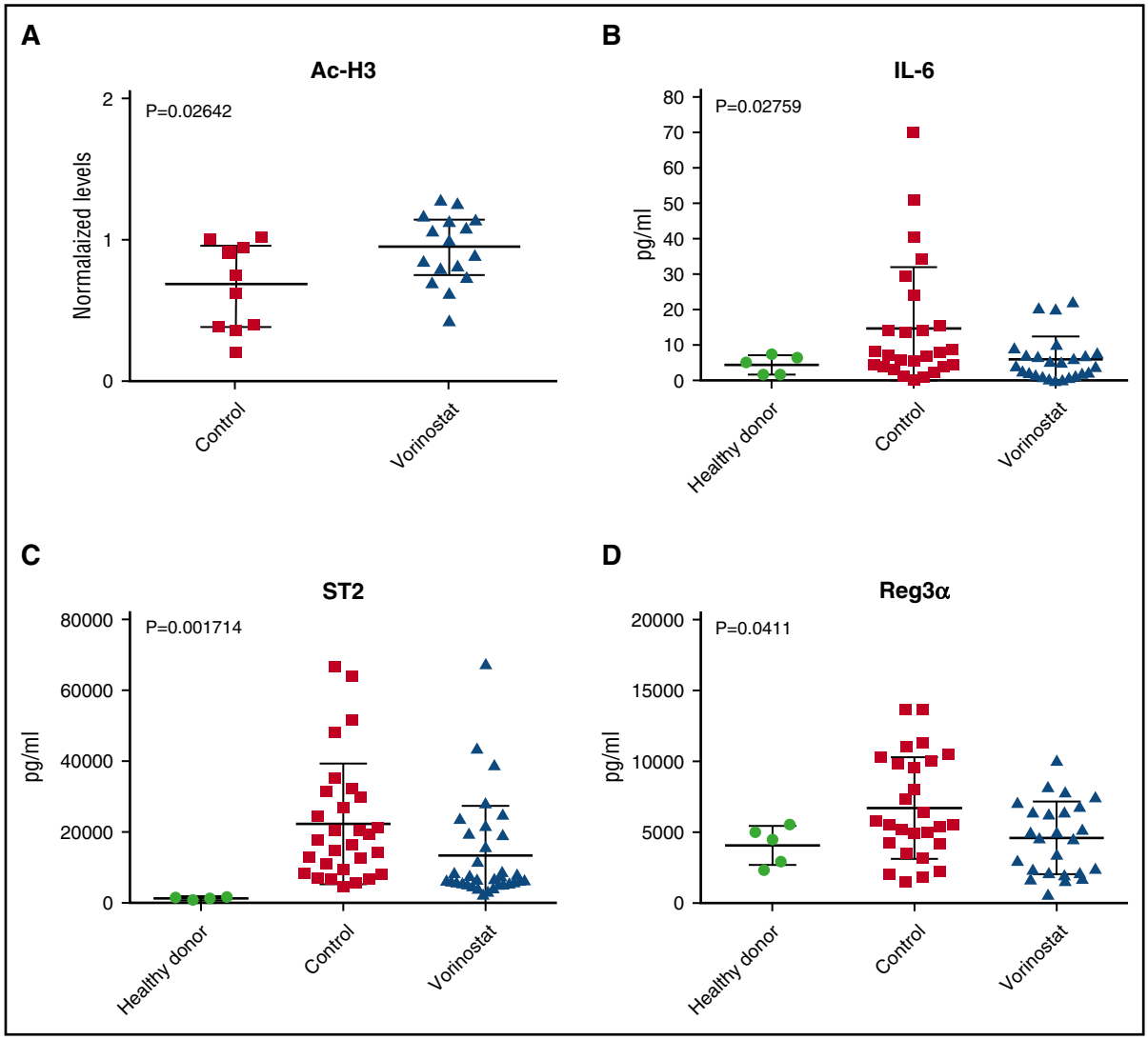

Figure 2. HDAC inhibition modulates histone acetylation. (A) levels of acetylated histone 3 (levels in each patient normalized to β-actin; ▲, n = 16; ■, n = 10) and (B) levels of IL-6, (C) Reg3α, and (D) ST2 on day 30 post-HCT (●, n = 5; ▲, n = 36; ■, n = 30). (▲) Patients in the study who received vorinostat. (■) Control patients who did not receive vorinostat. (●) Healthy donors. Median values ± interquartile range are plotted. Each data point represents a single patient.

In conclusion, vorinostat combined with standard tacrolimus and methotrexate GVHD prophylaxis for patients undergoing matched URD myeloablative conditioning HCT is safe and feasible. The incidence of grade 2 to 4 and grade 3 to 4 acute GVHD were low, and this study met its primary endpoint. Moreover, the relapse incidence at 1 year was 19%, which resulted in a GRFS of 47%, suggesting sufficient graft-versus-leukemia responses were preserved, consistent with preclinical data.[17] It is possible that vorinostat could decrease the risk for relapse in the posttransplant setting. Nonetheless, randomized trials are warranted to confirm the effect of HDAC inhibition across HCT settings.

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Authorship

Contribution: S.W.C., T.B., and P.R. designed the study; S.W.C. and P.R. analyzed the data and wrote the manuscript; I.H. conducted the pharmacodynamic experiments and performed analysis of laboratory correlative studies; E.G. collected and analyzed data; T.B. conducted the statistical analyses and performed statistical estimates of all clinical endpoints; J.M., B.P., A.P., M.R., G.Y., and C.A.D. provided assistance in study design and reviewed and edited the manuscript; and all authors vouch for the accuracy and completeness of the data and analyses and had access to primary clinical trial data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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