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Phase 1/2 trial of vorinostat plus tacrolimus and mycophenolate to prevent graft versus host disease following related donor reduced intensity conditioning allogeneic hematopoietic stem cell transplantation

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Abstract

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OIK, SWC, and PR designed the study. SWC and PR analyzed the data and wrote the manuscript. GH, IT, and YS conducted the pharmacodynamic experiments. JHB conducted the pharmacokinetic experiments. LC collected and analyzed data. TB conducted the statistical analyses. All other authors (JLMF, AP, JMM, JEL, SG, DRC, KSG, CK, GAY, MHL, SP, MYM, CAD, JFD) provided assistance in study design and reviewed and edited the manuscript. All authors vouch for the accuracy and completeness of the data and for the analyses.

CONFLICTS OF INTEREST

All authors declared no conflicts of interest.

BACKGROUND—Acute graft-versus-host disease (GVHD) remains a significant barrier to a more widespread application of allogeneic hematopoietic stem cell transplantation (HSCT). Vorinostat (suberoylanilide hydroxamic acid) is a histone deacetylases (HDAC) inhibitor that has been shown to attenuate GVHD in pre-clinical models. We aimed to study the safety and activity of vorinostat in combination with standard immunoprophylaxis for GVHD prevention in patients undergoing related donor reduced intensity conditioning HSCT.

METHODS—In this prospective, single-arm phase 1/2 study of vorinostat, we recruited patients with high-risk hematologic malignances at two centers in the USA. We enrolled patients aged 18 years or older who were candidates for a reduced intensity conditioning HSCT and had an available 8/8- or 7/8-Human Leukocyte Antigen (HLA) matched related donor. Disease status had to be adequately controlled at the time of transplant. All patients received a conditioning regimen consisting of fludarabine 40 mg/m^2 daily for four days (total dose 160 mg/m^2) and busulfan 3·2 mg/kg daily for two days (total dose 6·4 mg/kg). GVHD prophylaxis consisted of mycophenolate mofetil 1 gram three times daily from day 0 and through day 28 and tacrolimus beginning on day −3 pre-HSCT and tapered beginning on day 56 and discontinued by day 180 post-HSCT in the absence of GVHD. The investigational agent, vorinostat, was initiated on day −10 through day 100 post-HSCT. The primary endpoint of the study was grade 2–4 acute GVHD by day 100. We expected to reduce the incidence to 25% from 42% based on similarly treated patients from the study centers and published literature. Patients were assessed for both toxicity and the primary endpoint if at least 21 days of vorinostat were administered. Patients who received less than 21 days of therapy were still assessed for toxicity and were replaced in accordance to the protocol. The trial is registered with ClinicalTrials.gov, NCT00810602.

FINDINGS—Between March 2008 and February 2013, we enrolled 50 patients evaluable for both toxicity and response. All patients engrafted neutrophils and platelets at expected times post-HSCT. The median percentages of chimerism in whole-blood at day 100 and 1-year were 98% (interquartile range [IQR], 98–100) and 100% (IQR, 100–100), respectively. The primary endpoint of the study was met with a day 100 cumulative incidence of grade 2–4 acute GVHD of 22% (95% cumulative incidence: 13%, 36%). Eight additional patients enrolled were assessed for toxicity only, in accordance with the protocol, because they received less than 21 days of study drug. The most common non-hematologic adverse events were all grade 3 and included electrolyte disturbances (N=15), hyperglycemia (N=10), infections (N=4), mucositis (N=4), and elevated liver enzymes (N=3). There was one grade 4 hypokalemia event and two grade 4 infections. Non-symptomatic thrombocytopenia which occurred after engraftment was the most common hematologic grade 3 or 4 adverse event (N=9), but was transient and all cases resolved swiftly.

INTERPRETATION—Administration of vorinostat in combination with standard GVHD prophylaxis after related donor reduced intensity conditioning HSCT is safe and appears to reduce severe GVHD. Future studies are needed to assess the effect of vorinostat in the prevention of GVHD in broader HSCT settings.

Keywords

GVHD; hematopoietic stem cell transplantation; HDAC inhibitor; vorinostat

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for many patients with hematologic malignancies.[1,2] Despite advances, acute graft-versus-host disease (GVHD) remains a significant barrier to a more widespread application of allogeneic HSCT.[3] Reversible inhibitors of histone deacetylases (HDAC)modify gene expression[4] and reduce the production of inflammatory cytokines.[5] Vorinostat (suberoylanilide hydroxamic acid), a pan HDAC inhibitor, is approved for the treatment of cutaneous T-cell lymphoma.[6] We and others have demonstrated that vorinostat at lower, non-cytotoxic concentrations possesses immunoregulatory properties[5,7–9] and reduces GVHD in mice.[10] In GVHD models, vorinostat suppresses pro-inflammatory cytokines,[10,11] regulates antigen presenting cells (APCs) through induction of indoleamine 2,3-dioxygenase (IDO),[12] enhances T regulatory (Treg) functions,[13–15] and preserves graft-versus-leukemia (GVL) responses.[10,11,16] Based on these experimental observations, we performed a clinical study of HDAC inhibition in GVHD.[17] Here we report on the safety, clinical activity, pharmacokinetics, and pharmacodynamics of vorinostat from the multicenter, phase 1/2 clinical trial for prevention of GVHD. When the study was designed in 2007/2008, the incidence of grade 2–4 acute GVHD from published literature and similarly treated patients from the treating centers receiving related donor HSCT with a similar preparative regimen and standard GVHD prophylaxis was 42%.[20,21] We tested the hypothesis that the addition of vorinostat would reduce the incidence of grade 2–4 acute GVHD to ≤ 25%.

METHODS

Study Cohort

A prospective, single-arm clinical trial was conducted on an Institutional Review Board (IRB)-approved protocol under Investigational New Drug104159 from the Food and Drug Administration at the University of Michigan and Washington University. Adult patients diagnosed with a hematological malignancy who were 18 years of age or older and candidates for a reduced intensity conditioning (RIC) HSCT were eligible for study inclusion. Recipients were required to have a 7/8 or 8/8 HLA-A, -B, -C and -DRB1 matched related donor. For details, see Section 4.0 of the Protocol which is provided in the Supplementary Appendix Informed consent was sought from all study participants.

Study Design

All patients received a preparative regimen consisting of intravenous fludarabine (40 mg/m^2 on day −5 through day −2) and busulfan (3·2 mg/kg on days −5 and −4) (FluBu2) followed by the infusion of peripheral blood stem cells (PBSC) on day 0. GVHD prophylaxis consisted of tacrolimus initiated on day −3 and mycophenolate mofetil(MMF) on day 0 through day 28. Tacrolimus and MMF were initially administered intravenously, and were transitioned to an oral preparation once the patients tolerated solids without difficulty. Tacrolimus was tapered starting from day 56 post-HSCT and in the absence of GVHD was discontinued by day 180.

Oral vorinostat was administered daily starting ten days prior to the stem cell infusion and continued through day 100 post-HSCT. The phase 1 portion of the study tested two doses of vorinostat, 100 mg BID and 200 mg BID, based on the previous favorable safety profile with no reported grade 4 toxicities at either of these two doses using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version3·0 (CTCAE v. 3·0).[6] The first ten patients received vorinostat 100 mg BID, followed by nine patients who received the 200 mg BID dose. Based on the duration of study drug therapy (i.e., 111 days), dose modifications were designed with stringent criteria to avoid adverse toxicities. Briefly, the Protocol defined primary engraftment for neutrophils as the first of three consecutive days in which the absolute neutrophil count was ≥500/μL and for platelets as the first of three consecutive days in which the platelet count was ≥20,000/μL without transfusional support. If primary engraftment was not met by day 21 post-HSCT (e.g., ANC <500/μL or platelet count <20,000/μL), the following dose modifications were permitted per the Protocol. Vorinostat was to be held for ANC<500/μL (or platelet <30,000/μL). Following resolution of the first occurrence, vorinostat resumed at full dose. If subsequent occurrences of ANC to <500/μL (or platelet <30,000/μL) occurred, vorinostat was resumed at 50% dose once counts recovered. Vorinostat was to be further increased to full dose once the patient no longer required G-CSF.

The dose of vorinostat was also planned for and adjusted per the Protocol for serum creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and total bilirubin levels. The dose of vorinostat was reduced by 50% (or 75%) for serum creatinine >1.5 – 2.0 x (or >2.0 – 2.5 x upper limit of laboratory normal), AST or ALT > 200 – 399 U/L (or >400 U/L), or total bilirubin > 2.0 – 4.0 mg/dL (or 4.1 – 8.0 mg/dL). Vorinostat was held for serum creatinine > 2.5 x upper limit of laboratory normal, AST or ALT > 700 U/L, or total bilirubin > 8.1 mg/dL. Full dose was resumed once the levels normalized. For gastrointestinal (GI) symptoms felt to be related to vorinostat, the doses were also modified per the Protocol according to the severity of side effects. Briefly, in patients who developed CTCAE v.3.0 grade 2 or 3 GI symptoms, vorinostat was held after failure of anti-motility or anti-emetic agents. The dose was resumed at 50% once the symptoms resolved to CTCAE v. 3.0 grade 0 or 1. Vorinostat was discontinued permanently for any CTCAE v.3.0 grade 4 GI symptoms. According to the Protocol, vorinostat was continued at full dose if acute GVHD developed.

Because of potential interactions between vorinostat and the conditioning regimen, the Protocol was designed such that vorinostat would be held for any non-hematological CTCAE v.3.0 grade 4 or higher toxicities seen between the first dose of vorinostat and day 7 post-HSCT. If there was complete reversal of toxicity, vorinostat could be resumed after seven days and a minimum of one week off vorinostat. In the absence of complete reversal of toxicity within seven days, vorinostat would be discontinued permanently. For further details, Section 5·5 of the Protocol is available in the Supplementary Appendix.

Supportive care therapies, including anti-infectives and blood product transfusions, were administered according to institutional guidelines. Although no dose-limiting toxicities were reached at the 200 mg BID dose, there was an increased incidence of protocol-driven dose modifications, primarily due to non-symptomatic thrombocytopenia after engraftment.

Consequently, the 100 mg BID dose was selected as the phase 2 dose for the remaining patients. The study protocol was designed a priori such that patients who received more than 21 days of vorinostat were evaluable for toxicity and response, while those who did not receive vorinostat for at least 21 days were still evaluable for toxicity and would be replaced. For further details, Section 10.7 of the Protocol is available in the Supplementary Appendix.

Serum Pharmacokinetics

Pharmacokinetic studies were performed in five patients who received the 100 mg BID dose on day 1 after the stem cell infusion (for details, see Methods in the Supplementary Appendix).

Plasma Pharmacodynamics

In order to evaluate the pharmacodynamic activity of HDAC inhibition by vorinostat post-HSCT, we measured acetylation of histones (H3 and H4), TNF-α, Treg expansion, and FoxP3 and IDO expression in peripheral blood mononuclear cells (PBMCs) on day 30 (for details, see Methods in the Supplementary Appendix). PBMCs from patients who were similarly conditioned and prophylaxed without vorinostat were used as controls. IRB approval and informed consent applied for testing blood samples from controls (University of Michigan IRB #2001·0234, HUM00043287).

Outcomes

The primary endpoint was the incidence of grade 2–4 acute GVHD by day 100. Secondary outcomes included the safety and feasibility of vorinostat administration, incidence of relapse, chronic GVHD, infections, and overall survival. Acute GVHD was scored weekly by the modified Glucksberg criteria (for details, see Methods in the Supplementary Appendix).[18] Biopsies were obtained in all cases to confirm the diagnosis of acute GVHD. Clinically significant acute GVHD was treated with methylprednisolone 2 mg/kg/day. Vorinostat was continued during therapy for acute GVHD. Chronic GVHD was evaluated according to the National Institutes of Health Consensus Criteria.[19]

Statistical Analysis

The trial was designed to enroll 50 patients evaluable for safety and clinical outcomes. The null hypothesis of an incidence of grade 2–4 acute GVHD of 42% after matched related donor RIC HSCT by day 100 was based on institutional data as well as published studies.[20,21] The alternative hypothesis was an incidence of 25% with the use of vorinostat. The planned sample size of 50 patients provided 80% power to detect a 17% difference, assuming a one-sample test of a binomial proportion with a Type I error rate of 5%.

Overall survival was measured from the date of transplantation to the earlier of death from any cause or end of follow-up, and was estimated with the methods of Kaplan-Meier.[22] The cumulative incidence(±SE) of relapse, non-relapse mortality (NRM), and acute and chronic GVHD were estimated using the method of Gray.[23] Relapse and NRM were competing risks for each other and relapse and death were competing risks for GVHD. Since Kaplan-Meier methods regard patients with competing events as being censored for the event of interest,

we therefore used the methods of Gray[23], which regard patients with competing events to have no future risk of the event of interest, to estimate the cumulative incidence (±SE) of relapse, non-relapse mortality (NRM), and acute and chronic GVHD. Serum pharmacokinetic parameters, including area under the concentration versus time curve (AUC) were extracted from the data non-compartmentally with PK Solutions 2·0™ (Summit Research Services, Montrose, CO, USA). Student's t-tests were performed with SPSS 15·0 Windows (SPSS Inc., Chicago, IL, USA) to compare our pharmacokinetic results with published data (dose-normalized if necessary) after every day dosing of 200 mg vorinostat.[6] Pharmacodynamic outcomes were compared between study and samples collected from similarly treated patients who did not receive vorinostat with the Wilcoxon rank-sum test. These statistical analyses were performed in R (R Project for Statistical Computing at 222.r-project.org) and GraphPad Prism 6·0 (GraphPad Software, Inc., La Jolla, CA). A two-sided P value of less than 0·05 was considered to indicate statistical significance. This trial is registered with ClinicalTrials.gov number NCT00810602.

Role of the funding source

Merck and Co., Inc. supplied vorinostat, but had no other involvement in study design, undertaking, or management; data collection, analysis, or interpretation; or writing of the report. The authors provided Merck and Co., Inc. with a copy of the original report before submission. Merck and Co., Inc. made no amendments to the manuscript.

The vorinostat trial was designed by OIK, SWC, and PR. All other authors provided assistance in study design and reviewed and edited the manuscript. All authors vouch for the accuracy and completeness of the data and for the analyses. PR had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

RESULTS

Patient Cohort Characteristics

The first patient enrolled on March 31, 2009 and the last patient enrolled on February 8, 2013. A total of 61 patients were enrolled on the phase 1/2 study. Fifty patients completed 21 days of vorinostat and were considered evaluable for both toxicity and the primary outcome (Figure S1) Three patients withdrew consent prior to vorinostat initiation. Eight patients received at least one dose of study drug but withdrew consent prior to 21 days of therapy. However, all of them were still evaluable for toxicity and were replaced according to the study protocol (for details of the reasons for withdrawal, see Table S1).

Baseline demographic and clinical characteristics are presented in Table 1. Briefly, median age for study patients was 59 years (interquartile range[IQR],55–63). A significant proportion (84%) had high-to-intermediate comorbidity indices.[24] Forty-six patients received 8/8 HLA-A, −B, −C, and -DRB1 matched related grafts and four patients received one-locus HLA-mismatched grafts. All study patients engrafted neutrophils (absolute neutrophil count ≥ 500 per cubic millimeter) at a median time of 12 days (IQR, 11–13). The median time to platelet engraftment (≥20,000 per cubic millimeter without transfusions) was 12 days (IQR 11–13). There were no cases of primary or secondary graft failure. The

median percentages of donor chimerism in whole blood and T cells at 1-year post-HSCT were 100% (IQR 100–100) and 100% (IQR, 100– 100)(Table S2).

Pharmacokinetic Studies and Safety

The 100 mg BID dose of vorinostat was used for the pharmacokinetic studies, which demonstrated a mean half-life of 1·76 hours (h) and time to maximum concentration of 2·6 h. The observed clearancewas48·1 L/h and the maximum concentration was491 ng/mL (Table 2).

There were no deaths that were related or attributable to vorinostat treatment. A total of 33 serious adverse events (SAE) by NCI CTCAEv.3·0 criteria (Table 3) were reported in the 58 patients who received at least one dose of vorinostat. Of these SAEs, five were due to inpatient hospitalizations for GVHD and six for relapse. There were a total of 42non-hematologic grade 3 adverse events, which included electrolyte disturbances, hyperglycemia, infections, mucositis, and elevated liver enzymes. There was one grade 4 hypokalemia event and two grade 4 infection-related events (Table 3). The most common hematologic adverse event (grade 3 or 4) was non-symptomatic thrombocytopenia, which occurred after engraftment. QT prolongation, a known risk of vorinostat,[25] was monitored closely by a cardiologist (MHL). No significant increases in QTc intervals were noted (Table S3). Based on the stringent dose modification strategy, 29patients required dose adjustments according to the Protocol. However, there were no drug-related toxicities warranting discontinuation of vorinostat per the Protocol. Nonetheless,88% of the planned doses of vorinostat were administered.

Gram-positive bacteremia accounted for the majority of infections (N=17)in study patients. There were five cases of asymptomatic reactivation of CMV and one case of respiratory syncytial virus that resolved without adverse complications. Notably, there were no fungal infections. Overall, the location and severity of infectious complications were consistent with the target population (Table S4).

Efficacy

The cumulative incidence of grade 2–4 acute GVHD at day 100 (the primary endpoint) was 22% (95% confidence interval [CI]: 13%, 36%)in the study cohort with a median time to onset of 49 days (IQR,35·5–64·5). Five patients were censored for risk of GVHD due to recurrence of the underlying malignancy and one patient was censored due to death from pre-existing history of pulmonary hypertension. Six of the nine patients with GI GVHD had isolated upper GI tract involvement only. The three remaining cases involved the lower GI tract and accounted for the low cumulative incidence of grade 3–4 GVHD of 6±3% (Table 4 and Figure 1A). Eight of the nine patients responded completely to first-line corticosteroid therapy. The reduction in acute GVHD persisted through day 180 (28±7%) and 1-year (28±7%) (Table 4).

With a median follow-up of 26 months (IQR13·9–39·3), the cumulative incidence of moderate-to-severe chronic GVHD at 1-year in study patients was 45±7% (Table 4), resulting in a 1-year steroid-free survival of 37%. The cumulative incidence of relapse was 16±5%at 1-year and 2-years with a median time to occurrence of 98 days (IQR 54–147)

(Figure 1B). All-cause NRM and GVHD-related (acute and chronic) NRM were 10±5% and 6±6%, respectively, at 1-year and 2-years(Table 5 and Figure 1C), which resulted in an overall survival of 73±6%at 1-year and 2-years(Figure 1D). The median time to death from any cause was 161·5 days (IQR 87–295).

The protocol-specified plan was to limit the efficacy analysis of the primary endpoint to patients who received a minimum of 21 days of vorinostat. Because of the potential concern for introducing bias mechanisms, we also performed a sensitivity analysis of an intent-to-treat (ITT) estimate of grade 2–4 acute GVHD for all patients who received at least one dose of vorinostat, excluding the patients who never proceeded to HSCT (for details, Table S1). At day 100, 180, and 1 year, the incidence of grade 2–4 acute GVHD was 22% (95% CI: 13%, 35%), 27%, and 27%, respectively. The incidence of grade 3–4 acute GVHD at these time points was 5%, 11%, and 11%, respectively. The ITT estimate of relapse, NRM, and overall survival were 20%, 9%, and 70%, respectively, at both 1-year and 2-years. These outcomes are similar to the per-protocol analyses.

Pharmacodynamic and Correlative Studies

Laboratory studies were performed on samples obtained on day 30 post-HSCT and compared between study patients and in similarly treated patients who did not receive vorinostat. Significantly increased acetylation was observed in study patients, demonstrating HDAC inhibition (Figure 2A). A significant reduction in plasma levels of TNFR1 and intracellular expression of TNF-α in PBMCs was observed in vorinostat-treated patients (Figure 2B). There were no significant differences in $CD4^+$, $CD8^+$, and absolute lymphocyte counts (ALC) (Figure 2C and 2D, respectively). However, significantly greater absolute numbers of $CD4^+CD25^+CD127^-$ Tregs (Figure 2D) and increased expression of FoxP3 were observed in vorinostat-treated patients (Figure 2E). Furthermore, vorinostat-treated patients showed greater expression of IDO in PBMCs (Figure 2E). Thus, these immunomodulatory effects of HDAC inhibition with vorinostat following allogeneic HSCT were consistent with experimental observations.[10–14]

DISCUSSION

This study reports on the outcomes of patients with advanced hematologic malignancies enrolled prospectively in a clinical trial of vorinostat-based GVHD prevention after allogeneic HSCT and performed at two different institutions. To our knowledge, this is a first-in-human post-HSCT study that represents direct translation of HDAC inhibition derived from experimental observations.[10–14] We found that oral vorinostat can be administered safely in combination with standard immunoprophylaxis in the related donor RIC HSCT setting. The study met its hypothesized primary outcome by demonstrating a significant reduction in the incidence of grade 2–4 acute GVHD at day 100 (22%) compared to an incidence of 42% from published studies.[20,21] Importantly, with the ITT principle, this conservative estimate of treatment effect still resulted in a cumulative incidence of grade 2–4 acute GVHD of 22%. In addition, the study revealed an appreciably low incidence of severe grade 3–4 acute GVHD, despite a patient cohort that was older and with a high comorbidity index.

Although limited by the single-arm clinical trial design, we are encouraged with the lower incidence of grade 2–4 acute GVHD observed in this study. Further studies are warranted to confirm the efficacy of the drug within a prospective, randomized controlled trial. Currently, there are ongoing studies with panobinostat (LBH589) maintenance therapy following allogeneic HSCT in patients with high-risk hematologic malignancies and for treatment in patients with acute GVHD (ClinicalTrials.gov NCT01451268 and NCT01111526, respectively).

In a recent report of 611 patients with hematologic malignancies transplanted with low-dose total-body irradiation ± fludarabine from related donors followed by MMF and a calcineurin inhibitor, the incidence of acute GVHD by day 120 was 43% for grade 2–4 and 11% for grade 3–4 with a 2-year relapse incidence of 51%.[26] The incidence of grade 2–4 acute GVHD of 42%set as the null hypothesis appears to be consistent with that study[26] and also with other recently reported studies.[27,28] Thus, the decreased incidence of acute GVHD observed in the present study is unlikely due to changes in supportive care between the study and published literature.[26–28] In another recent phase1/2 study of RIC HSCT that combined maraviroc with standard GVHD prophylaxis, the incidence of grade 2–4 acute GVHD by 1-year was 29%, but the incidence of relapse at 1-year was 56%.[29] The data of our study show a 1-year incidence of acute GVHD (grade 2–4: 28% and grade 3–4: 12%), but much lower incidence of relapse (16%) in a very similar cohort of patients based on the distribution of diagnoses in the two studies. However, direct comparisons between studies are difficult and a prospective, randomized controlled trial comparing this newer approach with standard GVHD prophylaxis is needed.

The therapeutic potential of allogeneic HSCT relies on the GVL effect for eradication of residual malignant cells. GVL is tightly linked with GVHD.[30] Although experimental data suggest that GVL was maintained with vorinostat,[10] strategies that mitigate GVHD could be associated with increased relapse, impaired immune reconstitution, graft rejection, and increased infections. In the current study, despite the reduction in acute GVHD, we did not observe an increase in relapse relative to two other recent studies,[26,29] suggesting that GVL may not have been significantly altered. This could also be a consequence of the ability of an HDAC inhibitor, such as vorinostat, to enhance susceptibility of leukemia to immune-mediated rejection.[31] The vorinostat-mediated prevention strategy resulted in sustained engraftment in 100% of patients and there were no cases of graft failure. Moreover, HDAC inhibition in study patients did not appear to increase the risk for infectious complications. Importantly, there were no fungal infections within the first 180 days post-HSCT. These observations are comparable or even better to a recent published report in this target population.[29] The survival analysis from this single-arm clinical trial suggested a survival benefit when compared to published reports.[26–28] However, the trial was not powered to detect overall survival effects, only the primary endpoint. The sample size of 50 patients was selected a priori to have sufficient power for analyses of acute GVHD.

Unique to this trial were the pharmacodynamic studies informed by the experimental observations[10–14] and the analyses of pharmacokinetic parameters after allogeneic HSCT. Higher-than-expected drug exposures were observed with the 100 mg bid dose. No obvious interaction accounted for the observed increase in vorinostat exposure. Importantly,

however, there were no unexpected drug-related toxicities and adverse events were similar to those reported in other studies.[6] In addition, we measured plasma biomarkers of GVHD,[32] such as regenerating islet-derived 3-alpha, interleukin 2 receptor-alpha, elafin, and suppressor of tumorigenicity 2, on day 30 (data not shown). We found no clear correlation with clinical outcomes. This could be because of the consequence of the reduced intensity conditioning regimen, later time point of analyses, or smaller sample size of patients that actually developed acute GVHD on our study. The pharmacodynamic analyses demonstrated target HDAC inhibition, decreased pro-inflammatory cytokine production, increased Tregs, and increased IDO expression. These findings correlated with clinically significant reduced acute GVHD and were consistent with preclinical studies.[10–14] However, due to difficulty in obtaining sufficient number of sorted Tregs from individual patients at day 30, we have been unable to test the suppressive function of the cells that phenotypically appear as Tregs.

In conclusion, vorinostat combined with standard GVHD prophylaxis in the related donor RIC HSCT setting is safe and feasible. This regimen, consistent with experimental observations,[10–14] appears to reduce the incidence of clinically significant GVHD without major adverse events. Further studies are warranted to confirm these findings in randomized and also in broader HSCT settings. We are currently investigating the use of vorinostat combined with tacrolimus and methotrexate for GVHD prevention in the setting of unrelated donor transplant after myeloablative conditioning (ClinicalTrials.gov NCT01789255).

RESEARCH IN CONTEXT

Systematic Review

We conducted a systematic review of the scientific literature in PubMed with the search keywords “graft versus host disease (GVHD)”, “histone deacetylase (HDAC) inhibitor”, “suberoylanilide hydroxamic acid (SAHA)”, “vorinostat”, “reduced intensity conditioning (RIC)”, “related donor transplant”, “hematopoietic stem cell transplantation (HSCT)” before starting this trial. In addition to published literature obtained from the PubMed queries, we also conducted a thorough review of the University of Michigan and Washington University databases to determine an estimate of the incidence of grade 2–4 acute GVHD in patients undergoing related donor RIC HSCT. Based on institutional data as well as the published literature, we determined an estimate of 42% risk of developing grade 2–4 acute GVHD by day 100 in this patient population.[20,21] We cited the most relevant articles in this manuscript. Vorinostat is approved for use in the USA for the treatment of cutaneous T cell lymphoma and has emerged as a potential strategy for anti-cancer therapy. The existing evidence in this area of research in the setting of HSCT is primarily in murine models of GVHD. However, there are ongoing studies registered in ClinicalTrials.gov actively accruing patients for the use of other HDAC inhibitors in the HSCT setting.

Interpretation

At the time of the study design (2007/2008), there were no abstracts or manuscripts about the use of vorinostat for GVHD prevention in patients undergoing RIC HSCT. However, our pre-clinical data provided the rationale to translate our experimental observations into a

hypothesis-driven phase 1/2 trial. To our knowledge, this study represents the first phase 1/2 trial to use an HDAC inhibitor combined with standard immunoprophylaxis in the HSCT setting. Our study demonstrates safety and feasibility with the potential to reduce clinically meaningful acute GVHD. As a novel prophylaxis regimen, the use of vorinostat warrants further investigation in a prospective, randomized controlled trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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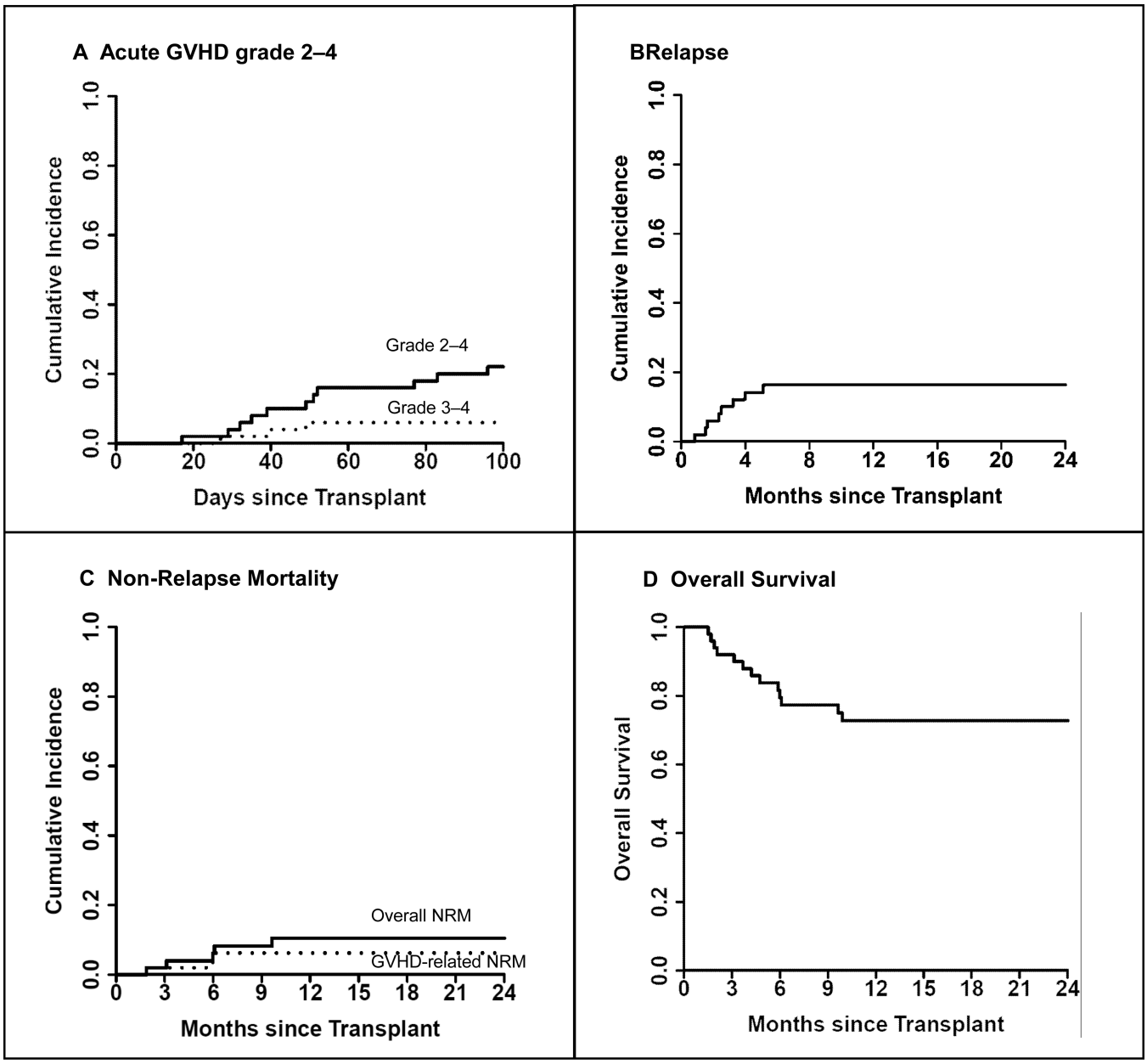
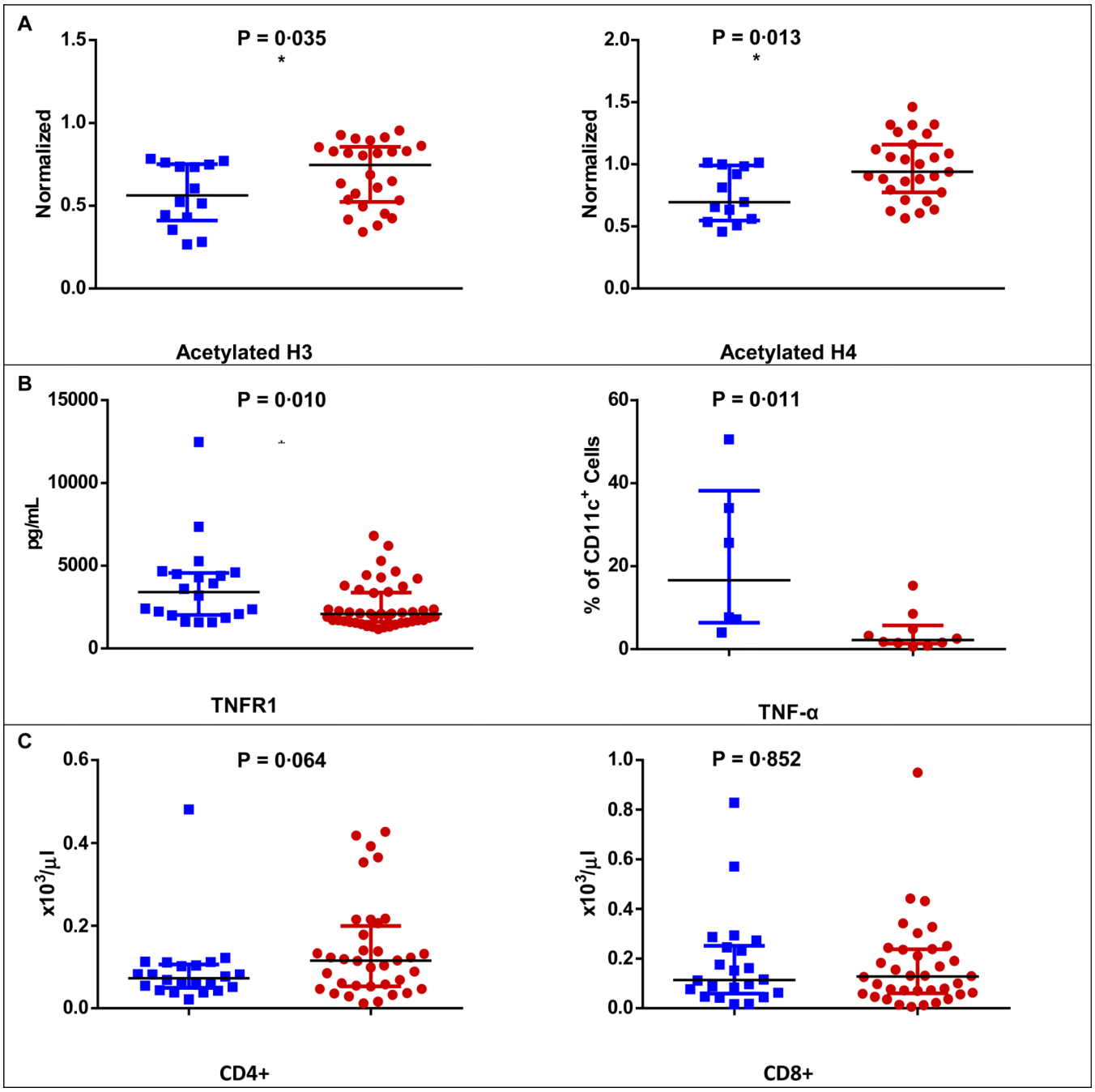


Figure 1. Clinical Outcomes of Acute GVHD, Relapse, Non-Relapse Mortality (NRM), and Overall Survival

Cumulative incidence of Grade 2–4 and Grade 3–4 acute GVHD (Panel A), Relapse (Panel B), GVHD-related NRM and overall NRM (Panel C), and Overall Survival (Panel D). The study population consisted of 50 patients whom underwent matched related donor, reduced intensity conditioning transplant with vorinostat combined with tacrolimus and mycophenolate mofetil for GVHD prevention.



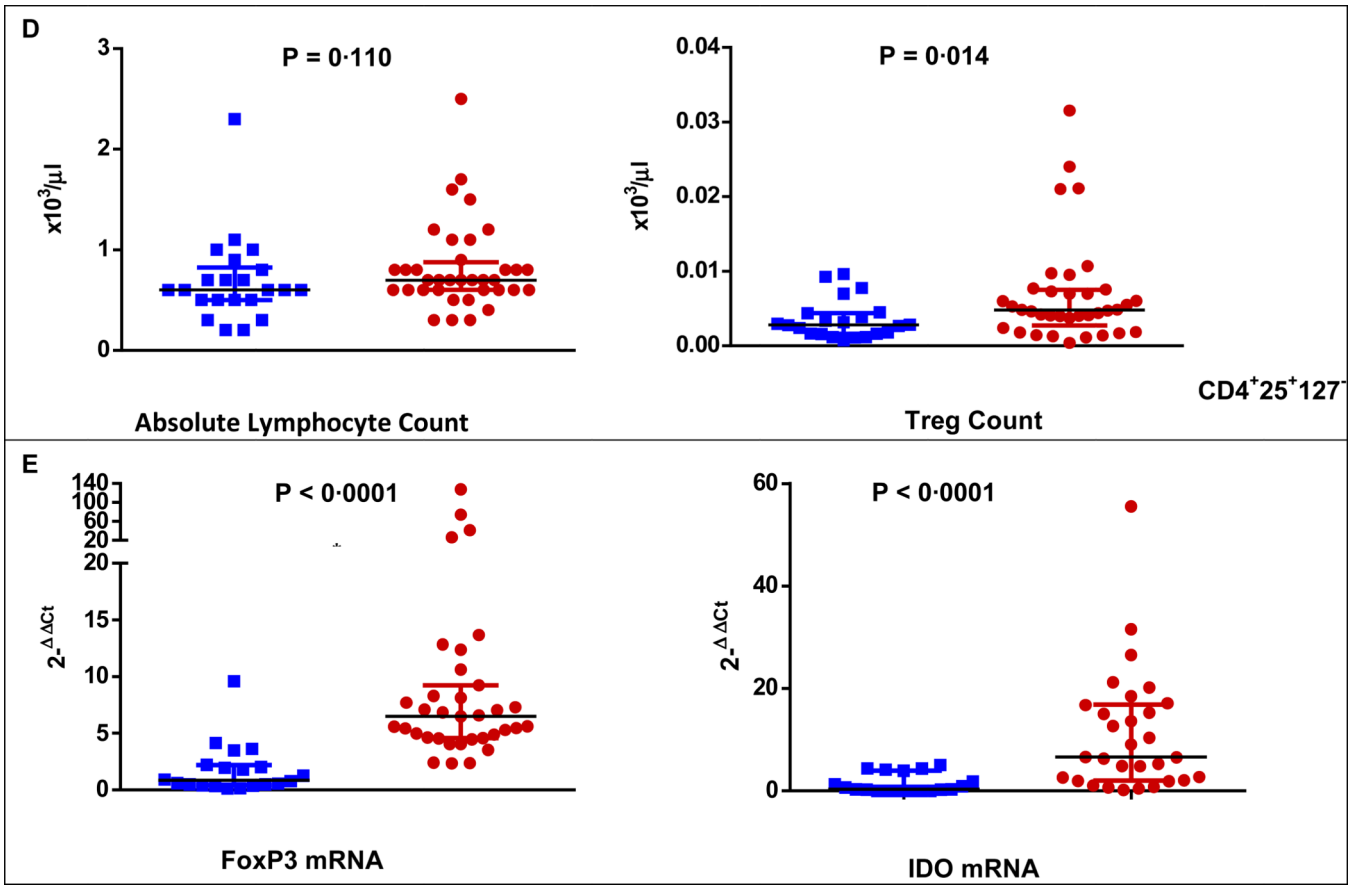


Figure 2. Pharmacodynamic Studies

Shown are controls (■)*versus* study patients (●). Panel A shows the results of Western blot analyses of histone acetylation in peripheral blood mononuclear cells (acetylated H3 ■n = 14, ●n = 26; acetylated H4 ■n = 13, ●n = 27). Panel B shows plasma pro-inflammatory cytokines as assessed by enzyme-linked immunosorbent (TNFR1 ■control n = 20, ●study n = 45) assay and by flow cytometry (TNF-α■n = 6, ●n = 10). Panel C shows the flow cytometric detection of $CD4^+$ (■n = 22, ●n = 36) and $CD8^+$ (■n = 22, ●n = 36) counts. Panel D shows the flow cytometric detection of absolute lymphocyte counts (■n = 22, ●n = 36) and $CD4^+25^+127^-$ Treg counts (■n = 22, ●n = 36). Panel D shows FoxP3 (■n = 20, ●n = 35) and indoleamine-2,3-dioxygenase (IDO■control n = 19, ●n = 30) expression as assessed by RT-PCR. All studies were conducted at day 30 post-transplant.

Table 1

Baseline Demographics and Clinical Characteristics of the 50 Study Participants

Characteristic		No. (%) or Median (Range, IQR)
Recipient Age (years)		59 (43–69, 7·8)
Gender	Male	28 (56)
	Female	22 (44)
Race/Ethnicity	White non-Hispanic	46 (92)
	White Hispanic	2 (4)
	White unknown	2 (4)
Diagnosis	Acute myelogenous leukemia	19 (38)
	Myelodysplastic syndrome	10 (20)
	Non-Hodgkin's lymphoma	12 (24)
	Chronic lymphocytic leukemia	4 (8)
	Myeloproliferative disorder or myelofibrosis	4 (8)
	Acute biphenotypic leukemia	1 (2)
Disease Status	Low	25 (50)
	Intermediate	20 (40)
	High	5 (10)
Comorbidity Index *	Low	8 (16)
	Intermediate	15 (30)
	High	27 (54)
Donor	Matched related	46 (92)
	One-antigen mismatched related	4 (8)
CMV Status	Recipient (R) or Donor (D) positive	29 (58)
	R+, D+	17 (34)
	R−, D+	6 (12)
	R+, D−	7 (14)
	Recipient and Donor negative	20 (40)
$CD34^+$ Count (10^6 cells/kg)		5·1 (1·9–8·0, 1·9)
Engraftment Day	Neutrophil	12 (8–19, 2)
	Platelet	12 (9–39, 2)

* HCT-Comorbidity Index: Low = 0, Intermediate = 1 or 2, High ≥ 3

Table 2

Pharmacokinetic Studies

	C_{max}* (ng/mL)	CL/F † (mL/min)	T_{max}‡ (h)	$T_{½}$§ (h)
Study	491	48·.1	2·6	1·76

*C_{max}: maximum concentration

†CL/F: clearance

‡T_{max}: time to maximum concentration

§$T_{½}$: mean half-life

Table 3

Serious Adverse Events and Adverse Events among the Study Participants*

	Frequency
Serious Adverse Event	
Anorexia †	1
Cystitis +	1
Diarrhea	1
Edema: limb ‡	1
Hemorrhoids §	1
Hypercalcemia +	1
Infection ‖	12
Pulmonary hypertension ¶	1
Rash **	1
Relapse ††	6
GVHD ‡‡	5
Thrombosis/thrombus/embolism	2
Adverse Events	
Elevated liver enzymes	3
Engraftment syndrome	1
Hyperbilirubinemia §§	1
Hyperglycemia ‖ ‖	11
Hyperkalemia	2
Hypermagnesemia ¶¶	1
Hypocalcemia ***	2
Hypokalemia†††	3
Hyponatremia	3
Hypophosphatemia	4
Hypotension	1
Infection ‡‡‡	6
Mucositis	4

*Event categories included serious adverse events (SAE) defined as satisfying one or more of the following criteria: inpatient hospitalization, prolongation of a hospital stay, permanent or severe disability, death, congenital anomaly in the offspring of a participant, overdose (either accidental or intentional) of the study medication, and life-threatening event. All SAE were grade 3 except for one grade 1 hypercalcemia, one grade 2 cystitis, one grade 3 hemorrhoids, one grade 5 pulmonary hypertension, and three grade 5 relapses. All adverse events were grade 3 except for one grade 4 hypokalemia event and two grade 4 infections.

†Pre-existing history of hemicolectomy

+Non-infectious cystitis and hypercalcemia occurred in the same patient during one admission post-relapse

‡Upper extremity swelling determined due to vancomycin

§Pre-existing history of hemorrhoids

‖ Infection–bacteremia (7), colitis (4), viremia (1). Two of the seven bacteremia events counted were regarded as possible contaminants (coagulase-negative *Staphylococcus aureus* and *Bacillus* species, not *antharacis* nor *cereus*)

¶ Pre-existing history of pulmonary hypertension

** Biopsy-confirmed dermal hypersensitivity likely due to oxycodone

†† Three relapse events were grade 3 and three were grade 5. One patient had upper respiratory and urinary tract infections and met sepsis criteria occurring at same admission as relapse grade 5. During one patient's relapse-related sepsis, the patient developed the following concurrent adverse events: grade 4 hypocalcemia, grade 3 hyperglycemia while also on steroids, and grade 3 hyponatremia.

‡‡ Graft versus host disease

§§ Pre-existing history of Gilbert's syndrome

‖ ‖ Of the 11 hyperglycemia events: eight occurred in patients with pre-existing diabetes mellitus, two occurred in patients on steroids.

¶¶ Hypermagnesemia occurred in one patient receiving daily magnesium sulfate while on tacrolimus

*** One hypocalcemia grade 3 adverse event occurred in a patient with pre-existing history of hypocalcemia.

††† One grade 4 hyporkalemia event: After a single serum K^+ measurement of 2·3 mmol/L, a repeat measurement within two hours demonstrated a normal serum K^+ level (3·7 mmol/L) without any change in management. Potassium levels remained within normal limits for remainder of admission.

‡‡‡ Infection – four grade 3 events: urinary tract (1), bacteremia (2), colitis (1); two grade 4 events: perirectal abscess (1), sinusitis (1)

Table 4

Cumulative Incidence of Graft-versus-Host Disease (GVHD)*

Variable	Incidence % (± SE)		
	Day 100	Day 180	1 Year
Acute GVHD			
Grade 2–4	22±6	28±7	28±7
Grade 3–4	6±3	12±5	12±5
Skin	6±3	8±4	8±4
Liver	2±2	6±4	6±4
Gut	18±5	24±6	24±6
Chronic GVHD			
Moderate–Severe	4±3	25±6	45±7

* Plus-minus values are means ± standard error (SE).

Table 5

Causes of Non-Relapse Mortality

Cause of Death *	Study No. (%)
Acute GVHD-related infection	2
Idiopathic pneumonia syndrome	–
Chronic GVHD-related infection	–
BOOP †	1
PEA arrest ‡	1
Pulmonary hypertension §	1

*2-year post-transplant causes of death. None of the deaths were considered to be related to vorinostat.

†Bronchiolitis obliterans with organizing pneumonia

‡Pulse less electrical activity

§Pre-existing history of pulmonary hypertension