Phase II Trial of Vorinostat plus Tacrolimus & Mycophenolate to prevent Graft versus Host Disease Following Reduced Intensity Conditioning Related Donor Allogeneic Transplant

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1.0 OVERVIEW

The proposed trial is an open label, non-randomized, phase II clinical trial investigating the use of vorinostat (Merck) for the prevention of severe acute GVHD following a reduced intensity allogeneic transplant. Historically, allogeneic hematopoietic stem cell (HSC) transplants have been associated with significant morbidity and mortality, either due to relapse of the primary disease, multi-organ dysfunction related to the intensity of the transplant preparative regimen, or from complications of acute or chronic graft versus host disease (GVHD). In an attempt to decrease morbidity from the transplant preparative therapy, reduced intensity transplant regimens have been developed at a number of transplant centers. Though reductions in toxicity have been noted, any success reported with these "less intensive" regimens have been partially offset by inferior tumor control.

The University of Michigan Blood and Marrow Transplantation Program has ten years of experience in performing reduced intensity transplants. Three approaches have been utilized, (1) a fludarabine/busulfan/TLI conditioning regimen followed by GVHD prophylaxis with tacrolimus/low dose mycophenolate, (2) the same conditioning regimen followed by GVHD prophylaxis with tacrolimus/mycophenolate, or (3) the same conditioning regimen followed by a rapid immunosuppression taper. Long term outcomes with these three regimens have been remarkably similar with rates of grade II-IV GVHD ranging from 38% to 54% and two-year survival of approximately 50%. Somewhat higher rates of GVHD and transplant-related mortality were observed in patients treated using a rapid immunosuppression taper offsetting any potential improvement in graft versus tumor effect. In order to maximize the portability of the results from this trial to other centers, we will build upon our experience of reduced intensity conditioning followed by tacrolimus/mycophenolate GVHD prophylaxis with a standard immunosuppression tapering schedule (beginning 2 months post-transplant and finishing at 6 months post-transplant). In our experience, this approach results in a 44% incidence of grades II-IV graft versus host disease (GVHD), with a 50% two-year survival rate. We will now build upon this experience, by adding a novel histone deacetylase inhibitor, vorinostat (Merck), in order to investigate whether we can achieve reductions in the incidence and severity of acute GVHD, while maintaining a graft versus leukemic / tumor effect. We will study up to two doses of vorinostat based on interim analyses of toxicity and efficacy.

If the proposed pilot trial is successful, we plan to expand the study to a multi institutional level via CTN (Clinical Trial Network).

Primary Objective: To assess if the addition of vorinostat to a standard GVHD prophylaxis regimen, can reduce the rate of grades 2-4 acute GVHD from 42% to 25%, as compared to historical control patients. Achieving the primary objective will support a reduction in toxicity while preserving the transplant process efficacy.

Secondary objectives will (a) establish the safety & feasibility of vorinostat, (b) determine steroid-free survival, 1 year relapse free survival, overall survival, (c) investigate plasma concentrations of inflammatory markers of acute GVHD, (d) assay protein acetylation in peripheral blood mononuclear cells (PBMC) before and after administration of vorinostat, and (e) assay levels of vorinostat and its metabolites before, during and after administration of the transplant conditioning regimen.

Patient Eligibility: All patients must have a hematologic malignancy, be suitable candidates to receive a reduced intensity transplant, and have a 7/8 or 8/8 HLA matched related donor at HLA A, B, C and DR loci. **Based on experience with previous trials, patients with acute myelogenous leukemia, myelodysplastic syndromes, and myeloma are expected to comprise the vast majority of the patient population in the proposed trial.** Eligibility criteria will be similar to that used in UMCC reduced intensity protocols (UMCC 9822 and 2002-61). Adult patients 18 yo and older will be eligible, provided they meet the disease status criteria listed in section 4.1.

Study Design:

- *Preparative regimen*: IV Busulfan 3.2 milligrams/kg on days -5 and -4, Fludarabine 40 milligrams/m^2 for 4 days (from day -5 to day -2).

- *Standard immunosuppression*: Mycophenolate mofetil (MMF) and tacrolimus. MMF will begin on day 0 and stop on day 28 (standard timing). Tacrolimus will be maintained at therapeutic levels through day 56, then tapered off by day 180 (standard taper).

- *Investigational agent*: Vorinostat 100 or 200 mg PO BID with dose determined by dose escalation/de-escalation schema. The vorinostat will continue until day 100 post-transplant, whether or not acute GVHD develops.

- *Biology samples*: Whole blood samples will be collected at serial time points, beginning approximately day -10 pre-transplant through the first 100 days of study. Samples will be collected from University of Michigan patients only.

Pharmacodynamic marker studies: Proteins acetylation and pro-inflammatory cytokine production by PBMC stimulated ex vivo by lipopolysaccharide (LPS) will be serially monitored as a surrogate pharmacodynamic marker of exposure to the study drug in subgroup of patients.

2.0 HYPOTHESES AND OBJECTIVES

2.1 Hypotheses: The histone deacetylase inhibitor (HDACi) vorinostat will result in a reduction of significant (grade II-IV) acute graft-versus-host disease (GVHD) for patients with hematologic malignancies undergoing reduced intensity allogeneic HSC transplantation.

2.2 Primary Objective: To assess if the addition of vorinostat to a standard GVHD prophylaxis regimen, can reduce the rate of grades 2-4 acute GVHD from 42% to 25%, as compared to historical control patients.

2.3 Secondary Objectives:

- To establish safety & feasibility of vorinostat administration in the above clinical setting
- To determine the rates of steroid-free survival and relapse at 1 year
- To determine overall survival rates (Long-Term Follow-up Study protocol IRB# 2001-0234)
- To correlate plasma concentrations of inflammatory markers of acute GVHD (including cytokines) with GVHD
- To assay protein acetylation in PBMC before and after administration of vorinostat

3.0 BACKGROUND AND RATIONALE

3.1 Introduction: HSCT, Reduced Intensity Transplant and Acute GVHD

For hematologic malignancies that can not be cured by standard chemotherapy/radiation alone, allogeneic HSCT represents the only curative option. In younger patients with less advanced leukemia or non-Hodgkins Lymphomas (NHL), conventional full intensity HCT from HLA-matched sibling results in prolonged disease-free survival (DFS) and cure in up to 80% of cases. Unfortunately, patients who are older or have more advanced diseases have a much less favorable outcome with DFS in the range 10-30%. Treatment failures occur most commonly due to GVHD, toxicity from the preparative regimen or relapse.

Low or reduced-intensity conditioning (RIC) transplant regimens were developed to lessen regimen-related toxicities and extend the application of allogeneic transplant to patients with hematologic malignancies who cannot tolerate full intensity transplants, especially older adults, (over 55 years) (1-3). This patient category now constitutes a large part of the transplant population at our institution and many others. Prolonged DFS in the range of 20-65% has been reported using RIC approach in this patient category (1-6).

Despite significant reduction of transplant-related mortality (TRM) achieved by using RIC (from 20-40% to 10-20%), relapse and GVHD continue to be major causes of morbidity and mortality. The rate of severe acute and chronic GVHD reported to be similar to that seen with full intensity, while onset of acute GVHD appears to be delayed. The severity of acute GVHD is graded clinically (I-IV) using a standardized system which takes into account changes in the three primary organs affected (gut, liver and skin) (7-9). Corticosteroids are the standard initial treatment of acute GVHD, but less than 50% of patients have a complete response to steroids. In the remaining patients, GVHD either fails to respond or recurs during the steroid taper. Studies have shown a mortality of 75%-100% in patients with grades III-IV GVHD (10, 11). Even if successful, high doses of corticosteroids are a major source of morbidity and mortality due to increased infections and deconditioning.

Investigator experience: RIC with Tacrolimus/low dose mycophenolate GVHD prophylaxis and standard taper:
In a previous phase II study (*UMCC 9822)* we prospectively tested whether a moderate reduction of the intensity of the preparative regimen would lead to significantly less regimen-related toxicity without compromising tumor control in a cohort of 44 patients. Patients received fludarabine/busulfan based conditioning and tacrolimus/*mycophenolate* were given as prophylaxis for GVHD. The dose of mycophenolate was 1500 mg/day, which is approximately half the dose of mycophenolate used currently. Donors were 5/6 or 6/6 matched family members. The median age was 61 years. Fatal regimen-related organ toxicity occurred in 3 patients. The cumulative incidence of grade 2 to 4 or grade 3 to 4 acute GVHD by day 100 was 38% (95% confidence interval [CI] = 25%, 55%) and 20% (95% CI = 10%, 39%), respectively, with a median time to onset of 66 days. By comparison, the median time to develop acute GVHD following full-intensity conditioning has historically ranged from 16 to 23 days.

For the entire cohort of reduced intensity recipients, 1-year overall survival, disease-free survival, and relapse rates were 54% (95% CI = 41%, 71%), 47% (95% CI = 35%, 65%), and 37% (95% CI = 19%,

51%), respectively. Median survival times were 138 days and 685 days for subjects with advanced (n=19) and non-advanced (n=25) disease, respectively (P =.005). After adjusting for age and comorbidity, disease stage continued to be significantly associated with overall survival (P =.005). (6)

In summary, the reduction in conditioning intensity, as used in UMCC 9822, was associated with similar GVHD rates as myeloablative transplant recipients, although onset was delayed. Patients with poorly controlled disease at time of transplant suffered from high rates of relapse and poor survival.

Investigator experience: RIC with Tacrolimus/Mycophenolate GVHD prophylaxis and standard taper:
In 2004, low dose mycophenolate was replaced by standard mycophenolate (3000 mg/d from day 0 to day 28) as standard GVHD prophylaxis for patients receiving non-protocol RIC HCT. Although a majority of patients during the time period between 2004-2007 were enrolled on a clinical trial (UMCC 2002-61), there were 26 patients who underwent RIC HCT using related donors and not enrolled on a clinical trial. All of these patients received tacrolimus/mycophenolate as GVHD prophylaxis. Compared to the UMCC 9822 study, there were similar proportions of patients with advanced disease (n=10) and non-advanced disease (n=16). GVHD grades 2-4 occurred in 42% of patients. The one-year cumulative incidence of relapse was 19% and two-year overall survival was 60%.

Investigator experience: RIC with Tacrolimus/Mycophenolate GVHD prophylaxis and rapid taper: UMCC 2002-61
In order to improve relapse-free survival in the setting of reduced intensity sibling donor transplants for patients with advanced hematologic malignancies, our group tested a modified Fludarabine / Busulfan based regimen with a more rapid post-transplant taper of immunosuppression, with the taper starting day 28, and completing by day +63 *(UMCC 2002-61)*. The rapid taper was intended to facilitate a GVL effect that hopefully would translate into improved DFS. Those patients who did not develop GVHD by day 100 received donor lymphocyte infusion (DLI). The interim results of this trial are (51 patients) presented here. The rate of acute GVHD grade 2-4 is 56%. The relapse rate at 1 year was 33% and overall 2-year survival is 47%. A comparison of acute GVHD rates, overall survival and relapse rates between the three different approaches (Tacro/low dose MMF, std taper; Tacro/MMF std taper; Tacro/MMF rapid taper) is shown in **Table 1.**

Table 1: Comparison of Different GVHD prophylaxis regimens in RIC HCT

	GVHD 2-4	1yr Relapse Rate	2yr OS
Tacro/lowMMF std taper (n=44)	38%	37%	30%
Tacro/MMF std taper (n=26)	42%	19%	60%
Tacro/MMF rapid taper (n=51)	56%	33%	47%

In summary, there were no statistically significant differences in GVHD rates, relapse rates, or survival between the three prophylaxis regimens. Given that the rapid taper does not result in superior outcomes compared to a standard taper, in this study we will build upon the widely accepted GVHD prophylaxis regimen of tacrolimus/mycophenolate utilizing a standard taper.

Acute GVHD results from a complex interaction of donor T cells and recipient antigen presenting cells (APC) that involves recognition of major and/or minor histocompatibility antigens in an inflammatory milieu. The target tissue damage is thought to derive from direct T cell injury and direct and indirect effects of cytokine such as TNF alpha. The critical role of the host-derived APC in initiation of acute GVHD, and the role of the donor derived APC in maintenance of acute GVHD has been well demonstrated in experimental models. HSCT recipients recently subjected to conditioning regimen toxicity present a pro-inflammatory milieu for T-cell recognition and activation. Several studies have confirmed that the dysregulation of pro-inflammatory cytokines and the loss of gastrointestinal tract integrity resulting in LPS leak contribute to GVHD, whereas the donor cytotoxic responses are critical for graft-versus-leukemia (GVL) preservation.

The ability to reduce GVHD that requires treatment with steroids while preserving alloreactivity directed to leukemic cells would represent a major advance in clinical HSCT and is one of the principal areas of consideration in this proposal.

3.2 Histone Deacetylase Inhibitors (HDACi) and Experimental GVHD

A new group of drugs inhibiting HDAC have been found to possess potent immunomodulatory, and anti-inflammatory properties at nanomolar concentrations in vitro and in vivo. At higher concentrations, anti-tumor activity in a wide variety of tumors has been reported (12-25). The mechanism of action is currently being elucidated, but is known to be mediated by histone acetylation that alters genes expression, and by histone acetylation-independent mechanisms that modify non-histone targets (STAT1, STAT3, RelA, tubulin, cell cycle regulators, Akt etc).

Vorinostat is an orally active, synthetic inhibitor of HDACs that reduces pro-inflammatory cytokine production in primary cells in vitro and exhibits anti-inflammatory effects in vivo (26-28). When cultured human PBMNCs were treated with non-apoptotic concentrations of vorinostat and then stimulated with LPS, a significant reduction TNFα, INF-γ, IL-1β, IL-1α and IL-6 levels were observed. In murine studies, oral administration of 10-25 mg/kg vorinostat has reduced LPS-induced serum TNFα and INFγ levels by > 50% (15). Of interest, anti-CD3-induced cytokines were not suppressed by vorinostat in PBMC either in vitro or in vivo.

Preliminary Studies - Investigator Experience: Given their intriguing anti-inflammatory properties, we tested the two synthetic hydroxamic acid derivatives, (1) vorinostat (SAHA) and (2) ITF 2357, another HDACi, in murine models of acute GVHD and GVL. We have previously demonstrated that brief administration of vorinostat after allogeneic BMT reduced serum levels of the pro-inflammatory cytokines, decreases intestinal histopathology, and reduces GVHD mortality and morbidity from acute GVHD compared with vehicle-treated animals. (29). We have performed a similar series of experiments with vorinostat and ITF 2357. Using the B6→B6D2F1 model of GVHD, we administered 35 mg/kg of vorinostat from day +3 to +7 after BMT and measured serum levels of TNFα, IL-1β and INFγ on day +7 (n=4 per group). **Figure 1** indicates that all three cytokines (TNFα, IL-1β and INFγ) were significantly decreased by administration of vorinostat.

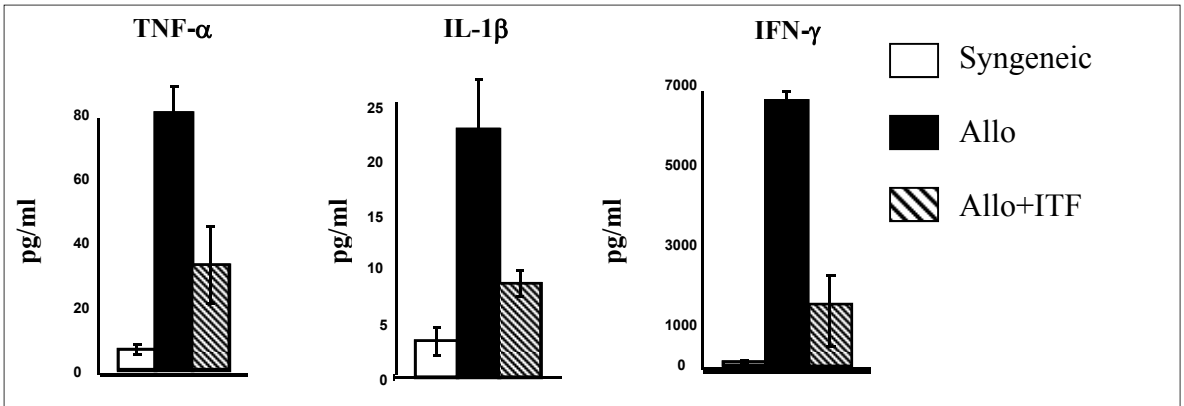


Figure 1. Vorinostat suppresses serum cytokine levels after experimental allogeneic HSCT

We subsequently evaluated the ability of vorinostat to prevent acute GVHD in two strain combinations B6 →B6D2F1 and Balb/c → B6, as shown in **Figure 2.** We administered 25 mg/kg of vorinostat from day +3 to +7 after BMT and evaluated the recipients for GVHD induced mortality. As shown in **Figure 2**, vorinostat significantly prevented the mortality from GVHD (circles, n=10) compared to controls (triangles, n=10) in both models. Syngeneic BMT recipients without GVHD (squares, n=5) had 100% survival.

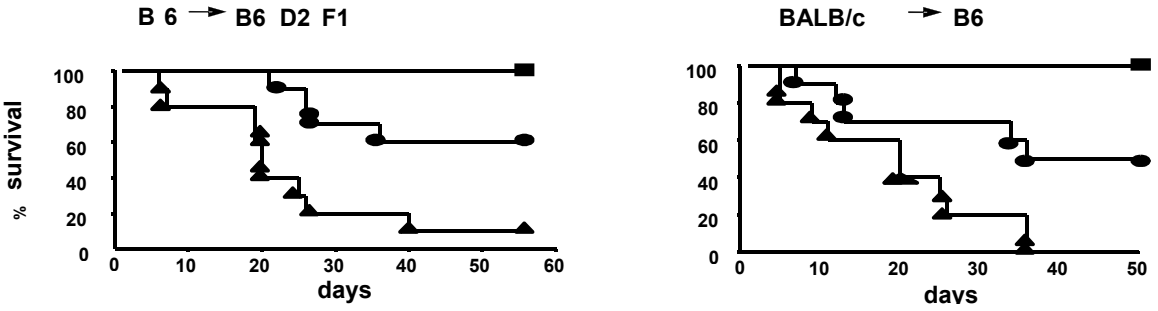


Figure 2. Vorinostat prevents GVHD mortality

In these same murine studies, neither vorinostat nor ITF affected donor T cell proliferative and cytotoxic responses to host antigens. Specifically, vorinostat or ITF did not suppress donor CD3+ cells in the spleen on either day +7 or day +14 after BMT. Additionally, vorinostat and ITF did not suppress the lysis of host type (P815) targets on day +14 after transplant (data not shown).

We tested the effects of vorinostat administration on GVL effects in this BMT model by adding 2,000 P815 cells syngeneic to the host to the BMT inoculum on day 0. The key results are shown in **Figure 3.** All syngeneic BMT recipients (dashed lines, n=10 per group) died of tumor by day 30 after transplant, regardless of treatment. Allogeneic BMT recipients treated with sterile water (thin solid line, n=10) died rapidly of GVHD, whereas those treated with vorinostat (thick solid line, n=10) showed significantly improved survival at 50% ($p < 0.02$). After day 50 all survival animals were killed and none had evidence of infiltration of the liver or the spleen, confirming the preservation of a potent GVL effects.

Further data suggest that the observed effect of HDACi is mediated, at least in part, via modulation of host–derived dendritic cells (DC). We determined that both HDAC inhibitors, vorinostat and ITF 2357, modulated LPS mediated responses of DC's which are professional APCs. Bone marrow cells were cultured with GM-CSF and IL-4 and then were harvested from B6 animals and normalized for CD11c expression. The cells were incubated with either the diluent or vorinostat (at 500 nM) or ITF 2357 (10 or 100 nM) for 16 hours and then stimulated over night with LPS. Pretreatment with either vorinostat or with ITF significantly reduced secretion of TNF-α by the BM derived DCs in response to LPS (**Figure 4**).

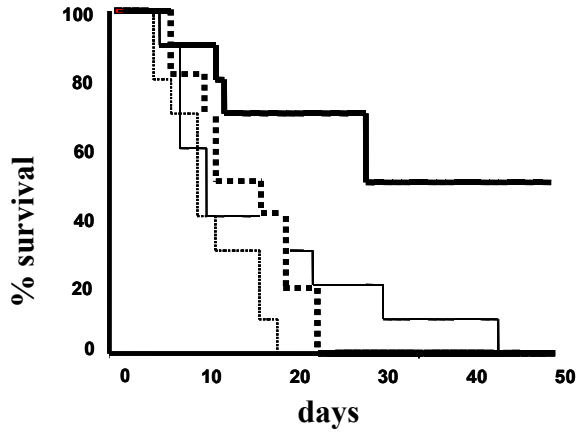


Figure 3. Vorinostat preserves GVL effects

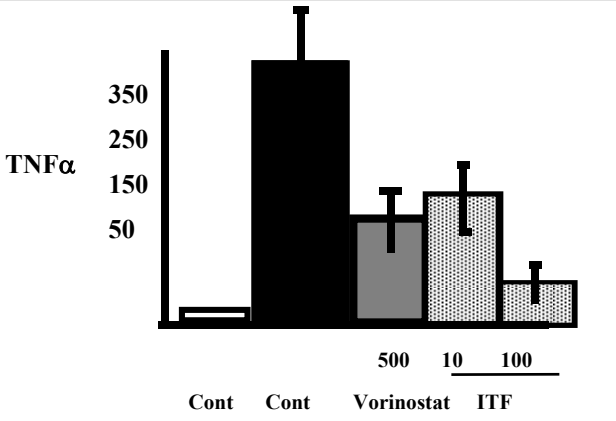
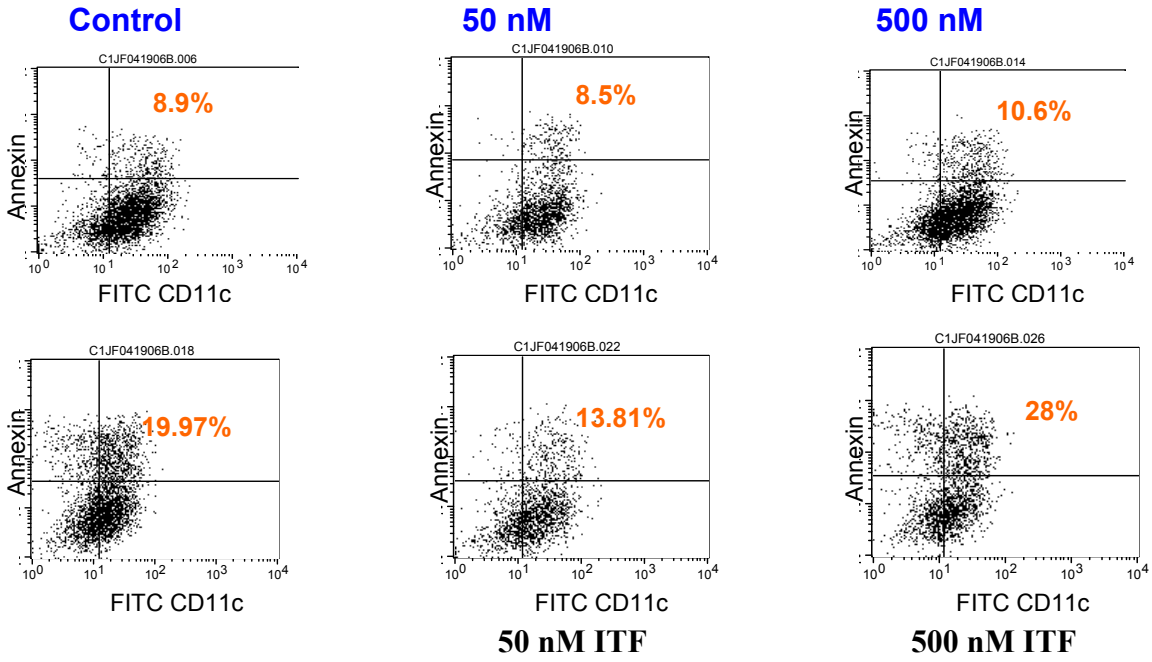


Figure 4: Vorinostat reduced DC derived TNF-α

The HDACi effect on DC also resulted in down-regulation of the surface co-stimulatory molecules (CD40, CD80 & CD86) as well as blunting of other Toll like-receptors (TLR-2,3, & 9)- induced TNF-alpha & IL-6 production (data not shown). Because HDAC inhibitors have been shown to induce cellular apoptosis, we then determined whether the regulation of DC function by vorinostat was secondary to loss of DC viability. As shown in **Figure 5**, we found no significant increase in annexin positive $CD11c^{+}$ cells when pretreated with 500nM of vorinostat or 50nM of ITF 2357, thus ruling out apoptosis as a significant cause of this inhibition.

Indoleamine 2,3-dioxygenase (IDO), an enzyme that degrades tryptophan, has recently been shown to suppress DC functions. We tested the hypothesis that treatment of DCs with HDAC inhibitors enhanced the expression of IDO. DCs were harvested from B6 BM and treated over night with increasing concentration of vorinostat or ITF 2357; the controls were treated with the diluent. The cells were harvested and analyzed for the induction of IDO mRNA by RT-PCR as described in Methods.

Figure 5: Annexin positive cells, vorinostat and ITF 2357



As shown in **Figure 6A**, both vorinostat and ITF 2357 increased the expression of IDO mRNA suggesting that HDAC inhibition increases the transcription of IDO. We next performed Western blot analysis and observed greater expression of IDO protein in these same cellular preparations **(Figure 6B).** We also found similar increase in IDO expression in the DCs harvested from BALB/c animals, thus ruling out any strain dependent artifacts (data not shown). These data demonstrate that the regulatory effects of HDAC inhibitors on DCs correlated with increased expression of the immuno-regulatory enzyme, IDO, both at the mRNA and protein levels.

Figure 6A:

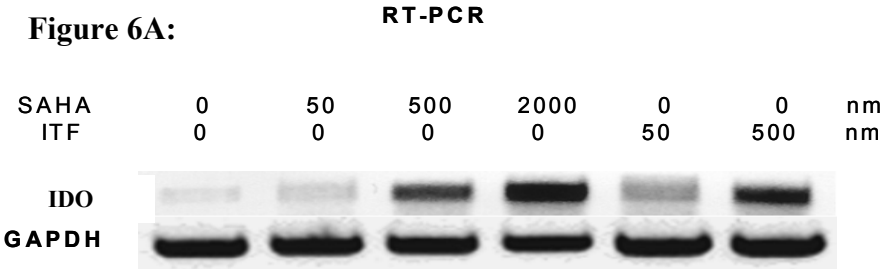
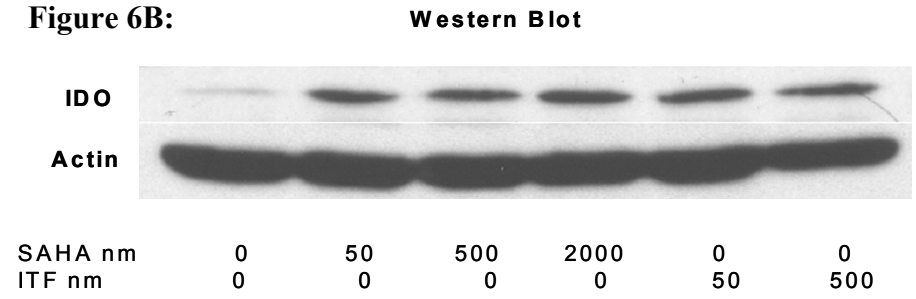


Figure 6B:



The central role of the DC in down-regulation of experimental GVHD was confirmed by the infusion of ex vivo HDACi pre-treated host DC to the experimental animals. We next utilized the well characterized mouse [BALB/c →B6] model of acute GVHD to determine the effect of HDACi-treated DCs on an ongoing GVHD reaction. On day -1, the recipient B6 animals received 11Gy and

were injected with 2 x 10^6 T cells and 5 x 10^6 BM cells on day 0, from either syngeneic B6 or allogeneic BALB/c donors. Injection of 5 million host type HDACi treated DCs to the recipients on days -1, 0, and +2 resulted in significantly better survival (60% vs. 10%, $P < 0.01$), reduced serum levels of TNF-α, donor T cell expansion in the spleen and the presence of GVHD histologically by day +7 after BMT (**Figure 7a-c**).

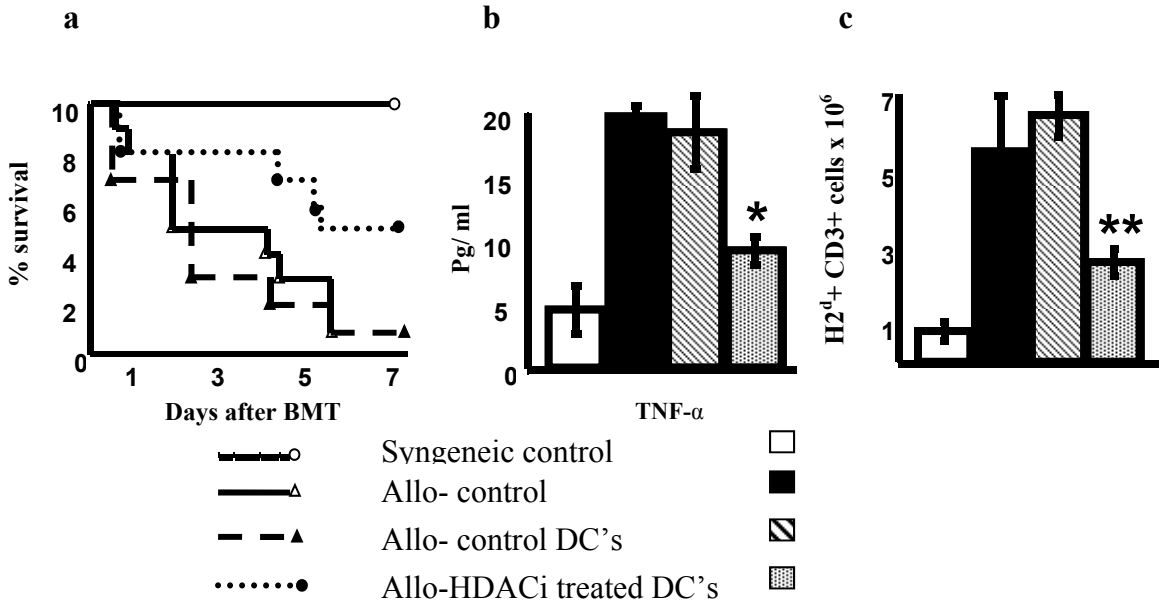


Figure 7. HDACi-treated DC's down-regulate experimental GVHD (BALB/c → B6)

Finally, to determine the clinical applicability of these findings we determined the effect of vorinostat on the DCs derived from the peripheral blood of normal healthy human volunteers and found that vorinostat significantly reduced allogeneic T cell proliferation.

Together our results demonstrate a critical role for histone deacetylase inhibition in the pro-inflammatory events contributing to GVHD and suggest that HDACi may provide a strategy to reduce GVHD while preserving cytotoxic T cell responses to host antigens and maintaining beneficial GVL effects. HDACi exert these favorable effects, at least in part via modulation of innate and allo-stimulatory functions of DCs in vitro and in vivo.

3.2.1 Dose selection

The in vivo data suggests that vorinostat induces acetylation in circulating PBMCs and is well tolerated. Of note, vorinostat is FDA approved and has a favorable safety profile with no reported grade V toxicities both at 100 mg and 200 mg PO BID dosing. Because vorinostat has not previously been tested in this setting, we will begin at 100 mg PO BID. If the lower dose appears safe, we will escalate the dose to 200 mg PO BID in an attempt to enhance efficacy. However, if the 200 mg PO BID dosing appears too toxic, we will de-escalate back to the 100 mg PO BID for the remainder of the study. Because patient safety is of paramount concern, we have adopted a strategy of dose

escalation/de-escalation that allows the potentially more efficacious higher dose to be tested without compromising patient safety.

4.0 STUDY POPULATION

4.1 Inclusion Criteria

4.1.1 Availability of a 7/8 or 8/8 HLA A, B, C and DR identical relative who is willing and able to donate allogeneic stem cells. Serological, low resolution, mid resolution or high resolution molecular typing will be sufficient for HLA-identical siblings. High resolution typing will be required for any mismatched or non-sibling related donor such as parent or child.

4.1.2 To be eligible a patient MUST meet at least **one** of the next three criteria:

a. Any patient ≥ **18 years of age** with a hematological malignancy not considered a candidate for allogeneic myeloablative transplant due to co-morbidities and/or advanced age (≥55 years).
b. Any patient ≥ **18 years of age** who has relapsed following prior autologous or allogeneic transplant for a hematologic malignancy.
c. Any patient ≥ **18 years of age** diagnosed with a hematological malignancy for which reduced intensity transplant is institutionally preferred over myeloablative transplant (eg, chronic lymphocytic leukemia).

4.1.3 Disease Status:

a. For patients with multiple myeloma, CLL, and lymphoma: must be in CR, PR, or stable disease.
b. For MDS, acute leukemia or CML: must have <20% blasts on marrow exam.
c. For all other diseases: must have non-refractory disease.

4.1.4
If the patient or the patient's partner is a woman of childbearing age, the patient and their sexual partner must agree to practice effective contraception.

4.2 Exclusion Criteria

4.2.1 HIV (seropositivity and/or PCR assay), HTLV1 / HTLV2 seropositivity.

4.2.2 Patients with positive serology for Hepatitis B or Hepatitis C must have PCR testing results available prior to admission. Patients may be tested for EBV PCR prior to admission but treatment with vorinostat may begin prior to results being available.

4.2.3 Pregnancy.

4.2.4 Organ function based exclusion criteria: Patients must meet current institutional Clinical Practice Guidelines for minimum reduced intensity transplant organ function; therefore patients with one or more of the following will not be permitted entry into the study:

a. Cardiac: Ejection fraction ≤ 40%

b. Renal: Estimated or actual GFR ≤ 40 ml/min (corrected for BSA)
c. Pulmonary: FEV1, FVC, or DLCO ≤ 40% predicted
d. Hepatic: Total bilirubin ≥3mg% and AST/ALT >5X institutional normal for age
e. Karnofsky score ≤50 (Requires considerable assistance and frequent medical care).

4.2.5 Persistent invasive infections not controlled by antimicrobials. Patients still under therapy for presumed or proven infection are eligible provided there is clear evidence (radiologic and/or culture) that the infection is well controlled. Patients under treatment for infection will be enrolled only after clearance from the Principal Investigator.

4.2.6 History of QT prolongation syndrome or prolonged QTc interval on ECG. Prolonged QTc is defined as > 0.45 sec in males and > 0.46 sec in females.

4.2.7 Any physical or psychological condition that, in the opinion of the investigator, would pose unacceptable risk to the patient.

4.2.8 Any patients that are currently taking any HDAC inhibitors or have taken an HDAC inhibitor within 30 days of the trial.

4.2.9 Any patient less than 18 years of age.

4.2.10 Nursing mothers are excluded from this study

4.3 Subject Screening and Registration Procedure

Patient registration for this trial will be centrally managed by the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log provided by the Clinical Trials Office.

It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the Clinical Trials Office. After patient eligibility has been determined, a copy of the **completed** Eligibility Worksheet together with all the pertinent de-identified source documents will be submitted by the requesting site to the Clinical Trials Office, either by fax (734-232-0744) or by email to CTO-Multisite@med.umich.edu.

A Multi-Site Coordinator of the Clinical Trials Office, who acts as the registrar, will review the submitted documents and process the registration. Sites should inform the Multi-Site Coordinator of a potential registration by 5 p.m. on the day prior to registration. Same day registrations cannot be guaranteed.

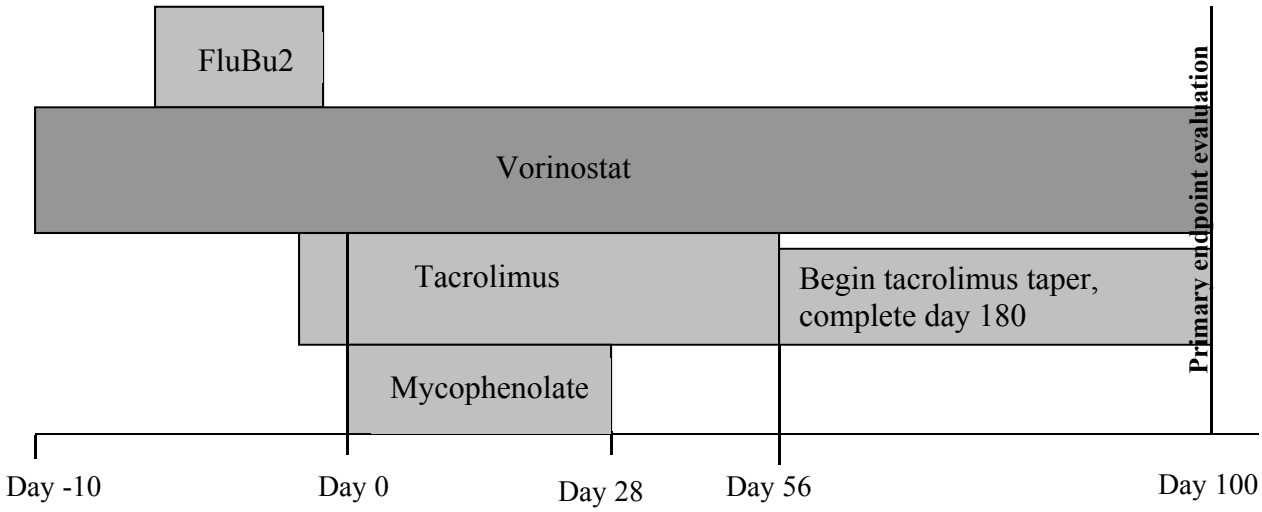
An email will be sent by the registrar to the requesting site registrar to confirm patient registration and to provide the study identification number that has been assigned to the patient. In addition, a copy of the completed Section Two of the Eligibility Worksheet signed and dated by the registrar, will be faxed back to the requesting site registrar.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not have study identification number assigned to them, and will not receive study treatment.

5.0 STUDY DESIGN

The proposed trial is an open label, multi-site, non-randomized, phase II clinical trial **comparing AGVHD rate in recipients of reduced intensity allogeneic transplant who received vorinostat along with standard post-transplant immunosupression to a historic control group. Up to two dose levels of vorinostat will be tested (see section 13).**

If the proposed pilot trial is successful, we plan to expand the study to a multi institutional level via CTN (Clinical Trial Network).



5.1 Patient Evaluations

See "pre-therapy" required observations (section 6.0). An IRB-approved informed consent must be obtained from patients (or legal guardians) prior to the initiation of treatment on this protocol. Patient demographics, including underlying disease, donor type, and eligibility criteria will be recorded at entry into the study. All screening evaluations will be completed as part of the standard work up for HSCT.

5.2 Allogeneic Stem Cell Collection and Donor Mobilization

Stem cells are to be collected and infused as per the institutional Clinical Practice Guidelines.

5.3 Conditioning Regimen

The transplant conditioning regimen will consist of a fludarabine plus busulfan ($FluBu_2$) regimen with adjustment of dosing for overweight patients based on institutional Clinical Practice Guidelines.

Fludarabine / Busulfan (FluBu2) regimen.

Fludarabine: 40 mg/m^2/day in 0.9 NS, administered IV on days -5 to day -2 pre-transplant for a total of 4 doses.

Busulfan: 3.2 mg/kg in 0.9 NS administered IV on days –5 and –4 for a total of 2 doses.

Fludarabine will be administered prior to the busulfan on days –5 and –4.

Total body irradiation 200 cGy delivered in a single fraction will be given on day 0 for patients receiving an HLA-mismatched transplant. Total lymphoid irradiation (TLI) 400 cGy can be substituted for TBI. Patients receiving an HLA-matched transplant may receive TLI or TBI per treating physician's discretion.

Seizure prophylaxis for busulfan will be administered according to the institutional Clinical Practice Guidelines.

5.4 Graft versus Host Disease (GVHD) Prophylaxis

5.4.1 Tacrolimus.

Tacrolimus begins on day -3 and is dosed IV or orally according to the institutional Clinical Practice Guidelines, with a desired trough level of 8-12 ng/ml in the absence of clinical indications for a different target (eg, hyperkalemia, elevated creatinine level). Tacrolimus levels outside of the desired range are not considered protocol deviations.

Cyclosporine can be substituted for tacrolimus in the event of patient intolerance to tacrolimus and will not be considered a protocol deviation.

It is suggested that tacrolimus be tapered starting around day 56 post-transplant, provided the patient has no evidence for GVHD at that time (**Table 2**).

Tacrolimus Taper Schedule:

Day post transplant Tacrolimus Dose
Day 57 - 89: 80% dose
Day 90-119: 60% dose
Day 120-149: 40% dose
Day 150-179: 20% dose
Day 180: Discontinue Tacrolimus

Table 2: Tacrolimus Dosing During Taper

Total daily dose (mg) on day 56	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
day 57	1.5	2.0	2.5	3.0	3.5	3.5	4.0	4.5	5.0	5.0	5.5	6.0	6.5
day 90	1.0	1.5	2.0	2.0	2.5	3.0	3.0	3.5	3.5	4.0	4.5	4.5	5.0
day 120	0.5	1.0	1.5	1.5	1.5	2.0	2.0	2.0	2.5	2.5	3.0	3.0	3.5
day 150	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5	1.5 qod
day 180	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The daily tacrolimus dose should generally be divided into two equal doses administered 12 hours apart.

5.4.2 CellCept (Mycophenolate): 1g PO TID starting on day 0 and ending on day 28 post-transplant. Mycophenolate can also be given intravenously at a dose of 10 mg/kg/dose maximum 1 gram/dose), administered every 8 hours if the patient is unable to tolerate oral medications. The conversion is 100% of the oral dose.

5.5 Administration of Vorinostat:

5.5.1 Vorinostat will be administered at a dose 100 mg PO BID starting day -10 in order to achieve steady state prior to beginning conditioning chemotherapy. Dose holds (of more than 14 days) due to a patient's inability to tolerate oral medications will be considered deviations. If tolerated, the vorinostat will be continued until day 100 post-transplant, whether or not acute GVHD develops.

5.5.2 Dose escalation/ de-escalation

Ten patients will be treated at a dose of 100 mg PO BID. If dosing modifications are not required, during the pre-engraftment period in more than 4 patients, **AND** there are no more than two observed toxicities CTCAE v. 3 grade 4 or higher considered probably or definitely related to the drug, then the dose of vorinostat will be escalated to 200 mg PO BID.

If dose escalation does not occur due to failure to meet the above criteria, then the entire study population will enroll at the 100 mg PO BID dosing subject to the stopping rules outlined in section 13.

If dose escalation occurs, patients will be treated at 200 mg PO BID dosing unless the probability of unacceptable toxicity exceeds the rules outlined in section 13.3. In that event, the dose of vorinostat will be de-escalated back to 100 mg PO BID for the remainder of study accrual.

5.5.3 Dosing modifications during pre-engraftment period: (engraftment defined: 10.8)

The following rules will be applied in the pre-engraftment period:

a. If ANC <500/uL on day 21: **HOLD** vorinostat until ANC > 1000, then resume at 100% dose.
b. If platelets <20,000/uL on day 21: **HOLD** vorinostat until platelet count > 30,000/ul (without platelet transfusion support x 3 consecutive days), then resume at 100% dosing.

5.5.4 Dosing modifications after primary engraftment:

a. If ANC decreases to 500 – 1000/uL, **CONTINUE** vorinostat at 100% dosing. Begin G-CSF.
b. If the ANC decreases to <500/uL, **HOLD** vorinostat until ANC recovers to >1000/uL, then **RESTART** vorinostat at 100% dosing.
c. If ANC decreases to < 500/uL again, **HOLD** vorinostat until the ANC recovers to >1000/uL, then **RESTART** vorinostat at 50% dosing. May resume 100% dosing of vorinostat when G-CSF is discontinued, provided the ANC remains >1000/uL.
d. If the platelet count decreases to < 30,000/uL, **HOLD** vorinostat until platelet count recovers to >30,000/uL (without platelet transfusion support x 3 consecutive days) then **RESTART** at 100% dosing.
e. If platelet count decreases to <30,000 /uL again, **HOLD** the vorinostat until the platelet count recovers to >30,000/uL (without platelet transfusion support x 3 consecutive days), then **RESTART** vorinostat at 50%. After one week of 50% dosing, may resume 100% dosing provided the platelet count remains >30,000/uL.

Depending on the patients' clinical status, dose adjustments may be made within 1 week from when the lab work is completed.

5.5.5 The dose of vorinostat will be adjusted based upon serum creatinine levels and liver function abnormalities (**Table 3**). Depending on the patients' clinical status, dose adjustments may be made within 1 week from when the lab work is completed. It can be anticipated that most cases of liver dysfunction after a reduced intensity conditioning will be due to acute GVHD rather than due to veno-occlusive disease (VOD), and continuation of vorinostat may be desirable in this setting. If other potential etiologies (infections, TPN, other medications etc.) are evident or strongly suspected, the drug can be held, and later resumed at the discretion of the treating physician and PI according to the parameters in **Table 3**.

Table 3. Vorinostat dosing modification based upon renal and hepatic function

	MILD	**MODERATE**	**SEVERE**
Serum creatinine	> 1.5 –2.0 x ULN	> 2.0 – 2.5 x ULN	> 2.5 x ULN
AST/ALT	>200 – 399 U/L	> 400 U/L	> 700 U/L
Total bilirubin	2 – 4 mg/dl	4.1 – 8 mg/dl	8.1 – 30 mg/dl
vorinostat Dose Reduction	50% reduction	75% reduction (if daily dose = 200 mg/d then 100 mg every other day, if daily dose = 400 mg/d then 100 mg/day)	hold

5.5.6 For gastrointestinal (GI) symptoms thought to be related to vorinostat: The vorinostat dose will be modified according to the severity of the GI side effects as described in **Table 4**. Depending on the patients' clinical status, dose adjustments may be made within 1 week from when the lab work is completed. Patients who require 50% dose reduction, will have their dose reduced for 7 days and may resume 100% dosing thereafter at physician discretion. A trial of anti-emetics and anti-motility agents

should be considered (institutional Clinical Practice Guidelines) before modifying the dose of vorinostat. Vorinostat should continue for patients who develop GI symptoms related to infection.

5.5.7 For GI GVHD (stage 1-4): Vorinostat will be continued at 100% dosing if acute gastrointestinal GVHD develops. If the patient develops GI GVHD symptoms (diarrhea, or nausea /vomiting) that persist > 5 days despite treatment using methylprednisolone (2mg/kg/day), hold the vorinostat. If no improvement in GI symptoms occurs after holding the vorinostat for 5 days, the symptoms will be deemed related to gastro-intestinal GVHD and the vorinostat can be resumed at 50-100% dosing at the discretion of treating physician and PI.

5.5.8 Special rule for toxicity observed up to day +7 post transplant: Because of potential interactions between vorinostat and the conditioning regimen, vorinostat will be **STOPPED** for any non-hematological CTCAE version 3.0, grade 4 or higher toxicities seen between first dose of study drug and day +7 post-transplant. If there is complete reversal of toxicity, vorinostat can be resumed after 7 days and a minimum of one week off study drug. In the absence of complete reversal of toxicity within 7 days, the study drug will be **DISCONTINUED** and the patient removed from the study. All such patients will remain evaluable for analysis and not replaced.

Table 4

Severity:	**Mild**	**Moderate**	**Severe**	**Life threatening**
Diarrhea	Grade 1: <4 stools/day over baseline	Grade 2: Increase 4-6 stools/day over baseline; not interfering with ADL.	Grade 3: After failure of antimotility agents and antiemetics (Increase >7 stools/day over baseline; TPN; interfering with ADL)	Grade 4: Life-threatening consequences
vorinostat Dose reduction	0%	No GI GVHD documented: Discontinue ONLY after failure of antimotility agents and antiemetics. Resume at 50% dose once the GI toxicity has resolved to grade 0-1. + GI GVHD: see guidelines 5.5.7		No GI GVHD: Discontinue permanently. + GI GVHD: see 5.5.7

Table 5

Severity:	**Mild**	**Moderate**	**Severe**	**Life threatening**
Nausea/Vomiting	Grade 1: Loss of appetite without alteration in eating habits; 1 episode of vomiting w/i 24 hrs.	Grade 2: Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated <24 hrs; 2 – 5 episodes of vomiting in 24 hrs	Grade 3: Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated ≥24 hrs; ≥6 episodes of vomiting in 24 hrs;	Grade 4: Life-threatening consequences
vorinostat Dose reduction	**0%**	No GI GVHD Documented: After failure of antimotility agents and antiemetics, may reduce dose by 50% (at treating	No GI GVHD Documented: After failure of antimotility agents and antiemetics,	No GI GVHD: Discontinue permanently. + GI GVHD: see

		physician's discretion) + GI GVHD: see 5.5.7	must reduce dose by 50% + GI GVHD: see 5.5.7	5.5.7

5.6 Post-transplant Supportive Care

5.6.1 Post-transplant supportive care, including infection prophylaxis for CMV and fungal infections will be conducted as per the Clinical Practice Guidelines.

5.6.2 To minimize drug induced myelosuppressive effects post-transplant, trimethoprim-sulfamethoxazole (Bactrim, Septra) will not be administered within the first 100 days post-transplant or until after the last dose of vorinostat, whichever comes first. Instead, intravenous or aerosolized pentamidine will be for PCP prophylaxis; other non-immunosuppressive prophylaxis may be substituted at physician discretion.

5.6.3 It is recommended that Vantin 200mg BID be used for bacterial prophylaxis in place of Levofloxacin (Levaquin) and to avoid medications that can prolong the QT interval.

5.6.4 Granulocyte colony stimulating factor (G-CSF, Neupogen) will be administered post-transplant per institutional Clinical Practice Guidelines.

5.6.5 It is recommended that the serum potassium be maintained > 4.0 mmol/L and magnesium > 2.0 meq/L, and that intermittent ECG monitoring for QT interval prolongation be performed as detailed in **Table 5**.

5.7 Pharmacokinetic (PK) studies

Pharmacokinetic (PK) studies will be collected on a total of 5 subjects who consent to the PK portion of the study (UM subjects only), preferably on day +1 following transplant. If day +1 falls on a weekend (Saturday or Sunday) or a holiday, the samples will be collected on the following regular business weekday, whenever possible, preferably on Monday when day +1 falls on the weekend.

5.7.1 Specimen Collection / Documentation

Prior to study drug (vorinostat) administration on day +1 following transplant, an indwelling heparin lock should be placed so that serial specimens can be collected. At each sampling time, 1 mL of blood will be withdrawn and discarded to assure that the solution used to maintain catheter patency does not dilute the sample. Even if a patient has a central venous catheter, it is preferable for PK samples to be withdrawn through a peripheral heparin lock. However, if the patient objects or has problems with peripheral venous access, the central venous catheter may be used for PK sampling. In the event that the central venous catheter is used, sufficient blood should be withdrawn before each PK sample to assure that the solution used to maintain catheter patency does not dilute the PK sample. It is important to document whether the sample was collected through a heparin lock or central venous catheter, especially for day 1 sampling.

Serum samples will be obtained on the following timepoints: Pre-dose (immediately before 1st dose of that day, then 0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, and 12 hr (immediately before 2nd dose of that day) following the 1st dose of that day. The acceptable time frame to obtain these samples will be ± 10 minutes.

5.7.2 Blood Sample Processing Procedures

1. 5 ml of blood will be collected in a red-top vacutainer tube (without anticoagulant) (BD Franklin Lakes, NJ) at the above-mentioned time-points.
2. Allow the blood sample to clot in room temperature for approximately 30 minutes.
3. Centrifuge the clotted sample at 2,000 X g for 15 minutes at 4 °C.
4. The resulting serum samples will be transferred to 3.6 mL NUNC internal thread round bottom cryotubes (NUNC 366524) with de-identified labels attached.
5. Samples will be immediately stored at -70C until transfer to central PK lab on DRY ICE.

At the time the samples are collected, they will be attached with a standard UMHS patient label which will include the patient's name, date of birth, and medical record. Once the clotted samples are centrifuged, the resulting serum samples will be transferred to a re-labeled cryotube that has been de-identified. Blood samples and all records associated with blood samples will be labeled only with a numeric code that contains no personal identifiers. A link will be created between the patient's name and the numeric code by using the last 5 or 6 digits (out of 8) of the patient's CPI number. This link will eliminate the possibility of transmission of privileged health information and will not be made available to the recipient investigator. This link between the de-identified numeric code label and the patient's identifiers will be maintained in a dedicated secure and HIPAA-compliant database that only the PI and Co-I have access to. The file will contain the patient's name, medical record number, date of birth, time of vorinostat administration, time of sample acquisition and any problems with sample acquisition. Samples will be stored at -70 degrees Celsius until shipment to the University of Pittsburgh Cancer Institute. Care will be taken to insure that labels are carefully attached to tubes and are not likely to fall off when frozen or during shipping.

5.7.3 Shipping Instructions

Samples will be de-identified and then shipped to the following name and address:

Dr. Jan H. Beumer
University of Pittsburgh Cancer Institute Room G27E, Hillman Research Pavilion
5117 Centre Avenue
Pittsburgh, PA 15213-1863

Samples will be shipped on dry ice without thawing. Samples will be wrapped in paper toweling or absorbent under-padding (benchkote) and then placed into the package; samples will not to be placed in direct contact with dry ice. They should not come into direct contact with dry ice, which might lead to cracking of the tubes. Samples will be accompanied by the appropriate sample acquisition and shipping form that indicates the material indicated in the above section of the protocol and also indicates the condition of the sample upon shipment, the name of the

individual shipping the samples and a phone number, fax number and e-mail contact for confirmation of sample receipt in Pittsburgh. Samples should be shipped to Pittsburgh by overnight express mail and should only be shipped on Monday, Tuesday, or Wednesday to insure that samples do not arrive on Saturday or Sunday. Before shipping samples, the PI or Co-I will notify Dr. Beumer's office by calling 412-623-1213 or Dr. Beumer's Laboratory by calling 412-623-3248 or by sending a fax to Dr. Beumer at 412-623-1212. Any residual sample will be destroyed at the end of this project or returned to the University of Michigan storage facility.

6.0 REQUIRED OBSERVATIONS

All pre-therapy studies should be performed within 21 days of study entry. **Table 6** indicates the observations required for each study timepoint:

Table 6: Required observations

	Pre-therapy	Days Post-Transplant[12]			
		30	100	180	1 year
History and physical exam	X	X	X	Routine Post-Transplant Monitoring	
CBC with differential and platelets[1]	X	X[1]	X[1]		
Serum chemistries[2]	X	X[2]	X[2]		
Viral screens [3]	X				
Pregnancy test (if applicable)	X				
Karnofsky performance status	X				
ECG[4]	X				
Disease status evaluation [per CPG]	Per CPG				
Acute GVHD assessment[Per CPG]	Per CPG				
Research samples[7] (plasma protein assays)	X	X	X		
Pharmacokinetic samples[10]	X				

See footnotes 9, 10, 11,12

Footnotes:

1. CBC: Obtain pre-therapy, as clinically indicated while admitted. Upon discharge CBC will be drawn every 7 to 14 days, through day 60, and as clinically indicated thereafter. Dose adjustments will be made as per section 5.5. Patients no longer receiving study medication will be monitored as clinically indicated.

2. Serum chemistries: Serum creatinine, AST, ALT, and total bilirubin need to be obtained pre-therapy and as clinically indicated while admitted. Upon discharge Serum Chemistries will be drawn every 7 to 14 days, through day 60, and as clinically indicated thereafter. Dose adjustments will be made as per section 5.5. Patients no longer receiving study medication will be monitored as clinically indicated.

3. Serologic and/or PCR based viral screens: Obtain pre-therapy according to institutional standard practice guidelines, which may include testing for *HIV, HTLV1, HTLV2, Hepatitis B, Hepatitis C, and EBV* .

4. *ECG*: Obtain pre-therapy.
4.1 ECG monitoring will be performed at least once a week during the first 30 days of vorinostat therapy or every 2 weeks during the first 60 days of vorinostat therapy.

5. *Disease status evaluation:* Guidelines for disease status evaluation, including myeloma studies, cerebrospinal fluid analysis for acute leukemias, and radiographic imaging for lymphomas (CT, MRI, FDG PET scans, as appropriate) shall follow institutional standard practice guidelines.

6. *GVHD assessment:* (see section 8.0)

7. *Research samples for plasma protein assays*: Collect 12 - 18 ml of peripheral blood into heparinized green top tubes starting on approximately day pre-therapy, day +30, and +100. All samples will be processed, frozen and banked in the Core Immunology Lab. Analyses will be performed for protein levels of a panel of inflammatory mediators, including but not limited to the TNF pathway. (Samples will be collected from University of Michigan patients only).

8. *Research samples for pharmaco-dynamic (PD) / acetylation studies:* PBMC will be isolated and frozen for pharmaco-dynamic assays of acetylation according to the protocol (see Appendix II). The time of administration of the drug and the time of sample collection for PD/acetylation status should be recorded in the "Sample collection form" (see Appendix V). This form is to be sent with the sample when it goes for analysis. Samples will be collected in heparanized green tops for a total of approximately 15ml any time prior to the first dose of vorinostat and on day 30, and day 100 post-transplant. (Samples will be collected from University of Michigan patients only).

Samples will be batched and run simultaneously in order to maximize efficiency and enhance comparison of samples.

9. *Additional Research samples:* Additional samples will be collected whenever a new diagnosis of acute or chronic GVHD is made, whenever there a significant change in the patient's medical condition (in the opinion of the investigator), including potential relapse. (Samples will be collected from University of Michigan patients only).

Obtaining samples for research purposes are secondary, not primary objectives of this study. Thus, failure to have a research sample drawn at any time point will not be considered a protocol violation.

10. *Pharmacokinetic samples:* Pharmacokinetic (PK) studies will be collected on a total of 5 subjects who consent to the PK portion of the study. Prior to study drug (vorinostat) administration on day +1 following transplant, serum samples (5 mL) will be obtained on the following timepoints: Pre-dose (immediately before 1st dose of that day, then 0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, and 12 hr (immediately before 2nd dose of that day) following the 1st dose of that day. (Samples will be collected from University of Michigan patients only).

11. *Post transplant observations:* Patient condition and scheduling issues may impact the timing of post-transplant observations. The acceptable time frame for completing these observations is ± 10 days for day 30 observations, ± 14 days for day 100 observations, ± 30 days for day 180 observations, and ±

45 days for annual observations. Failure to obtain research blood collections in accordance with the above schedule will not be considered a protocol deviation.

12. Procedures performed prior to study target ranges, for clinical reasons (such as bone marrow biopsies), will be accepted at the PI's discretion and not considered a deviation.

7.0 DRUG INFORMATION

7.1 Fludarabine (Fludara, FAMP)

7.1.1 Formulation

The chemical name for fludarabine phosphate is 9H-Purin 6-amine, 2-fluoro-9-(5-O-phosphono-D-arabinofuranosyl). The molecular formula of fludarabine phosphate is C10H13FN5O7P (MW 365.2). A fluorinated nucleotide analog of the antiviral agent vidarabine, 9-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase.

7.1.2 Availability

Each vial of sterile lyophilized solid base contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of sterile water for injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration.

Fludarabine is available as a 25 mg/ml solution for injection and as powder for injection (Fludara). Both products are stored in the refrigerator (2° to 8° C / 36° to 46° F).

7.1.3 Storage and stability

Reconstituted Fludarabine IV is chemically and physically stable at room temperature or 48 hours if refrigerated. In addition, reconstituted fludarabine (IV) contains no antimicrobial preservative and thus care must be taken to ensure that there is sterility of the prepared solutions. Prepared solutions should be discarded 8 hours after initial use.

Reconstituted powder vials are stable for 16 days at room temperature or refrigerated. Solution diluted in 0.9%NaCl or dextrose in water are stable for 48 hours at room temperature or the refrigerator. However, as stated above, reconstituted and diluted solution do not contain antimicrobial preservative and should be used within 8 hours.

7.1.4 Administration

Fludarabine should be administered intravenously over a period of approximately 30 minutes.

7.1.5 Expected toxicity, dose modifications, and management

Fludarabine can cause diarrhea, nausea, vomiting, and skin rash. Fludarabine can temporarily lower the number of white blood cells, which help defend the body against infection and other diseases in your blood. This can increase patient's chance of getting an infection. It can also lower the number of platelets, which are necessary for proper blood clotting, resulting in easy bruising and excessive bleeding from wounds. Less common side effects are aching muscles, general feeling of discomfort or illness, headache and loss of appetite. Rare side effects include cough or hoarseness, fever or chills, loss of vision, lower back or side pain, painful or difficult urination. Rarely, fludarabine can cause a temporary loss of hair in some people.

7.2 Busulfan (Myleran)

7.2.1 Formulation

Busulfan is a bifunctional alkylating agent known chemically as 1,4-butanediol, dimethanesulfonate with a molecular formula of H3SO2O(CH2)4OSO2CH3 and a molecular weight of 246.

7.2.2 Availability

Busulfan is commercially available for either intravenous (Busulfex) administration or orally in 2 mg oral tablets.

7.2.3 Administration

Intravenous Busulfan (Busulfex) should be diluted in 0.9NS and administered by intravenous infusion over 3 hours. Busulfan is a potent cytotoxic drug that causes profound myelosuppression and emesis at the recommended dosage. It should be administered under the supervision of a qualified physician who is experienced in allogeneic hematopoietic stem cell transplantation, the use of cancer chemotherapeutic drugs and the management of patients with severe pancytopenia.

7.2.4 Expected toxicity, dose modifications, and management

Busulfan can cause scarring of the lung tissue, nausea and vomiting, seizures, diarrhea, impotence, sterility, generalized skin pigmentation, scarring of the heart muscle, testicular atrophy, cataracts and the development of a new cancer.

7.3 Tacrolimus (Prograf, FK506)

7.3.1 Formulation

Tacrolimus, previously known as FK506, is the active ingredient in Prograf. Tacrolimus is a macrolide immunosuppressant produced by Streptomyces Tsukubaensis. Tacrolimus has an empirical formula of C44H69NO12.H2O and a formula weight of 822.05. Tacrolimus appears as white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol, and very soluble in methanol and chloroform. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and

calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the generation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

7.3.2 Availability and administration

Prograf is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 1-mg capsule shell contains gelatin and titanium dioxide, and the 0.5 mg and 5-mg capsules shell contains gelatin, titanium dioxide and ferric oxide. Prograf is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL for administration by intravenous infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v. Prograf injection must be diluted with 0.9% sodium chloride injection or 5% dextrose injection before use.

Intravenous administration will be given by continuous infusion. Oral preparation will be administered on empty stomach every 12 hours.

7.3.3 Potential Side Effects

a. Increased susceptibility to infection and the possible development of lymphoma may result from immunosuppression.
b. Nephrotoxicity has been noted in 40% and 33% of liver transplantation patients receiving Prograf in the U.S. and European randomized trials, respectively. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. A lymphoproliferative disorder (LPD) related to Epstein - Barr virus (EBV) infection has been reported in immunosuppressed organ transplant recipients. The risk of LPD appears greatest in young children who are at risk for primary EBV infection while immunosuppressed or who are switched to Prograf following long-term immunosuppression therapy.
c. Mild to severe hyperkalemia has been noted in 44% and 10% of liver transplant recipients treated with Prograf in the U.S. and European randomized trials and may require treatment.
d. Neurotoxicity, including tremor, headache, and other changes in motor function, mental status, and sensory function were reported in approximately 55% of liver transplant recipients in the two randomized studies. Tremor and headache have been associated with high whole-blood concentrations of tacrolimus and may respond to dosage adjustment. Seizures have occurred in adult and pediatric patients receiving Prograf. Coma and delirium also have been associated with high plasma concentrations of tacrolimus.
e. Hypertension is a common adverse effect of Prograf therapy. Mild or moderate hypertension is more frequently reported than severe hypertension. Antihypertensive therapy may be required; the control of blood pressure can be accomplished with any of the common antihypertensive agents. Since tacrolimus can cause hyperkalemia, potassium-sparing diuretics should be avoided. While calcium-channel blocking agents can be effective in treating Prograf-associated

hypertension, care should be taken since interference with tacrolimus metabolism may require a dosage reduction.

f. Hyperglycemia was associated with the use of Prograf in 47% and 29% of liver transplant recipients in the U.S. and European randomized studies, respectively and may require treatment.

7.4 Mycophenolate (CellCept)

7.4.1 Formulation and mechanism of action

CellCept (mycophenolate mofetil) is the 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent; inosine monophosphate dehydrogenase (IMPDH) inhibitor. The chemical name for mycophenolate mofetil (MMF) is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. It has an empirical formula of C23H31NO7, a molecular weight of 433.50.

Mycophenolate mofetil is a white to off-white crystalline powder. It is slightly soluble in water (43 μg/mL at pH 7.4); the solubility increases in acidic medium (4.27 mg/mL at pH 3.6). It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol. Mycophenolate mofetil hydrochloride has a solubility of 65.8 mg/mL in 5% dextrose injection USP (D5W). The pH of the reconstituted solution is 2.4 to 4.1.

Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to form MPA, which is the active metabolite. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Addition of guanosine or deoxyguanosine reverses the cytostatic effects of MPA on lymphocytes. MPA also suppresses antibody formation by B-lymphocytes. MPA prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 and interleukin-2, but did block the coupling of these events to DNA synthesis and proliferation.

7.4.2 Availability

CellCept is available for intravenous or oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/mL mycophenolate mofetil.

Inactive ingredients in CellCept 250 mg capsules include croscarmellose sodium, magnesium stearate, povidone (K-90) and pregelatinized starch. The capsule shells contain black iron oxide, FD&C blue #2, gelatin, red iron oxide, silicon dioxide, sodium lauryl sulfate, titanium dioxide, and yellow iron oxide.

Inactive ingredients in CellCept 500 mg tablets include black iron oxide, croscarmellose sodium, FD&C blue #2 aluminum lake, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, povidone (K-90), red iron oxide, talc, and titanium dioxide; may also contain ammonium hydroxide, ethyl alcohol, methyl alcohol, n-butyl alcohol, propylene glycol, and shellac.

Inactive ingredients in CellCept Oral Suspension include aspartame, citric acid anhydrous, colloidal silicon dioxide, methylparaben, mixed fruit flavor, sodium citrate dihydrate, sorbitol, soybean lecithin, and xanthan gum.

CellCept Intravenous is the hydrochloride salt of mycophenolate mofetil. The chemical name for the hydrochloride salt of mycophenolate mofetil is 2-morpholinoethyl (E)-6- (1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride. It has an empirical formula of C23H31NO7 HCl and a molecular weight of 469.96.

CellCept Intravenous is available as a sterile white to off-white lyophilized powder in vials containing mycophenolate mofetil hydrochloride for administration by intravenous infusion only. Each vial of CellCept Intravenous contains the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt. The inactive ingredients are polysorbate 80, 25 mg, and citric acid, 5 mg. Sodium hydroxide may have been used in the manufacture of CellCept Intravenous to adjust the pH. Reconstitution and dilution with 5% dextrose injection USP yields a slightly yellow solution of mycophenolate mofetil, 6 mg/mL.

7.4.3 Administration

The dosing for oral and intravenous mycophenolate are the same. For oral dosing, doses should be rounded to the nearest 250 mg. The oral solution (200 mg/ml) may be substituted for capsules or tablets at patient preference or physician discretion, in which case, the dose should be rounded to the nearest 100 mg (0.5 ml).

7.4.4 Adverse events

The principal adverse reactions associated with the administration of CellCept include diarrhea, leukopenia, sepsis, vomiting, and a higher frequency of infections, including opportunistic infection. The adverse event profile is similar regardless of the form administered.

7.5 Vorinostat

Vorinostat will be supplied by Merck for patients participating in this clinical trial.

7.5.1 Formulation

The chemical name for vorinostat is suberoylinalide hydroxamic acid.

7.5.2 Availability

Vorinostat is available for clinical use only in oral formulations.

7.5.3 Storage and stability

The samples will be stored per the manufacturer's recommendation. Samples will be collected from University of Michigan patients only.

7.5.4 Pharmacokinetics

Drug Concentration Levels
Peak Concentration: The peak concentrations (Cmax), at steady state in the fed-state following oral administration of multiple 400-mg doses of vorinostat, was 1.2 +/- 0.53 micromolar (Prod Info ZOLINZA(TM) oral capsules, 2006).

Time to Peak Concentration: The median time to maximum concentration (Tmax), at steady state in the fed-state following oral administration of multiple 400-mg doses of vorinostat, was 4 hours (range, 0.5 to 14 hours) (Prod Info ZOLINZA(TM) oral capsules, 2006).

Area Under the Curve: The mean area under the curve (AUC), at steady state in the fed-state following oral administration of multiple 400-mg doses of vorinostat, was 6 +/- 2 micromolar x hour (mcM x h) (Prod Info ZOLINZA(TM) oral capsules, 2006).

Absorption: High-fat meal resulted in a 33% increase in the extent of absorption and a 2.5-hour delay in the rate of absorption (time to maximum concentration (Tmax)) compared to the fasted state (Prod Info ZOLINZA(TM) oral capsules, 2006).

Distribution: Vorinostat is approximately 71% bound to human plasma protein (Prod Info ZOLINZA(TM) oral capsules, 2006).

Metabolism: Vorinostat is metabolized via glucuronidation and hydrolysis followed by beta-oxidation into 2 inactive metabolites. Biotransformation by cytochrome P450 is negligible (Prod Info ZOLINZA(TM) oral capsules, 2006).

Excretion: Vorinostat is mainly eliminated through metabolism with < 1% of the dose recovered as unchanged drug in the urine. The mean urinary excretion of the 2 pharmacologically inactive metabolites at steady state was 52 +/- 13.3% of vorinostat dose; 16 +/- 5.8% of the dose as O-glucuronide and 36 +/- 8.6% of the dose as 4-anilino-4-oxobutanoic acid of vorinostat (Prod Info ZOLINZA(TM) oral capsules, 2006).

Elimination Half-life: The mean terminal half-life of vorinostat is approximately 2 hours (Prod Info ZOLINZA(TM) oral capsules, 2006).

7.5.5 Administration

Vorinostat 100-200 mg/dose administered orally BID will begin on day -10 and will continue until day 100 post transplant. The drug will be administered with food when feasible.

7.5.6 Adverse events

The principal adverse reactions observed in patients who received vorinostat 400 mg daily for a median of 97.5 days included: Peripheral edema (12.8%), prolonged QT interval (3.5% to 6%), alopecia (18.6%), pruritus (11.6%), weight loss (20.9%), anorexia (24.4%), constipation (15.1%), diarrhea (52.3%), nausea (40.7%), dysgeusia (27.9%), vomiting (15.1%), xerostomia (16.3%), anemia (14%), thrombocytopenia (25.6%), muscle spasms (19.8%), dizziness (15.1%), headache (11.6%), chills (16.3%), fatigue (52.3%), increased serum creatinine (16.3%), cough (10.5%), and fever (10.5%). Most of the adverse reactions were considered to be of mild to moderate severity. The incidence of grade 3-4 (severe to life threatening) adverse reactions was: Prolonged QT interval (<1%), weight loss (1.2%), anorexia (2.3%), nausea (3.5%), vomiting (1.2%), anemia (2.3%), thrombocytopenia (5.8%), muscle spasms (2.3%), dizziness (1.2%), chills (1.2%), fatigue (3.5%), and fever (1.2%). No drug related deaths have been reported. Thus the overall incidence of severe adverse reactions when vorinostat is administered for prolonged duration is low.

Hyperglycemia has been observed in patients receiving vorinostat. Serum glucose should be monitored, especially in diabetic or potentially diabetic patients. Adjustment of diet and/or therapy for increased glucose may be necessary.
Other adverse events include deep vein thrombosis during treatment with vorinostat, particularly in patients with previous thromboembolic events. Unpublished data from a University of Michigan clinical trial of 27 patients with advanced prostate cancer treated with vorinostat 400 mg daily demonstrated one grade 4 toxicity – a deep vein thrombosis. Almost half of the patients (48%) developed grade 3 toxicities, in all cases one of the toxicities described in the principal adverse reactions listed above, resulting in dose reductions in the majority of patients.

Relevant to a hematopoietic cell transplantation study, inhibition of platelet fragmentation rather than myelosupression is believed to be responsible for thrombocytopenia.

8.0 ASSESSMENT OF GVHD

Acute GVHD will be evaluated using the institutional Clinical Practice Guidelines (Appendix I). GVHD severity will be determined clinically; however, biopsies of affected organs are encouraged whenever possible. Overall GVHD will be graded weekly during the first two months after transplant, then every other week to day 100. Response to GVHD therapy will be measured through the use of the consensus and IBMTR GVHD severity indices based on physical exam and laboratory serum values.

Individual organ stages will be scored by the attending physician. Stages will be reviewed by the protocol chair, including reasons for declaring an individual organ potentially inevaluable. Discrepant scores will be reviewed by at least one investigator who will resolve the discrepancies (which may/may not include the use of a third, independent evaluation).

9.0 GVHD TREATMENT

If a patient develops acute GVHD, he/she will be treated according to institutional Clinical Practice Guidelines. If a patient develops acute GVHD before the scheduled vorinostat dosing has been completed, in addition to standard treatment for acute GVHD, he/she will continue to receive vorinostat therapy until its scheduled completion or unless the patient experiences toxicity attributable to vorinostat. Other GVHD therapy, including immunosuppressive can be added at the discretion of the treating physician.

In the event of GVHD responsive to therapy, the first immunosuppressive medication to be tapered should be the steroid therapy so long as clinical situation allows. The steroid taper should follow the institutional Clinical Practice Guidelines.

10.0 STUDY DEFINITIONS

10.1 Definition of "screening": A patient is considered to be in the screening period from the time they signed consent until the date their eligibility criteria have been determined as either "eligible" or "ineligible (screen fail).

10.2 Definition of "Enrolled": A patient is considered to be enrolled onto the study once they have signed consent and have successfully met all screening criteria, as documented by the inclusion/exclusion document., and the eligibility criteria has been reviewed and accepted by either the P.I. or a Co.-I. The date of enrollment will be documented as the date the P.I. or Co-I. has reviewed and approved eligibility.

10.3 Treatment Period: The study treatment period is defined as the first day of treatment with vorinostat through the last day of treatment with Vorinostat. This period is expected to last from day -10 through day 100, however may be extended, due to a postponement of the patient's BMT procedure, without constituting a deviation.

10.4 Follow Up Period: A patient is considered to be in the "follow up period" from the 1st day they no longer receive vorinostat therapy through at least day +365 post transplant and until the study is closed.

10.5 On Study: Patients are considered "On Study" from the time they are enrolled until they meet one of the "Off Study" criteria listed below.

10.6 Off Study: Patients are considered "Off study" once they meet one or more of the following criteria:

a. Death
b. Lost to follow up
c. Entry onto a competing trial
d. Withdrawal of consent by patient or investigator for any further treatment and follow up observations
e. Relapse of underlying malignancy or development of new malignancy

f. Unacceptable or dose limiting toxicity or complication

Patients have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of intercurrent illness, adverse events, treatment failure, protocol violation, or other reasons. Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation should be made at the time of the patient's withdrawal with an explanation of why the patient is withdrawing and every effort should be made to perform follow-up evaluations.

The assessment and reporting period for all adverse events, reportable under this protocol, will occur from the first day the treatment with vorinostat is administered until day +100 post transplant or until 30 days after the last dose of vorinostat is administered, whichever comes first.

After day +100, post transplant, (or after 30 days after the last dose of vorinostat whichever comes first), subjects will be followed only for GVHD, relapse, and survival for at least one year and until the study is terminated.

10.7 Definition of an "evaluable" patient: Patients removed from study who receive vorinostat for at least 21 days will be considered fully evaluable for both toxicity and response. Patients removed prior to 21 days of vorinostat will be evaluable for toxicity only and will therefore be replaced. The number of patients removed from study prior to being fully evaluable will be monitored regularly by the study's DSMB in order to identify and address problems that may develop with respect to patient accrual. We estimate that 10% of subjects may need to be replaced. Patients who die before day 14 will be counted towards the endpoints but additional patients will be enrolled to make up for the very short follow-up on such patients. During the first 21 days of study drug treatment, if the subject misses the study drug for more than 14 days, then this will be considered a deviation and the subject will be replaced.

10.8 Definition of engraftment and delayed engraftment:

10.8.1 Engraftment: Engraftment for neutrophils is defined as the first of three consecutive days in which the absolute neutrophil count (ANC) is > 500/uL. Engraftment for platelets is defined as the first of three consecutive days in which the platelet count is > 20,000/uL, without transfusional support.

10.8.2 Delayed engraftment: In preceding reduced intensity HCT transplant trials we observed engraftment of WBC (defined as ANC >500 for 3 days) in 97% of patients by day 14, while engraftment of platelets (defined as platelets transfusion independence) occurred in 88% of patients by day 18. Therefore, in the current trial delayed engraftment will be defined as either ongoing ANC <500/uL or ongoing dependence of platelet transfusions on or after day 21. Patients who engraft prior to day 21 and later have low ANC or platelet transfusion requirements, as occasionally occurs in HCT patients due to infection and other complications will not be considered to have delayed engraftment.

11.0 ADVERSE EVENTS AND REPORTING CRITERIA

An adverse event (AE) is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product. As such, the AEs will be followed and reported after the study subjects have been started on the investigational drug.

These events may be:
a. *Definitely related:* clearly associated with study drug/treatment
b. *Probably related*: likely associated with study drug/treatment
c. *Possibly related*: may be associated with study drug or other treatment
d. *Unlikely to be related*, or
e. *Definitely not related* to the study drug/treatment

For reporting purposes, an AE should be regarded as definitely or probably related to the regimen if the investigator believes that at least one of following criteria are met:

a. There is a clinically plausible time sequence between onset of the AE and the administration of the study drug or treatment.
b. There is a biologically plausible mechanism for the study drug or treatment causing or contributing to the AE.
c. The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.
d. A potential alternative cause does not exist.

Serious Adverse Drug Experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes:
- Death,
- a life-threatening adverse drug experience,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Expected Event: - An event that is expected in that it has been addressed or described in one or more of the following: Informed consent document(s) for this study, IRB application for this study, grant application or study agreement, protocol or procedures for this study, investigators' brochure or

equivalent (for FDA regulated drugs or devices), DSMB/DSC Reports, published literature, other documentation, or characteristics of the study population.

Unexpected *adverse events are those that:*

a. those that do not fall in to the above criteria
b. are not described in the Investigator's Brochure as far as vorinostat is concerned.
c. are not anticipated in the study informed consent or the BMT Program clinical transplant consent. This includes adverse events for which the specificity or severity is not consistent with the description in the informed consent.

The severity or grade of an adverse event may be measured using the following definitions:

Mild: Noticeable to the subject, but does not interfere with the subject's expected daily activities, usually does not require additional therapy or intervention, dose reduction, or discontinuation of the study. In the transplant setting CTC grades 1 and 2.

Moderate: Interferes with the subject's expected daily activities, may require some additional therapy or intervention but does not require discontinuation of the study. In the transplant setting using the CTCAE v. 3.0 version some grade 2 and most grade 3.

Severe: Extremely limits the subject's daily activities and may require discontinuation of study therapy, and/or additional treatment or intervention to resolve. In the transplant setting using the CTCAE version 3.0 some grades 3 and all grade 4.

Event reporting for Bone Marrow Transplant Protocols can be complicated and confusing to investigators, data managers, and regulatory oversight bodies because patients typically develop numerous complications such as infections, chemotherapy-related organ damage, medication side effects, etc as part of the typical course of a bone marrow transplant and not related to the study therapy. Furthermore, transplant-related complications often occur both simultaneously and in series, as one complication leads to a series of additional downstream events, making time-sensitive reporting of events difficult. Therefore, a well-conceived event reporting plan will separate complications that might be seen with any transplant, from study-related events that are relevant to subject safety. In order to achieve this goal, the DSM plan for this study will focus on rapid and specific identification and reporting of the following as SAEs:

a. Events which are serious and likely (probably or definitely) related to the investigational component of study therapy.
b. Events occurring at unusual frequency or severity in study subjects compared to non-study subjects undergoing similar transplants.
c. Events resulting in death regardless of attribution.
d. Events that are serious and unexpected (unexpected is defined as not included in the last version of the Investigator's Brochure, in the study consent or the transplant consent.

Therefore, we will not report as SAEs events that are expected and coincident with a typical transplant course unless they are either fatal or related to the investigational therapy.

Event grading: The NCI Common Terminology Criteria (Version 3.0) will be used to grade intensity of adverse events and assist in reporting adverse events.

Events not reported: The large majority of patients undergoing unrelated stem cell transplant will frequently experience hematologic events (i.e. anemia, thrombocytopenia, leukopenia, neutropenia), infections, electrolyte abnormalities, and organ toxicities. Given the frequency with which these occur in transplant patients, we will not report events that coincide with events that typically occur during the pre-and post transplant related therapy regardless of severity unless they otherwise meet the criteria for reporting as detailed above and below. An event is regarded as typical if it is specified in the study consent or the BMT Program transplant consent and if it occurs in more than 5% of transplant patients.

Events reported as adverse events in tabular format annually: Events that are classified as severe (all CTCAE V. 3 grade 4, some grade 3) will be reported to the Cancer Center DSMB with each routine report (quarterly) and in the annual IRB report so long as the events do not meet the criteria for expedited reporting as defined above. Events determined to be a CTCAE V. 3 grade 1 or 2 will not be captured as part of this protocol.

Events reported as serious adverse events to the FDA within 7 days of recognition of the event if ANY of the 2 criteria are met: (1) All events that are serious, related, and unexpected require expedited reporting and a narrative. (2) All deaths during the ON treatment period and all deaths within 30 days after the last dose of the study drug.

Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-993-1220) will be provided with copies of all serious adverse experiences within two working days of first awareness of the event by the study team. Additionally, any pregnancy occurring in association with use of a Merck Product will be reported to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220).

Merck contact information:
Cesar Sanz-Rodriguez, MD, PhD
Global Director Scientific Affairs
Chair Zolinza IISP committee
Merck & Co., Inc.
One Merck Drive
P.O. Box 100
Whitehouse Station, NJ 08889-0100 USA

A copy of all 15 Day Reports and Annual Progress Reports is to be submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators, by the investigator. This submission will be cross referenced according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, a copy of these reports will be submitted to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

In studies involving human subjects, serious adverse experience means any experience that suggest a significant hazard, contraindication, side effect or precaution. A serious adverse experience includes

any experience that is fatal or immediately life threatening, results in a persistent or significant disability/incapacity, requires or prolongs in-patient hospitalization, or is a congenital anomaly, cancer, or overdose.

Other important medical events that may not result in death, not be life-threatening, or not require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject/patient and may require medical or surgical intervention to prevent one of the outcomes listed previously.

Adverse Event data will be collected at all times and will be reviewed by the PI and/or a sub-I as well as the data manager every 2 weeks. Events occurring at unusual frequency or severity: Any event that might be occurring at an unusual frequency or severity will be discussed at the BMT Program DSMC and the results of the discussion will be reported to the Cancer Center DSMB and IRB in the regular DSMB submission. Any event that is clearly occurring at an unusual frequency or severity will be reported to the DSMB within 14 days of recognition of the problem by the PI and data manager.

11.1 Multi-site Serious Adverse Event (SAE) Reporting to Merck and The University of Michigan

Within 2 working days of first awareness of the event (immediately if the event is fatal or life-threatening), the Principal Investigator at the site of the event will report to the University of Michigan any SAE ("SAE," as defined above) that occurs during the SAE reporting period (from the first day the treatment with vorinostat is administered until day +100 posttransplant). The University of Michigan will report to Merck by facsimile (see below).

SAEs should be reported on the MERCK Co. Forms for SAE Reporting Appendix IV.

University of Michigan Contact:
Clinical Trials Office
Attn: Multi-Site Coordinator
Fax 734-232-0744 or email: CTO-Multisite@med.umich.edu

Merck & Co., Inc. Contact:
Attn: Worldwide Product Safety
Fax number: 215-993-1220

12.0 DATA SAFETY MONITORING PLAN (DSMP)

The following are the procedures for data and safety monitoring of this clinical trial to be conducted within the Blood and Marrow Transplant program as required by the NIH policy. This is to insure the safety of participants, the validity of research data, and the appropriate termination of studies for which significant benefits or risks have been uncovered or when it appears that the trial cannot be concluded successfully. This protocol will conduct a data and safety monitoring process as described in the plan below.

12.1 Trained and Certified Personnel

All of the research protocol personnel who will work with study subjects, study subject data or subjects' research samples have completed training in the protection of human research participants per guidelines issued by the U. S. Department of Health and Human Services, Office of Human Research Protections. The documentation of completion of the certification is maintained in the UMCCC Clinical Trials Office or the Blood and Marrow Transplantation Office.

The investigator and designated associates have attended an IRB sponsored HIPAA research presentation in accordance with the policy of the study site. Each participant in this research trial will be listed by study specific numbers, without initials or date of birth; however, the date of transplant may be included when corresponding with the IRB or outside agencies.

Designation of Responsibilities: The Principal investigator(s) are solely responsible for the implementation and conduct of this trial. The principal investigator has however, designated associates to assist with the protocol implementation which includes but is not limited to the following:

1. BMT Physicians – have been designated to assist with participant education, informed consent process, study implementation and compliance, recording of primary source documentation, AE assessment and reporting, adherence to all regulations.
2. BMT Research Nurse(s) - have been designated to assist with participant education, informed consent process, study implementation and compliance, recording of primary source documentation and adherence to all regulations.
3. BMT Data Manager(s) - have been designated to assist with patient enrollment/eligibility, verification of protocol compliance, all data collection and recording from primary source, AE reporting, DSM reports and adherence to all regulations.
4. BMT Clinical Team - The members of the BMT clinical team that have been designated to assist the investigator in any aspect of this protocol will be listed on the protocol specific designation log.
5. The BMT internal Data and Safety Monitoring Committee (DSMC) will meet at least quarterly to review the data, safety and monitoring reports and all SAEs that have been filed. The report will be submitted to the UMCCC DSMB and to the UM IRB at least quarterly. Whenever an unanticipated data, safety and monitoring board meeting takes place or when a new development occurs the IRB will be notified of the occurrence.

12.2 Multi-site Data Safety and Monitoring Plan

The UMCCC will serve as the the DSMB for this study. This committee is responsible for the review and monitoring the study's scientific progress, accrual rate and any serious adverse events.

Each participating site is required to have its own DSMC for the study. This committee will be composed of the local site principal investigator, site co-investigator(s), site data manager or study coordinator and other members of the study staff involved in the conduct of the trial.

The BMT program's internal study specific DSMC will minimally consist of the principal investigator or a Co-Investigator and the assigned data manager. Scheduled meetings will take place quarterly or more frequently depending on the activity of the protocol.

Responsibility for compliance with this DSM plan rests solely with the principal investigator. A written summary will be made of each meeting and signed by one of the principal investigators, or a sub-investigator designated by the principal investigator, and the data manager. The written summary will then be reported to the UMCCC DSMB and to the UM IRBMed in order to avert or manage any potential conflicts of interest that may arise from the inclusion of investigators in the program's internal Data and Safety Monitoring committee. Data and safety monitoring reports of these regular meetings will be kept on file in the Cancer Center Clinical Trials Office. The data manager assigned to the trial will complete the report. The BMT internal DSMC will meet at least quarterly to review the data, safety and monitoring reports and all SAEs that have been filed. The report will be submitted to the UMCCC DSMB and to the UM IRB at least quarterly. Whenever an unanticipated data, safety and monitoring board meeting takes place or when a new development occurs the IRB will be notified of the occurrence.

During the committee's quarterly meeting, the principal investigator will discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

These meetings are to be documented by the site data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the site principal investigator or co-investigator. Each site is required to submit the completed DSMR to the Multi-Site Coordinator at the University of Michigan Clinical Trials Office on a quarterly basis together with other pertinent documents.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of both the sites data manager or study coordinator and the site principal investigator or co-investigator. These reports are to be sent to the University of Michigan Clinical Trials Office within 7 calendar days of awareness of the event and on a quarterly basis with the Protocol Specific Data and Safety Monitoring Report.

12.3 Storage and Dissemination of Reports

The Clinical Trials Office (CTO) is responsible for collating all the Data and safety Monitoring Reports from all the participating sites, and providing the information to Data Safety Monitoring Board. The CTO will coordinate the reporting process between the Investigator and the IRBMED and UM DSMB as well as other applicable reporting agencies (FDA, and study sponsors). Copies of all related correspondence and reporting documents will be maintained in a locked file by the CTO regulatory team and the research data file will be maintained in a file by the BMT data management team.

The Principal Investigator is committed to report to MERCK Co. the following data on a quarterly basis:

- Patients' recruitment/withdrawals/drop-outs
- Relevant efficacy findings
- Safety data (i.e. listing of all reportable AEs [as defined at para 11.0 of the protocol], irrespective of severity and drug-relatedness)

12.4 Clinical Monitoring Procedures

Clinical studies coordinated by UMCCC must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements.

This study will be monitored by a representative of the CTO of the UMCCC. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the CTO. This will be done as an actual site visit; teleconference, videoconference, or web-based meeting after the site has been given access to the study database and assembled a study reference binder. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting. Study–related questions or issues identified during the site initiation meeting will be followed-up by the appropriate UMCCC personnel until they have been answered and resolved.

The first annual monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The initial annual visit is not justified unless there is at least one participant enrolled on a study. At a minimum, a routine monitoring visit will be done at least once every 12 months, or once during the course of the study if the study duration is less than 12 months. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

Monitoring visits may be in the form of a site visit or a review of the documents at the CTO. During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the CTO representative conducting the monitoring visit to verify consistency of data

collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The CTO expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner. For review of study-related documents at the CTO, the site will be required to ship or fax documents to be reviewed.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit if all of the following apply:

- No patient has signed the Informed Consent Form and has enrolled into the study
- Investigational agent has not been dispensed
- All investigational agent and materials have been returned as defined for the study or destroyed and accounted for properly.

12.5 Quality Assurance and Audits

The Quality Assurance Review Committee (QARC) of the UMCCC performs quality assurance audits of investigator-initiated clinical trials. Audits provide assurance that trials are conducted in compliance with the protocol. Further, they ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements.

A QARC audit of each clinical trial is conducted annually. Audits occur within the month of the study's initial IRB approval.

Audits may be conducted in one of two ways:
- On-site audit of study records, including source documents
- Audit of study records and source documents at the UMCCC Clinical Trials Office

In the latter case, participating sites would be required to provide the documents to be audited to the UMCCC Clinical Trials Office.

All audit findings are reported by QARC to the UMCCC DSMB. These findings are followed-up by the DSMB until they have been resolved.

The DSMB can also request QARC for a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Clinical Trials Office that such a request has been made.

13.0 STATISTICAL CONSIDERATIONS

13.1 Sample Size Justification

We will enroll a maximum of 50 evaluable patients in this study, which will give us 80% power to detect a difference in Day 100 Grade 2-4 acute GVHD rates of 0.25 versus 0.42, assuming a Type I error rate of 5%. Anticipated accrual time is 2 years.

13.2 Study Endpoint

The primary study endpoint is the incidence of grades 2-4 acute GVHD by day 100 post-transplant. All patients who develop grades 2-4 acute GVHD between day 0 and day 100 will be counted towards this endpoint.

13.3 Dose-Finding Portion of Study

Two doses of vorinostat, 100 mg and 200 mg, each PO BID, will be studied. The first ten patients enrolled will receive the lower dose of vorinostat. If dosing modifications are not required during the pre-engraftment period in more than four patients **AND** there are no more than two patients observed with toxicities CTCAE v. 3 grade 4 or higher considered probably or definitely related to the drug, then the dose of vorinostat will be escalated to 200 mg PO BID. We defined three CTCAE v.3 grade 4 toxicities as the threshold for limiting dose escalation as three of ten patients with CTCAE v. 3 grade 4 toxicity indicates that the true CTCAE v.3 grade 4 toxicity rate is above 5% and reaches our upper bound on a tolerable rate of CTCAE v.3 grade 4 toxicity.

If dose escalation does not occur due to failure to meet the above criteria, then the entire study population will enroll at the 100 mg PO BID dosing subject to the stopping rules outlined later. If dose escalation occurs, the next ten patients will be treated at 200 mg PO BID dosing. If dosing modifications are not required during the pre-engraftment period in more than four of those ten patients **AND** there are no more than two of those ten patients observed with toxicities CTCAE v. 3 grade 4 or higher considered probably or definitely related to the drug, then the remaining 30 patients will be assigned 200 mg PO BID. Otherwise, the remaining 30 patients will be assigned to 100 mg PO BID. If escalation from 100 mg to 200 mg occurs, the actual number of subjects receiving the same dose of vorinostat will be 40 patients rather than 50 patients. However, all 50 patients will be used to assess the lack of efficacy stopping rules described next, regardless of the dose each receives.

13.4 Stopping Rule for Toxicity

There is no formal stopping rule that will be used during the dose-finding portion of the study. Once we have completed the dose finding portion of the study, we will continue to monitor for excessive toxicity after additional cohorts of 10 subjects have been assigned to the same dose using the same definition of toxicity in the dose-finding portion of the study. Specifically, at toxicity interim analysis

K=1,2,3, we will have enrolled n_K=30, 40 and 50 subjects, respectively. We will consider a dose safe if no more than $0.2n_K$ patients are observed with toxicities CTCAE v.3 grade 4 or higher considered probably or definitely related to the drug. For example, once 30 subjects receive 200mg, we will terminate the study if more than 6 patients have toxicities CTCAE V.3 grade 4 or higher probably or definitely related to the drug. For true toxicity rates of 0.05, 0.10, 0.15, 0.20, and 0.25, the probabilities of early termination are <0.001, 0.05, 0.35, 0.79, and 0.97, respectively.

13.5 Stopping Rule for Lack of Efficacy

Once we have enrolled 20 subjects (i.e completed the dose-finding portion of the study) and observed all 20 subjects for 100 days, we will perform an interim analysis to assess for an unacceptably high rate of Day 100 Grade 2-4 acute GVHD. Future interim analyses (if necessary) are planned each time the study proceeds and enrolls five additional subjects and each completes 100 days of observation. Based upon prior results collected on 40 patients, 16 patients experienced Grade 2-4 acute GVHD by Day 100, giving us a prior rate of 42%. We therefore assume that p, the probability of Grade 2-4 acute GVHD by Day 100, has a prior Beta (16, 24) distribution. We will stop the current trial for lack of efficacy (excessive GVHD) if the data collected give us 90% posterior probability that p is greater than 0.42. The actual values satisfying this stopping rule are given in **Table 6**. If the actual rate of Day 100 Grade 2-4 acute GVHD is 0.25 as hypothesized, we have only a 0.02 probability of stopping the trial, while if the actual rate of Day 100 Grade 2-4 acute GVHD is 0.50, we have a 0.88 probability of stopping the trial. Although this stopping rule requires 100 days of follow-up for each subject, we will not halt accrual of future subject to wait for full follow-up of previously enrolled subjects needed for the interim analysis.

Table 7 Stopping Rules for Lack of Efficacy

Number of Enrolled Subjects Observed for 100 Days	Number of Subjects with Grade 2-4 aGVHD
20	10
25	12
30	14
35	16
40	18
45	20

13.6 Stopping Rule for Excessive Graft Failure

Stopping rules for excessive graft failure will apply to all subjects entered on study. Engraftment failure will be defined as the inability to achieve an ANC > 500/uL within 28 days post transplant.

We consider engraftment failure in more than 5% of subjects within 28 days of transplant (EF28) to be unacceptable. Once 10 subjects have been enrolled and followed for 28 days, we will continually monitor the rate of EF28 and stop if we have strong evidence that the actual rate of EF28 is above 5%. Subjects who have not engrafted and die within 28 days of transplant will be considered to be in graft failure and contribute to the number of events of EF28. The official stopping rules are displayed in the second column of **Table 7** and are the number of events that would produce a 95% confidence interval whose lower bound exceeds our upper limit of 5%. If EF28 equals or exceeds the numbers in **Table 7**,

enrollment will be halted and the protocol will be re-evaluated and either closed or modified and re-approved before re-opening. If the true rate of EF28 is 0.05, 0.10, 0.15, and 0.20, the probabilities of stopping the study for excessive EF28 are 0.02, 0.34, 0.87, and 0.99, respectively.

13.7 Stopping Rule for Excessive Mortality

Stopping rules for excessive mortality will apply to all subjects entered on study. We consider all-cause mortality in more than 30% of subjects within 100 days of transplant (ACM100) to be unacceptable. Once ten subjects have been enrolled and followed for 100 days, we will continually monitor the rate of ACM100 and stop if we have strong evidence that the actual rate of ACM100 is above 30%.

The official stopping rules are displayed in the third column of **Table 7** and are the number of events that would produce a 95% confidence interval whose lower bound exceeds our upper limit of 30%. If ACM100 equals or exceeds the numbers below, enrollment will be halted and the protocol will be re-evaluated and either closed or modified and re-approved before re-opening.

Although this stopping rule requires 100 days of follow-up for each subject, we will not halt accrual to wait for full follow-up of previously-enrolled subjects. For example, we will enroll the 11th subject as soon as they are eligible, regardless of whether the first ten subjects have all been followed for 100 days. With a projected enrollment of about two subjects per month, we would expect to enroll, for example, approximately five additional subjects before the 10th subject is fully followed for ACM100, or five additional subjects before the 15th subject is fully followed for ACM100. If the true rate of ACM100 is 0.25, 0.30, 0.35, and 0.40, the probabilities of stopping the study for excessive EF28 are 0.02, 0.11, 0.38, and 0.74, respectively.

Table 8 Stopping Rules for Engraftment Failure and All-Cause Mortality

Number of Enrolled Subjects Observed for 28 or 100 Days	Number of Subjects with EF28	Number of Subjects with ACM100
10	3	7
15	4	9
20	4	11
25	5	13
30	5	15
35	6	17
40	6	19
45	7	21

13.8 Analysis Methods for Primary Outcome

The rate of Day 100 Grade 2-4 acute GVHD will be summarized as the proportion of all subjects who experience Grade 2-4 acute GVHD within 100 days of transplant, with a corresponding 95% confidence interval. If some subjects do not develop Grade 2-4 acute GVHD and die within 100 days of transplant, we will instead summarize the rate of Day 100 Grade 2-4 acute GVHD using cumulative incidence, treating death as a competing risk.

13.9 Analysis Methods for Secondary Outcomes

Steroid-free survival, overall survival, and relapse will be estimated with Kaplan-Meier methods. The multiple vorinostat levels will be analyzed with paired t-tests and linear mixed models as necessary, while histone acetylation in PBMC will be analyzed with a paired t-test. Plasma concentrations of inflammatory GVHD markers will be summarized with means and 95% confidence intervals.

The serial PK data collected on five UM subjects will be summarized with means and 95% confidence intervals. Trends over time in the PK data will also be estimated with a linear mixed model; no formal hypothesis testing will be performed.

APPENDIX I: GVHD ASSESSMENT GUIDELINES

GVHD STAGING

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dl	Adult: < 500 ml/day Child: < 10 ml/kg/day
1	Maculopapular rash < 25% BSA	2-3 mg/dl	Adult: 500–999 ml/day Child: 10 -19.9 ml/kg/day Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.
2	Maculopapular rash 25 – 50% BSA	3.1-6 mg/dl	Adult: 1000-1500 ml/day Child: 20 – 30 ml/kg/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dl	Adult: >1500 ml/day Child: > 30 ml/kg/day
4	Generalized erythroderma plus bullous formation and desquamation > 5% BSA	>15 mg/dl	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

- For GI staging: The "adult" stool output values should be used for patients ≥ 50 kg in weight.
- Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is estimated to be 50% of total stool/urine mix.
- For stage 4 GI: the term "severe abdominal pain" will be defined as:
  (a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
  (b) Pain that significantly impacts performance status, as determined by the treating MD.
- If colon or rectal biopsy is +, but stool output is <500 ml/day (<10 ml/kg/day), then consider as GI stage 0.
- There is no modification of liver staging for other causes of hyperbilirubinemia (see appendix A).

Overall Clinical Grade:

Grade 0	No stage 1-4 of any organ
Grade I	Stage 1-2 rash and no liver or gut involvement
Grade II	Stage 3 rash, or Stage 1 liver involvement, or Stage 1 GI
Grade III	Stage 0-3 skin, with Stage 2-3 liver, or Stage 2-3 GI
Grade IV	Stage 4 skin, liver or GI involvement

APPENIDX II: SAMPLE PROCUREMENT FOR PHARMACO-DYNAMIC STUDIES

1.0 Objectives

Evaluation of the pharmacodynamic activity of vorinostat by measuring in PBMC and plasma using the following parameters:

a. intracellular acetylation of different proteins (histones H3 and H4 and tubulin).
b. the amount of apoptotic cells.
c. the total intracellular acetylase activity by measuring histone acetyl transferase (HAT) and the histone deacetylase (HDAC) activities.
d. the cellular expression of the different Class I and Class II HDAC isoforms.
e. the level of circulating cytokines.

2.0 Materials

a. Tissue Culture Medium (RPMI 1640 plus 1% FCS)
b. Solution C (0.2% DMSO)
c. Solution 1 (41 µM vorinostat in 0.2% DMSO)
d. Solution 2 (13.6 µM vorinostat in 0.2% DMSO)
e. RBC lysing solution (BD PharmLyse 1x, BD BioSciences, Cod. 555899)
f. Fixing solution (BD Cytofix, BD BioSciences, Cod. 554655)
g. Freezing solution (10% DMSO plus 90% FCS)

3.0 Sampling Prior to Vorinostat Therapy:

A sample of 15-20 ml of heparinized blood (5-10 ml for children < 50kg), will be drawn before starting vorinostat treatment for each enrolled patient. The blood will be collected in a green top tube labelled as tube 0. The blood sample will processed in the Core Immunology Lab as follows:

a. Label 3 tubes (15 ml) as: tube C, tube 1 and tube 2.
b. Add 1 ml of the blood from tube 0 to each tube.
c. Add 1 ml of tissue culture medium.
d. Add 50 µl of Solution C to tube C.
e. Add 50 µl of Solution 1 to tube 1.
f. Add 50 µl of Solution 2 to tube 2.
g. Incubate the tubes at 37°C (without stirring) for 24 hours.
h. Blood in tube 0 will be treated as indicated at point 15.
i. At the end, draw the supernatant from each tube (tubes 1, 2 and 3) and discard.
j. The remaining cellular pellet of each tube will be added to 10 ml of RBC lysing solution, vortex and incubate at room temperature for 15 min maintaining the tube in the dark.
k. Centrifuge at 200 x g for 5 min at +4°C then aspirate and discard the supernatant.
l. Add 2 ml of fixing solution to the cellular pellet, vortex and incubate at +4°C for 20-30 min.
m. Centrifuge at 200 x g for 5 min at +4°C then aspirate and discard the supernatant.

n. Suspend the cellular pellet by gentle mixing with 2 ml of freezing solution and store the sample at –80°C until FACS analysis.
o. Centrifuge tube 0 at 200 x g for 5 min at +4°C then draw supernatant (plasma) and store at –80°C until cytokine measurement.
p. Separate peripheral blood mononuclear cells (PBMC) from the blood in tube 0 by centrifugation over a Ficoll density gradient according the standard procedures.
q. Wash twice the PBMC pellet with saline.
r. Aspirate the supernatant and freeze the PBMC pellet at –80°C until biochemical analysis.

4.0 Sampling During Vorinostat Treatment

On days of required blood draws, draw 1 ml of heparinized blood. The blood will then be processed in the Core Immunology Lab as follows:

1. Centrifuge at 200 x g for 5 min at +4°C then draw supernatant (plasma) and store at 80°C until cytokine measurement.
2. The remaining cellular pellet of each tube is added with 10 ml of RBC lysing solution, vortex and incubate at room temperature for 15 min maintaining the tube in the dark.
3. Centrifuge at 200 x g for 5 min at +4°C then aspirate and discard the supernatant.
4. Add 2 ml of fixing solution to the cellular pellet, vortex and incubate at +4°C for 20-30 minutes.
5. Centrifuge at 200 x g for 5 min then aspirate and discard the supernatant.
6. Suspend the cellular pellet by gentle mixing with 2 ml of freezing solution and store the sample at –80°C until FACS analysis.

APPENDIX III: DEFINITIONS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME, SEPSIS AND SEPTIC SHOCK

SIRS criteria:
Two or more of the following criteria:
Core temperature ≥38ºC (100.4ºF) or ≤36ºC (96.8ºF)
Heart rate ≥90 beats/min
Respiratory rate ≥20 breaths/min or PaCO2 of ≤32 mmHg
White-cell count of ≥12,000/mm^3 or ≤4,000/mm^3 or >10 percent immature neutrophils.

Sepsis:

Evidence of SIRS accompanied by known or suspected infection.

Severe sepsis:
Sepsis accompanied by hypoperfusion or organ dysfunction.

Cardiovascular:
Systolic blood pressure ≤90 mmHg or MAP ≤70 mmHg for at least 1 hour despite adequate volume resuscitation, or the use of vasopressors to achieve the same goals.

Renal:
Urine output <0.5 mL/kg of body weight/hr, or acute renal failure.

Pulmonary:
PaO2/FiO2 ≤250 if other organ dysfunction present or ≤200 if the lung is the only dysfunctional organ.

Gastrointestinal:
Hepatic dysfunction (eg, hyperbilirubinemia, transaminitis).

Central nervous system:
Acute alteration in mental status (eg, delirium).

Hematologic:
Platelet count <80,000/mm^3 or decreased by 50 percent over three days, or disseminated intravascular coagulation.

Metabolic:
pH ≤7.30 or base deficit >5.0 mmol/L **AND**
Plasma lactate >1.5 x upper limit of normal.

Septic shock:

Severe sepsis with persistent hypoperfusion or hypotension despite adequate fluid resuscitation.

SIRS: systemic inflammatory response syndrome; MAP: mean arterial pressure; PaO2: partial pressure of arterial oxygen; PaCO2: partial pressure of arterial carbon dioxide; FiO2: fraction of inspired oxygen.

APPENDIX IV: FORMS FOR SAE REPORTING


MERCK Co.

SERIOUS ADVERSE EVENT REPORT		
Study Protocol (title/no.)	**Patient no.** |__|__|	**Patient Initials** |__|__|
Initial Report ☐	**Follow Up Report** ☐ **Date of the Initial report:** |__|__| |__|__| |__|__| dd mm yy	
Principal Investigator Name: **Centre Address:** **Telephone No.** **Fax No.**		
Patient Gender: ☐ M ☐ F	**Height (cm):** |__|__|__|	**Weight (kg):** |__|__|__|. |__|
Date of birth (dd/mm/yyyy): |__|__| |__|__| |__|__|__|__|		
EVENT Medical term(s): _____ _____ **NARRATIVE:** _____ _____ _____ _____ **RELEVANT TEST/LAB DATA** _____ _____ _____		
Event Start Date (dd/mm/yy) |__|__| |__|__| |__|__|	**Event End Date (dd/mm/yy)** |__|__| |__|__| |__|__|	**Ongoing** ☐

SEVERITY	ACTION TAKEN WITH STUDY DRUG	CRITERION FOR SERIOUSNESS	OUTCOME
1. |__| Mild 2. |__| Moderate 3. |__| Severe	1. |__| Dose not changed 2. |__| Dose reduced 3. |__| Temporarily withdrawn 4. |__| Definitely withdrawn 5. |__| Dose increased	1. |__| Death 2. |__| Life-threatening 3. |__| Persistent or significant disability/incapacity 4. |__| Hospitalization / prolongation of existing hospitalization 5. |__| Congenital anomaly/birth defect 6. |__| Medically relevant	1. |__| Resolved 2. |__| Resolved with sequelae 3. |__| Fatal * 4. |__| Not resolved 5. |__| Unknown

*Autopsy: Yes □ No □ If yes, please attach the report.

Study drug:
Vorinostat

Dosage: |__|__|__|
mg /day

Administration Route: oral

Date of the first administration:
|___|___| |___|___| |___|___|
dd mm yy

Date of the treatment administration associated to this report:
|___|___| |___|___| |___|___|
dd mm yy

Dechallenge and Rechallenge			
Did reaction abate after stopping study drug?	□ No	□ Yes	□ NA
Did reaction reappear after study drug reintroduction?	□ No	□Yes	□ NA

CAUSAL RELATIONSHIP TO STUDY DRUG:

Definite |__| Probable |__| Possible |__| Unlikely |__| Not related |__| Unknown |__|

MEDICAL HISTORY:

_____
_____
_____
_____
_____
_____

CONCOMITANT DISEASES/PREDISPOSING FACTORS:

_____
_____
_____
_____
_____

_____
_____
_____

CONCOMITANT THERAPY (Treatment of the event excluded)	**Dose/day Route**	**Date** (dd/mm/yy)	**Other suspect drug** *Is there a reasonable causal relationship between the event and the concomitant therapy?*
1. _____ Indication: _____	**Dose/day :** _____ **Route:** _____	**Start Date**|__|__| |__|__| |__|__| **End Date** |__|__| |__|__| |__|__| **Ongoing:** ☐	☐ no ☐ yes
2. _____ **Indication:** _____	**Dose/day:** _____ **Route:** _____	**Start Date**|__|__| |__|__| |__|__| **End Date** |__|__| |__|__| |__|__| **Ongoing:** ☐	☐ no ☐ yes
3. _____ **Indication:** _____	**Dose/day:** _____ **Route:** _____	**Start Date**|__|__| |__|__| |__|__| **End Date** |__|__| |__|__| |__|__| **Ongoing:** ☐	☐ no ☐ yes
4. _____ **Indication:** _____	**Dose/day:** _____ **Route:** _____	**Start Date**|__|__| |__|__| |__|__| **End Date** |__|__| |__|__| |__|__| **Ongoing:** ☐	☐ no ☐ yes
5. _____ **Indication:** _____	**Dose/day:** _____ **Route:** _____	**Start Date**|__|__| |__|__| |__|__| **End Date** |__|__| |__|__| |__|__| **Ongoing:** ☐	☐ no ☐ yes

Date of this report _____

Investigator name (printed letters)_____

Investigator Signature _____

APPENDIX V:

SAMPLE COLLECTION FORM	
Pt # ……. Pt initials …… Sample collected for ☐ PD/acetylation status ☐ Other	
Vorinostat Administration *	Sample collection
Date |__|__|__|__|__|__| mm dd yy	Date |__|__|__|__|__|__| mm dd yy

*Record the date of the last drug administration prior to the sample collection

Appendix VI. THROMBOEMBOLIC EVENT FORM

Patient Information
Name or initials_____
Gender_____Date of Birth_____
Age_____Weight (kg)_____Ht (ft)_____

Onset date of event (dd/mm/yr)_____
Please indicate the type of Thromboembolic Event the patient experienced by checking the appropriate boxes.

□ **Deep Vein Thrombosis** □ distal leg (calf) □ proximal leg (popliteal or higher) □ distal arm (basilic, cephalic, brachial) □ proximal arm or shoulder (axillary, subclavian) □ neck (IJ, EJ) □ **Pulmonary Embolism** □ pulmonary artery □ lobar □ segmental □ subsegmental □ more distal	□ **Arterial Thrombotic event** □ Cerebral vascular accident with permanent sequelae (i.e. Stroke) □ Cerebral vascular accident with reversible sequelae (i.e. TIA) □ Peripheral Arterial event □ **Central Venous Catheter (line) related** □ **Other Thrombotic event (Describe)**

□ Asymptomatic
□ Symptomatic **Describe:**

LAB WORK Specify/Attach results if available.
□ PT_____
□ PTT_____
□ Platelets_____
□ d-dimer _____
□ factor V Leiden_____
Deficiencies of
- □ anti-thrombin III
- □ Other_____
- □ protein C
- □ protein S

□ Prothrombin gene mutation
□ Hyperhomocysteinemia
□ Urinalysis_____
□ Increased CRP_____
□ Other_____

CLINICAL EVIDENCE confirmed by:
□ Ultrasound
□ CT Scan
□ MRI
□ Angiography
□ VQ Scan

Zolinza™ INFORMATION

□ 400 mg daily Start Date_____
□ Other _____ Stop Date_____

Was the Zolinza™ dose reduced or therapy discontinued? □ Yes □ No If yes, please provide dosing history:

_____
_____
_____
_____

Indication

□ CTCL
□ Stage_____
□ Other_____
□ Sites of disease_____

MEDICATION HISTORY

Concurrent and/or medications patient received within 30 days of TE event.

□ Oral contraceptives
□ HRT
□ Megestrol acetate
□ Anti-VEGF
□ Chemotherapy
□ Thalidomide
□ Lenalidomide
□ Antithrombotics
  □ ASA
  □ Clopidogrel
  □ Warfarin
□ Other :
□ Growth factors_____

CONCURRENT CONDITIONS **PAST MEDICAL HISTORY**

Does the patient have any of the following concurrent conditions (≤ 4-6 weeks) and/or past medical history?

Check all that apply

□ Inpatient treatment
□ Fever >100.4°F/38°C
□ Immobility
□ Cancer
□ Pregnancy (<1mo)
□ Tumor Compression of venous return
□ Family history premature (onset <60yo) CAD
□ Venous Stasis, varicose leg veins
□ Endocarditis
□ Infection
□ Prosthetic valve
□ Vena caval filter
□ DVT
□ Pulmonary embolism
□ Coagulopathy
□ Family history DVT/PE/thrombophilia
□ Myocardial Infarction
□ Recent prolonged air travel
□ Recent abdominal/pelvic surgery
□ Atrial fibrillation
□ Ischemic Stroke
□ Congestive Heart Failure
□ Diabetes Mellitus
□ Cardiac stent
□ Autoimmune disease (e.g. RA, SLE, Antiphospholipid antibody syndrome, lupus anticoagulant)
□ Indwelling long term Central venous catheter
□ Obesity or BMI > 30
□ Recent hip/knee/leg fracture
□ Recent sitting/standing for prolonged periods of time
□ Nephrotic syndrome/Proteinuria
□ Heparin-induced thrombocytopenia
□ Recent major trauma
□ Recent hip/knee replacement
□ Other_____

TREATMENT

□ IV unfractionated heparin (UH)
□ Low molecular weight heparin (LMWH)
□ Arixtra (fondaparinux)
□ Thrombolytics
□ Warfarin
□ ASA
□ Other_____

OUTCOME of the Adverse Event

□ Recovered/Resolved
□ Recovered/Resolved with sequelae
□ Not Recovered
□ Recovering
□ Fatal
□ Unknown

Date (mm/dd/yr)_____

Contact Information

Name_____ Phone_____
Business address_____ E-mail_____
_____
Date Completed_____

Fax form to: Worldwide Product Safety
(215) 993-1220

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