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Translational phase I trial of vorinostat (suberoylanilide hydroxamic acid) combined with cytarabine and etoposide in patients with relapsed, refractory, or high-risk acute myeloid leukemia

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Abbreviations: HDAC, histone deacetylase; cytarabine, ara-C or cytosine arabinoside; AHD, antecedent hematologic disease; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasm other than CML; MDS, myelodysplastic syndrome; MTD, maximum tolerable dose; DLT, dose-limiting toxicity; CR, complete remission; PD, progressive disease; NE, non-evaluable; OS, overall survival, defined as the date entered on study until the day of death; SCT, stem cell transplant; ECOG, Eastern Cooperative Oncology Group; TRAIL, tumor necrosis factor (TNF)-related apoptosis inducing ligand; IV, intra-venously; ANC, absolute neutrophil count; PO, orally; BID, twice a day; TID, three times a day; FLT3-ITD, FMS-like tyrosine kinase 3 internal tandem duplication mutation; BCRP, breast cancer resistance protein, ABCG2; Pgp, P-

glycoprotein, the product of the Multidrug Resistance 1 (MDR1) gene, ABCB1; MRP1, multidrug resistance-associated protein 1, ABCC1.

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Ivana Gojo: *I do not have any conflicts to disclose.*

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Mariola Sadowska: *Except for partial salary support for this project* [see Dr. Ross' statement below], *I do not have any other conflict of interest to disclose*.

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Igor Espinoza-Delgado: Sadly, Dr. Espinoza-Delgado died a few weeks before our original manuscript was submitted, and hence cannot give a statement regarding COI. For 19 years, Dr. Espinoza-Delgado was a Senior Investigator at the Investigational Drug Branch of CTEP, where he oversaw CTEP-sponsored clinical trials of histone deacetylase inhibitors, including vorinostat. Although a statement regarding COI cannot be made for Dr. Espinoza-Delgado at this time, it is reasonable to state that he was sufficiently vetted for COI by NCI/CTEP for his role as monitor for CTEP-sponsored clinical trials of vorinostat. Dr. Espinoza-Delgado retired from the NCI on October 1, 2011. At the time of his death, he was employed by Millennium Pharmaceuticals in Cambridge, MA.

Douglas D. Ross: The translational studies described in this manuscript were supported by a grant from Merck, Inc., the manufacturer of vorinostat. These funds were requested from Merck after CTEP discontinued the Translational Research Initiative (TRI) funding program, which had originally supported this trial's translational studies. The funds from Merck were used by the Translational Laboratory Shared Service of the UMGCC (directed by Dr. Lapidus), and included partial salary support for Dr. Sadowska. Additionally, Dr. Ross attended a number of "Vorinostat Investigator Meetings" which were held in the evenings during the ASCO and ASH meetings. These investigator meetings were jointly sponsored by NCI/CTEP and by Merck. Their purpose was to update investigators conducting clinical trials with vorinostat on other ongoing or completed vorinostat trials. In general, dinner was served during these meetings; however, Merck did not pay for travel or lodging for Dr. Ross to attend the dinner meetings.

STATEMENT OF TRANSLATIONAL RELEVANCE: (148 words – limit 150 words)

This work tests previous *in vitro* observations that, while concurrent administration of vorinostat with cytarabine is antagonistic because of reduction of cells in S-phase by vorinostat, sequential administration of vorinostat followed by cytarabine results in synergistic activity against cultured acute leukemia cell lines. Other *in vitro* observations predicted that vorinostat followed by etoposide would be synergistic. We report a phase I trial in which escalating doses of vorinostat were given orally for seven days followed by fixed doses of cytarabine and etoposide given intravenously on days 11-14. Overall, percentages of patient-derived blast cells in S-phase did not change significantly following vorinostat treatment. Of 13 patients with high-risk leukemias treated at the maximum tolerated dose of vorinostat (200 mg, orally, twice a day), 6 obtained a complete remission (CR) with median duration of 7 months. The relatively high CR rate in this poor-risk AML patient group warrants further study.

ABSTRACT – 247 words as written (limit 250 words)

PURPOSE. To determine the maximum tolerated dose (MTD) of the histone deacetylase inhibitor vorinostat combined with fixed doses of cytarabine and etoposide in patients with poor-risk or advanced acute leukemia; to obtain preliminary efficacy data, describe pharmacokinetics and *in vivo* pharmacodynamic effects of vorinostat in leukemia blasts.

EXPERIMENTAL DESIGN. In this open-label phase I study vorinostat was given orally on days 1-7 at 3 escalating dose levels: 200 mg BID, 200 mg TID, and 300 mg BID. On days 11-14 etoposide (100 mg/m^2) and cytarabine (1 or 2 g/m^2 BID if ≥65 or <65 years old, respectively) were given. The study used a standard 3+3 dose escalation design.

RESULTS. Eighteen of 21 AML patients treated on study completed planned therapy. Dose-limiting toxicities (hyperbilirubinemia/septic death [1] and anorexia/fatigue [1]) were encountered at the 200 mg TID level; thus the MTD was established to be vorinostat 200 mg BID. Of 21 patients enrolled, 7 attained a complete remission (CR) or CR with incomplete platelet recovery, including 6 of 13 patients treated at the MTD. The median remission duration was 7 months. No differences in % S-phase cells or multidrug resistance transporter (MDR1 or BCRP) expression or function were observed *in vivo* in leukemia blasts upon vorinostat treatment.

CONCLUSIONS. Vorinostat 200 mg BID can be given safely for 7 days prior to treatment with cytarabine and etoposide. The relatively high CR rate seen at the MTD in this poor-risk group of AML patients warrants further studies to confirm these findings.

INTRODUCTION

The observation of aberrant histone deacetylase (HDAC) activity in a variety of human cancers – resulting in epigenetically-mediated enhancement of the expression of genes favorable to cancer progression and repression of genes regulating differentiation and apoptosis – led to the development of HDAC inhibitors as an anticancer therapeutic strategy. Vorinostat (suberoylanilide hydroxamic acid, NSC 701852) is a small molecule HDAC inhibitor that targets most human Class 1 and Class 2 HDAC enzymes, but does not affect the activity of Class 3 HDACs. Vorinostat has excellent oral bioavailability; it is currently the most potent HDAC inhibitor available clinically (1). Vorinostat is approved by the US Food and Drug Administration "for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies."

In addition to its efficacy in producing growth arrest and inducing differentiation or apoptosis in a variety of cancer cells, vorinostat was found to enhance the expression of TRAIL death receptors DR4 and DR5 in human leukemia cell lines, thereby synergizing with TRAIL in stimulating apoptosis by both the death receptor and mitochondrial pathways (2). In a few reports, P-glycoprotein (Pgp) or multidrug resistance protein (MRP2) was down-regulated in response to HDAC inhibitors (3, 4), whereas HDAC inhibitors upregulated breast cancer resistance protein (BCRP) and/or Pgp expression in other reports (5-9). These findings have all been observed in cultured cancer cell lines; to date, there are no published reports of alterations in TRAIL death receptor or multidrug resistance-associated transporter expression in tumor cells taken from patients undergoing treatment with vorinostat.

Vorinostat is being investigated for use in combination with other chemotherapeutics in a number of diseases, including the acute leukemias. In *in vitro* cytotoxicity studies, vorinostat interacted additively or synergistically with anthracyclines and etoposide (10). Studies in our laboratory confirmed these findings for etoposide; however, for cytarabine, a mainstay of AML therapy, we found that vorinostat was antagonistic in this combination because vorinostat diminished cells in S-phase, the cell cycle phase in which cells are most vulnerable to cytarabine toxicity (11). In contrast, the sequential administration of vorinostat followed by cytarabine produced synergy, particularly when a 72-hour interval was interposed between exposure to vorinostat and

exposure to cytarabine to allow re-entry of cells into S-phase (11).

The present work was conceived to test the safety of vorinostat given in a sequential combination with fixed doses of cytarabine and etoposide to patients with newly diagnosed poor-risk or advanced leukemias, and to define its maximum tolerated dose (MTD) in this regimen. Since vorinostat was administered as a single agent prior to cytarabine and etoposide, this provided the opportunity to study the effects of vorinostat on percentage of S-phase cells, expression of TRAIL death/decoy receptors, and expression and function of BCRP and MDR1/Pgp in patient-derived leukemic blast cells by sampling prior to vorinostat and during its administration.

MATERIALS AND METHODS

Study population

Patients ≥18 years old having relapsed or refractory AML or ALL, secondary AML (therapy-related or arising from antecedent hematologic disorder [AHD]), or CML in accelerated or blastic phase failing or intolerant of tyrosine kinase inhibitors were eligible for the study. A list of eligibility criteria is provided in Supplemental Table S1. A leukemic blast count $<30x10^9$/L was required at initiation of study treatment. Hydroxyurea or leukapheresis had to be discontinued at least 24 hours prior to initiation of treatment, however, their use was allowed on treatment days 1 through 10 (i.e., prior to the start of cytarabine and etoposide) if it became necessary to control a rising blast count ($>30x10^9$/L) or leukostasis.

Study design

This was a National Cancer Institute (NCI)-sponsored phase I dose-escalation study (NCT00357305) of vorinostat given in combination with fixed doses of cytarabine and etoposide to patients with poor-risk or advanced acute leukemias. The primary objective was to define the MTD of vorinostat given in combination with ara-C and etoposide for two strata of participants: those ≥65 years old, and those <65 years old. Vorinostat was administered orally (PO) on days 1-7 at the starting dose level (DL1) of 200 mg twice a day (BID). Dose escalation of vorinostat was planned to proceed to 200 mg three times a day (TID) (DL2) and 300 mg BID (DL3) until the MTD was defined. Patients <65 years old were given cytarabine 2 g/m^2 and patients ≥65 years old were given cytarabine 1 g/m^2 intravenously (IV) over three hours every 12 hours on days 11-14, for a total of 8 doses. Etoposide 100 mg/m^2 IV over 1 hour once a day on days 11-14 was given to all patients.

The classic 3+3 design was used for each age stratum with provision for cohort expansion to 6 evaluable patients if a dose-limiting toxicity (DLT) was observed among the initial 3 patients. If ≥2 DLTs were observed at a given DL, dose-escalation was halted and dose-finding continued at a lower DL until the MTD was defined (the highest DL with <33% first-cycle DLTs). For dose escalation, a patient was considered “evaluable” if the patient completed Cycle 1 treatment or was withdrawn from the protocol due to drug toxicity. If a patient was withdrawn from the study without meeting these criteria, the patient was replaced in that cohort, if needed, to have three

evaluable patients (or six if cohort needs to be expanded due to DLT). If the patient was removed from the study due to progressive disease before finishing treatment, this patient was replaced by enrollment of new patient in the cohort. Patients who achieved a complete remission (CR) or CR with incomplete platelet recovery (CRp) could receive a second cycle of treatment as consolidation. No intra-patient dose escalation was allowed. Secondary objectives included preliminary evaluation of efficacy, and evaluation of vorinostat pharmacokinetics and pharmacodynamics. Before any trial-specific activity was conducted, all patients signed a University of Maryland Institutional Review Board (IRB)-approved informed consent form. The study was monitored by the Greenebaum Cancer Center's Data and Safety Monitoring Committee (DSMC), and was conducted in accordance with the Declaration of Helsinki and in compliance with International Conference on Harmonization Good Clinical Practice Guidelines.

Safety assessment

Clinical and laboratory monitoring of the study participants was performed according to the standards of practice for adults with leukemia undergoing intensive anti-leukemia therapies. Toxicities were graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A DLT was defined as any grade ≥4 drug-related non-hematologic toxicity or any grade 3 drug-related non-hematologic toxicity lasting >24 hours, with the following exceptions: grade 3 elevation in bilirubin, SGOT, SGPT or alkaline phosphatase of any duration; fatigue and anorexia if lasting >7 days were considered DLT; grade 3 diarrhea or mucositis was considered DLT during vorinostat treatment but if encountered during or following cytarabine/etoposide treatment was considered DLT only if resolution to ≤grade 2 required more than 2 days; grade ≥3 neurologic toxicity of any duration was considered DLT. Hematologic DLT was defined as myelosuppression (grade 4) for ≥42 days following the start of cytarabine and etoposide, with a bone marrow (BM) cellularity of ≤5% and no evidence of leukemia.

Response

A BM examination was performed at the time of hematologic recovery (within one week of absolute neutrophil count ≥1.5x10^9/L and platelets ≥100x10^9L) or at any time that leukemia re-growth was suspected (12).

Response was determined according to the International Working Group criteria for AML (12).

Pharmacokinetic studies

Peripheral blood (PB) (5 mL/time point) was collected in a red-top vacutainer before, and at 0.5, 1, 2, 2.5, 3, 4, 6 and 8 h after the first vorinostat dose on day 1. The blood was allowed to clot at 4°C for 20-30 min, then centrifuged at 2,000x*g* for 15 min at 4°C, and serum was aspirated and stored at -70°C until analysis. Vorinostat was quantitated using a previously published, FDA-validated LC-MS/MS assay (13). Serum pharmacokinetic parameters, including area-under-the-concentration-versus-time curve (AUC) were extracted from the data by non-compartmental methods with PK Solutions 2.0™ (Summit Research Services, Montrose, CO, USA). Descriptive statistics were calculated with Microsoft Excel 2010.

Translational (pharmacodynamic) studies

Depending upon the number of cells available for study, the pharmacodynamic studies were conducted with the following order of priority (from highest to lowest): cell cycle studies, DR4/DR5 determination, DcR1/DcR2 determination, multidrug resistance transporter expression and function.

BM (or PB if the marrow was inaspirable, provided that the absolute blast count was >5x10^9/L), was collected prior to treatment ("pre" vorinostat sample) and once again between days 4-7 during vorinostat treatment approximately 2 hours following the most recent vorinostat dose ("on" vorinostat sample). Mononuclear cells were purified by gradient sedimentation using ACCUSPIN™ System-Histopaque®-1077 (Sigma-Aldrich, St. Louis, MO, USA). Leukemia cells isolated by this method were routinely >90% pure as determined by Wright-Giemsa staining (Sigma-Aldrich) of cytospin preparations. The cells were then cryopreserved in 7.5% DMSO/92.5% FBS and stored in liquid nitrogen vapor until use. Fresh or thawed cryopreserved cells were used for analysis. Preliminary studies revealed similar results for fresh and cryopreserved cells from the same individual.

Buccal mucosal cells were studied to determine the extent to which vorinostat treatment altered normal tissue. Buccal mucosal cells were obtained at times of BM/PB collection by swabbing the buccal mucosa four times with 4 to 6 sterile brushes (Cytobrush Plus GT, Medscand Medical, Hollywood, FL, USA), then collecting cells in RPMI 1640 medium (GIBCO Life Technologies, Grand Island, NY, USA), treating with Collagenase A (Roche Applied

Sciences, Indianapolis, IN, USA), 100 µg/ml for 1.5 h at 37°C, washing with PBS, sieve-filtering, re-washing with ice-cold PBS twice, then counting. Typically, this yielded 0.2 to 1x10^6 nucleated cells.

Cell cycle phase distribution determination and analysis of S-phase fraction: Cells were fixed with cold 70% ethanol, stained with propidium iodide/RNase staining buffer (BD Pharmingen, San Jose, CA, USA) and analyzed by flow cytometry (FACSCcanto, Becton Dickinson, San Jose, CA, USA) as described previously (11). Cell cycle phase distribution was determined using FlowJo Software (Tree Star, Inc., Ashland OR, USA) or ModFit (Verity Software House, Topsham, ME, USA).

Cell lines used for assay development and as positive and negative controls: HL-60/W cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) in 2005. HL-6O/Vinc cells (14), which were selected with vincristine and stably overexpress Pgp, were obtained from Dr. Melvin Center, Kansas State University, Manhattan, KS in 1992. HL-60/W and HL-60/Vinc cells were maintained in suspension culture in Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO), 10% (v/v) heat-inactivated fetal bovine serum (Lonza, Walkersville, MD), and were passaged weekly. K562 and K562/BCRP cells were kindly obtained from Dr. Yoshikazu Sugimoto of the Japanese Foundation for Cancer Research (15) in 2004, were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, and were passaged twice weekly. K562/MX10 cells are K562/BCRP cells that we selected with mitoxantrone to increase BCRP expression (16). After receipt in our laboratory, all cell lines were grown in quantity, then stored frozen in liquid nitrogen vapor in 10% dimethyl sulfoxide, 90% fetal calf serum until time of further use. Cell lines were used within 20 passages of initial cryopreservation. Cells were used in logarithmic growth phase, with cell viability routinely >95% by trypan blue dye exclusion. Cell cultures were tested twice yearly to assure the absence of contamination by Mycoplasma (Lonza). The authenticity of these cell lines in comparison to ATCC data for these lines was verified by short tandem repeat profiling (StemElite ID System, Promega, Madison WI). HL-60, HL-60/Vinc, K562 and K562/BCRP, all shared 13 of 13 human autosomal alleles (100%) with the ATCC reference for HL-60 or K562. Each cell line tested negative for the mouse marker included in the StemElite ID kit.

Determination of TRAIL death and decoy receptor protein expression: Leukemia cells were washed twice with PBS/2%FBS, then stained with antibodies. Up to 5x10^5 mononuclear cells or buccal mucosal cells were suspended in 100 µL of PBS. The antibodies used were PE-conjugated and obtained from R&D Systems,

Minneapolis, MN, USA: TRAIL-R1/DR4 antibody, cat. no. FAB347P; TRAIL-R1/DR5 antibody, cat. no. FAB6311P; TRAIL-R3/DcR1 antibody, cat. no. FAB6302P; TRAIL-R4/DcR2 antibody, cat. no. FAB633P, and the corresponding isotype controls for the PE-labeled antibodies (IgG1 cat. no. IC002P or IgG2b cat. no. IC004P). Human FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany), cat. 130-059-901) was used for each reaction to eliminate nonspecific antibody binding (20µL/1x10^7 cells). 10 µL of the antibody were added to 100 µL of cell suspension. Following incubation on ice for 1 h, the cells were washed twice with PBS, fixed in 2% paraformaldehyde, and then analyzed by flow cytometry. HL-60 cells untreated and treated with vorinostat (1 µM for 24 hours) were used as positive controls for DR4, DR5, DcR1 and DcR2.

Assessment of MDR1/Pgp and BCRP transcripts, protein, and function: Total RNA was extracted from at least 10^6 leukemic blasts cells using NucleoSpin® RNA II (Macherey-Nagel, Bethlehem, PA). The RNA was used for Real Time Quantitative-PCR analysis of MDR1, BCRP, and β-actin transcript levels. Up to 500 µg of total RNA were converted into cDNA using qScript™ cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA). The RT-PCR reactions were prepared using PerfeCTa® SYBR® Green FastMix® for iQ™ (Quanta Biosciences) and detected on the BioRad MyiQ™ instrument (Biorad, Hercules, CA, USA). Expression of MDR1, BCRP, and β-actin mRNA was measured using quantitative, real-time PCR methods with primers as described previously (16).

Pgp (ABCB1) and BCRP (ABCG2) proteins were detected by flow cytometry using the PE-conjugated anti-MDR1 (clone UIC2, cat. 12-2439-41 eBioscience, San Diego, CA, USA) or anti-BCRP (clone 5D3, cat. FAB995A, R&D Systems). PE-conjugated mouse IgG2a κappa and mouse IgG2b, were used as isotype control antibodies for Pgp and BCRP determinations respectively. Cell staining with antibody and isotype control was compared by the Kolmogorov-Smirnov (KS) statistic, which generates D-values ranging from 0 (no difference) to 1 (no overlap) (17). A D-value of <0.15 is considered to indicate no overexpression of the transporter (18).

Functional expression of BCRP was determined as outlined previously (19) by measuring the effects of a specific inhibitor of BCRP (Ko143 10 µmol/L, Sigma-Aldrich) on the accumulation/retention of a specific BCRP substrate (pheophorbide A at 1 µM, Frontier Scientific, Inc., Logan, UT, USA); functional expression of Pgp was measured by the effects of a specific Pgp inhibitor (PSC833 2.5 µg/mL, Novartis, Basel, Switzerland) on the accumulation/retention of a specific substrate of Pgp (3,3'-diethyloxacarbocyanine iodide, DiOC2, Sigma-Aldrich) at 0.6 ng/mL. Intracellular dye accumulation (3h) and retention (20h) were measured by flow cytometry and analyzed

using FlowJo software (Tree Star, Inc.).

HL-60/Vinc cells and their corresponding parental cell line HL-60/W were used as positive and negative controls for MDR1/Pgp, respectively (14); K562/MX10 cells and their corresponding parental cell line K562/W were used as positive and negative controls for BCRP (16).

Statistical analysis

Overall survival (OS) was measured from the time of enrollment onto this study to the time of death. Progression free survival (PFS) was measured from the time of enrollment to the time of documented disease progression. Survival (overall and progression-free) was estimated using the Kaplan-Meier method. CR duration was measured from the time when criteria were first met for CR until the first date that recurrent disease was objectively documented. Exact confidence intervals were computed for proportions such as response rate.

The two-sample paired t-test and the Wilcoxon Signed-Rank Test were used to compare the secondary endpoint data in the “pre” vorinostat samples with those of the “on” vorinostat sample. For comparison of the “pre” vorinostat sample with the “on” vorinostat sample for a given patient, the 2-tailed t-test was used.

RESULTS

Patient characteristics

Twenty-one patients were treated on this study; their characteristics are summarized in Table 1 and in Supplemental Table S2. Their median age was 61 years and 11 (52%) patients were 60 and older. All patients had high-risk AML, including relapsed or refractory AML, AML arising from AHD or therapy-related. Among 10 patients with prior remission, nine had CR1 duration of <12 months. Overall, 10 patients had AML arising from AHD (4 therapy-related; 2 arising from myeloproliferative disease and 8 from myelodysplastic syndrome) and 1 patient had newly diagnosed therapy-related AML. Nine of the 10 patients received prior treatment for AHD such as hydroxyurea and erythropoietin (1 patient), azacitidine or decitabine (7 patients), and allogeneic stem cell transplant (SCT) (1 patient). An additional patient treated on study received allogeneic SCT for AML in CR2. Overall, 4 (19%) patients had favorable karyotype (all with additional karyotypic abnormalities and 1 patient with documented c-kit mutation). Eight patients (43%) had unfavorable karyotype including complex karyotype (≥ 3 abnormalities) in 5 (24%) patients, del 7/7q in 6 (29%) patients, del 5/5q in 5 (24%) patients (both in 4 patients); 9 patients had intermediate-risk karyotype. Among 4 patients with normal karyotype, 3 had a FLT3-ITD mutation.

Toxicity and determination of MTD

Eighteen of 21 patients completed the assigned first cycle of therapy. Two patients completed only the vorinostat portion and withdrew consent before starting cytarabine/etoposide on day 11; of these, one patient developed progressive bone pain and increasing WBC while on vorinostat and requested to proceed with an alternative chemotherapy on day 8; another refused any chemotherapy on day 9. An additional patient age >65 was inadvertently given a higher dose of cytarabine (2 g/m^2 instead 1 g/m^2) and hence is evaluable only for vorinostat toxicity. The most frequent toxicities judged "possibly" related to the full treatment regimen are listed in Table 2A and 2B, and include diarrhea, nausea, fever, vomiting, and fatigue. Of the 2 patients treated with vorinostat only, none experienced a DLT.

Of the 7 patients treated at DL1 (200 mg vorinostat PO BID), one patient opted off protocol after receiving only vorinostat. This patient was evaluable for toxicity but not dose escalation. The remaining six of 7 patients

initially treated on DL1 completed therapy and were evaluable for the dose escalation. No DLTs were observed and other than fever, no grade ≥3 adverse events were noted (3 patients in each age stratum). Eight patients were then treated at DL2 (200 mg vorinostat TID; 6 patients <65 years old and 2 patients ≥65 years old) but only 5 were evaluable for dose escalation. Three patients were not evaluable for dose escalation for the following reasons: incomplete treatment (<65 years old), incorrect cytarabine dose and death due to sepsis unrelated to study treatment on day 36 (>65 years old), and fungal sepsis and death before count recovery on day 39 (<65 years old, not considered DLT). However, 2 DLTs were observed at DL2 (Table 2A and 2B). Amongst the <65 year old age stratum, dose-limiting hyperbilirubinemia was observed in one patient. This patient subsequently experienced multi-organ failure and death on day 49 in the setting of neutropenia and fever which were considered to be possibly treatment-related. In the ≥65 year old age stratum, one patient experienced dose-limiting grade 3 anorexia, fatigue, and muscle weakness attributable to vorinostat. Given 2 DLTs among 5 dose-escalation-evaluable patients at DL2, and an additional patient with neutropenic death, the DSMC recommended dose-deescalation to DL1, as stipulated in the protocol design. Six more patients were treated at DL1 (3 in each age stratum) with no DLT. Hence, the MTD of vorinostat in combination with fixed doses of cytarabine and etoposide was established to be 200 mg PO BID.

Median times to count recovery are shown in Table 2C. Overall, 16 (77%) patients experienced grade ≥3 febrile neutropenia, 7 (33%) patients had documented bacteremia, and 9 (43%) had pneumonia. Two patients received a second course of protocol treatment as consolidation. Five patients had high or rising blast counts during or before vorinostat therapy; most required hydroxyurea.

Antitumor properties

Clinical outcomes are summarized in Table 3A. Analyzed on an "intent-to-treat basis," of the 21 patients enrolled, 5 achieved CR and 2 CRp, giving an overall response rate (ORR) of 33%. Of these 21 patients, 5 were not evaluable for treatment response because of early death (2 patients), protocol violation (1 patient), or not receiving the full course of treatment (2 patients–Table 3A). The median remission duration was 7 months (range 1 to 19+ months). The ORR at the MTD was 46% (Table 3A, B). OS was 193 days (95% CI 96-519 days) and progression-free survival was 45.5 days (95% CI 42-342 days), Figure 1. The characteristics of patients achieving CR

or CRp, including post-remission treatments, is given in Table 3B. One patient is currently alive who had an allogeneic SCT following achievement of CR. The diagnosis, age, initial WBC, need for hydroxyurea, mutational status and karyotypes of the 21 patients enrolled are provided in Supplemental Table S2. In the CR group, 3 patients had favorable karyotypes and no patient required hydroxyurea to control circulating blasts during vorinostat treatment. The median age of the CR group was 61 years (range 46-71 years) compared to 69 years for the PD group (range 20-77 years) and 57 years for the not evaluable group (NE, range 35-67 years). There were no statistically significant differences in age among the groups (2-tailed t-test, $P>0.05$).

Pharmacokinetics

All patients provided pharmacokinetic samples. A summary of the pharmacokinetic data is given in Table 4. Vorinostat pharmacokinetics were similar at DL 1 and 2, and between patients younger or older than 65 years. The C_{max} values for DL1 averaged 196.8±90.4 ng/mL, with a t_{max} of 2.1±1.9 hours. The C_{max} values for DL2 averaged 165.2±64.1 ng/mL, with a t_{max} of 3.5±2.0 hours. The C_{max} values for the <65 yo cohort averaged 173.8±173.8 ng/mL, with a t_{max} of 3.3±3.3 hours. The C_{max} values for the ≥65 age group averaged 202.6±101.4 ng/mL, with a t_{max} of 1.6±1.1 hours.

Vorinostat effects on the cell cycle in patient-derived blasts and buccal mucosal cells

Cell cycle phase distribution was determined in leukemic blasts (16 patients) and/or buccal mucosal cells (17 patients) sampled “pre” and “on” vorinostat. For buccal mucosal cells, there was no statistically significant alteration in any cell cycle phase upon treatment (Table 5A). Similarly, leukemia blasts showed no statistically significant change in cell cycle phase in aggregate (Table 5A), or when analyzed by treatment response category using the two-sample paired t-test (Supplemental Table S3).

TRAIL death and decoy receptors

The percentages of cells expressing the TRAIL death receptors DR4 and DR5 and the TRAIL decoy receptors DcR1 and DcR2 were determined by flow cytometry. All death receptor data for paired “pre” and “on” vorinostat samples are provided in Supplemental Table S4. Although the mean values increased slightly for DR4,

DR5, DcR1, and DcR2 (Table 5B-D), due to the small sample size and relatively large variance no significant differences were seen amongst the leukemic blasts between the "pre" and "on" vorinostat samples, using the two-sample paired t-test or the Wilcoxon Signed-Rank test. Furthermore, there were no statistically significant differences in death receptor expression in the "pre" and "on" vorinostat samples amongst the CR and PD groups (data not shown). The buccal mucosal samples available for death receptor studies were limited; however, no statistically significant differences between "pre" and "on" vorinostat samples were observed for DR4 or DR5 expression in buccal mucosal cells (Table 5B, C).

Table 5E demonstrates the validity of our assays using HL-60 cells treated with vorinostat as positive controls for DR4, DR5, DcR1 and DcR2. Such controls were run concomitantly with each assay of patient samples. These studies reveal that 24-hour exposure of HL-60 cells to 1 μM vorinostat significantly increases DR4, DR5, DcR1 and DcR2 expression, (P<0.02, t-test).

Vorinostat effects on the expression and function of MDR1-Pgp/ABCB1 and BCRP/ABCG2

Eight paired patient samples were evaluated for MDR1 or BCRP mRNA expression. None of these patients achieved a response from protocol treatment. Data are expressed as amount of mRNA present relative to beta-actin x 10^{-4}. In the pretreatment samples (Table 5F), MDR1 mRNA expression was comparable to that of K562/W cells with the exception of cells of patients 22 and 24, which appeared to express a higher level of MDR1 mRNA; additionally, cells of patients 2, 22, and 24 appeared to express levels of BCRP mRNA that were >3 times the expression level of the parental HL-60/W cell line. MDR1 expression increased in the "on" treatment samples in 7 patients (88%, 95% CI 50%-99%) suggesting a trend toward increased expression of MDR1 mRNA caused by vorinostat (Table 5F). Within patients, using the 2-sample t-test, MDR1 mRNA expression levels increased in the "on" vorinostat samples from 4 patients and decreased in 1 patient (P≤0.05). BCRP mRNA did not differ significantly between "pre" and "on" vorinostat samples except for patient 13 (Table 5F). Among all patients, there was no significant alteration in MDR1 or BCRP mRNA expression in the "on" vorinostat sample compared to the "pre" vorinostat samples using the paired t-test.

Only 3 paired patient samples were studied for transporter protein expression. Of these, patients 17 and 24 met Kolmogorov-Smirnov D-value criteria for BCRP protein expression in the pretreatment samples, with slight

decreases following treatment (Table 5F).

Six paired patient samples were studied by functional assays; of these, pretreatment samples from patients 7, 13, 17, 18, 22, and 24 appeared to show enhanced accumulation or retention of DiOC2 in the presence of the Pgp inhibitor PSC833, consistent with functional expression of Pgp (Table 5F). None of the pretreatment samples appeared to express functional BCRP. There was no difference in function for either Pgp or BCRP in the "on" vorinostat sample compared to the "pre" vorinostat samples using the paired t-test.

DISCUSSION

This phase I clinical trial achieved its primary objective of determining the MTD of vorinostat given on days 1-7 prior to the administration of fixed doses of cytarabine and etoposide on days 11-14 in patients with relapsed, refractory, or high-risk newly diagnosed acute leukemias. Furthermore, it provides preliminary descriptive data regarding the treatment efficacy of this combination. At the MTD of vorinostat – 200 mg BID – the regimen was well tolerated with common adverse events of fatigue, nausea, vomiting, and diarrhea occurring at grade 1 or 2 levels. Higher dosing of vorinostat (200 mg TID) was associated with more severe fatigue, anorexia, muscle weakness, neutropenia and severe infections. In patients who responded to treatment, the median time to blood count recovery was within the time-frame expected in AML patients treated with intensive chemotherapy. Using intent-to-treat analysis, the ORR was 33% in this population of high-risk patients, and was 46% in patients treated at the MTD level, with median remission duration of 7 months.

HDAC inhibitors have demonstrated modest antileukemic activity as single agents. In a phase I study of MS-275 (entinostat), a benzamide HDAC inhibitor, in 38 adults with advanced acute leukemias, increased acetylation of histone H3/H4 was demonstrated, but no clinical responses were seen, using “classical criteria” (20). A low (5%) response rate was observed in frail or elderly AML patients treated with valproic acid alone or in combination with all-trans retinoic acid (21). In a phase I study of depsipeptide in 10 AML patients, some antitumor activity was seen, but there were no CRs (22). Severe constitutional symptoms limited the extent to which depsipeptide could be administered in that study. A subsequent phase II investigation of depsipeptide in 20 AML patients found antileukemic activity (but no CRs) only in patients with rearrangements involving core binding factor (CBF), predominantly the t(8;21) translocation (9). In our present study, of the 7 responders, 3 had CBF rearrangements, two with inv(16)(p13.1;q22); one with t(3;21)(q26.1;q22). All 3 of these patients achieved a CR, with remission durations of 10, 7 and 4 months. In contrast, only one of the 9 patients in the nonresponding group had CBF AML, with t(8;21)(q22;q22).

Vorinostat as a single agent appears to have better antileukemic activity than MS-275, valproic acid, or depsipeptide, and is better tolerated than the latter. A phase I trial of vorinostat in 41 patients with advanced leukemias and myelodysplastic syndromes documented 4 CRs in AML patients, none of whom had CBF AML. However, there was only one case of CBF AML amongst the 31 AML patients studied (23). In that study, vorinostat

was administered for 14 days in 21-day treatment cycles. The MTD was found to be 250 mg TID, or 200 mg BID. As with our study, the common toxicities observed were gastrointestinal and fatigue. Based on preclinical data suggesting synergism between HDAC inhibitors and anthracyclines (10), another phase I investigation examined vorinostat given for 3 vs. 14 days in combination with fixed doses of idarubicin in 41 patients with relapsed or refractory leukemias (24). DLT was encountered in 2 patients on the 14-day arm, including prolonged myelosuppression and mucositis. The MTD was found to be 400 mg TID for 3 days, with idarubicin given at 12 mg/m^2 IV days 1-3. Clinical responses were seen in 7 of the 41 patients (17%); including 2 CRs and one CRp. Recently, very encouraging results were reported for a phase II study by Garcia-Manero et al using vorinostat at 500 mg TID x 3 days followed by cytarabine and idarubicin 24 hours later. Patients were given a shortened consolidation cycle followed by vorinostat maintenance. Among 75 newly diagnosed patients, the CR/CRp rate was 86% with median survival of 15.7 months. Interestingly, among 11 FLT3-mutated patients, the ORR was 100% with 1-yr survival of 91% (25).

The serum exposures of vorinostat observed in the current study appear somewhat lower than that reported previously in the literature for a 200 mg dose of vorinostat under fed conditions (26); however, given the large variability in vorinostat exposure, our observed values are not at odds with previously published data.

The present study was based on our preclinical evaluation of the combination of vorinostat with cytarabine and etoposide in human leukemia cell lines (11) which demonstrated synergy between vorinostat and etoposide, but antagonism for the concomitant administration of cytarabine and vorinostat. This antagonism was attributed to vorinostat-induced diminution of the percentage of cells in S-phase, and was overcome by sequential administration of vorinostat followed by cytarabine, which produced synergism. Vorinostat is thought to affect the cell cycle by blocking cell entry into S-phase (27). In our previous study, significant reduction in the percentage of S-phase cells was found within 24 hours after *in vitro* exposure to vorinostat at concentrations of 1 μM or greater. In the present study, following a 200 mg oral dose of vorinostat, we found the mean C_{max} to be 185 ng/mL (0.7 μM), with a T_{max} of 2.6 h (Table 4). With these plasma concentrations, we did not observe a significant alteration in the percentage of buccal mucosal or leukemia blast cells in S-phase of the cell cycle in the “on” vorinostat samples, compared to the ”pre” vorinostat samples. Hence for the MTD dose level, adverse cytokinetic interactions between vorinostat and cytarabine may be of less concern than predicted initially (11).

HDAC inhibitors are known to promote apoptosis by both the intrinsic and extrinsic pathways, and to enhance expression of the TRAIL death receptors DR4 and DR5 in cultured cell lines. Specifically, HL-60 cells were previously found to exhibit increased DR4 and DR5 expression, but not DcR1 or DcR2 expression, following 24-hour exposure to 1 μM vorinostat (2). Our present control studies with HL-60 cells confirm the published findings for DR4 and DR5, but we found that these conditions also increased the expression of the decoy receptors DcR1 and DcR2 (Table 5E), in contrast to the previous report. Peak plasma levels of vorinostat approached 1 μM in our clinical trial (C_{max} 0.7 μM) but, in aggregate, there was no difference in death or decoy receptor expression in the "pre" vs. the "on" vorinostat samples.

In the limited number of patient samples studied, heterogeneity of expression of MDR1 or BCRP mRNA and Pgp function was observed. Although good correlation between mRNA, protein and functional assays was seen for the MDR1/Pgp and BCRP transporters in the control cell lines, there was poor correlation between these measures in the patient samples, as was observed in a previous study of AML samples (28). In aggregate, we did not observe an increase in MDR1 expression in the "on" vorinostat samples compared to "pre;" however, when change within individual patients was considered, we did observe a trend for higher MDR mRNA expression in the "on" vorinostat samples, as was reported previously for bone marrow blast cells in a clinical trial with another HDAC inhibitor, depsipeptide (9).

We have found that vorinostat administered on days 1-7 at a dose of 200 mg PO BID can be safely and effectively combined with cytarabine (2 g/m^2 for patients <65 years old or 1 g/m^2 for patients ≥65 years old, IV Q12H) and etoposide (100 mg/m^2/day IV QD) given on days 11-14. On an "intent-to-treat" basis, among 13 high risk AML patients treated at the MTD level, a CR rate (5 CR + 1 CRp) of 46% was observed. Encouraging results reported by Garcia-Manero also support the validity of this approach (25). Given the potential need for hydroxyurea to control blast counts during vorinostat treatment and lack of confirmation of *in vitro* pharmacodynamic effects on S-phase cells, it is possible that higher doses of vorinostat given initially but for a shorter period of time may be as effective and easier to administer in patients with rapidly proliferating disease. Confirmation of the efficacy of this approach in a future phase II trial is warranted.

Table 1: Patient characteristics

Total, *N*	21
Completed full treatment regimen, n	18
Completed only vorinostat, n	3
Male/Female, n	11/10
Median Age, y	61
Age range, y	20-77
Age < 60 years	10
Age ≥ 60 years	11
Ethnicity, n (%)	
White	16 (76)
Black	3 (14.5)
Hispanic	2 (9.5)
Age stratum 1 (<65 years), n	13
Male/Female, n	7/6
Median age, y	54
Evaluable for response	9
Evaluable for toxicity	13
Age stratum 2 (≥65 years), n	8
Male/Female, n	4/4
Median age, y	71
Evaluable for response	7
Evaluable for toxicity	8
ECOG performance status, n	
0	14
1	5
2	2
Diagnosis, n (%)	
AML-newly diagnosed	6 (28.5)
2°AML and/or t-AML	6
AML-relapsed	8 (38)
2°AML	2
AML-refractory (primary or at relapse)	7 (33.5)
2°AML and/or t-AML	3
Median No of prior Rx for AML, range	1 (0-3)
Karyotype	
Favorable*	4
Intermediate*	9
Normal	4
Unfavorable*	8
Complex	5

*Karyotype prognostic categories based on classification as described in (29, 30)

Table 2. Vorinostat + ara-C + etoposide: Toxicity

A. Treatment-related adverse events with frequency > 10%, n, (%); DLTs in bold

	DL 1 (N=13)		DL 2 (N=8)		
Adverse event (drug related)	Gr 1/2	Gr 3-4	Gr 1/2	Gr 3-4	Gr 5
Anorexia	1 (8%)	-	3 (38%)	**1 (13%)**	-
Fatigue	5 (39%)	-	1 (13%)	**2 (25%)**[+]	-
Muscle weakness	3 (23%)	-	-	**2 (25%)**[+]	-
Multi organ failure	-	-	-	-	**1 (13%)***
Fever-infection	-	5 (39%)	-	3 (38%)	**1 (13%)***
Nausea	5 (39%)	-	5 (63%)	-	-
Diarrhea	4 (31%)	-	5 (63%)	-	-
Vomiting	5 (39%)	-	3 (38%)	-	-
Rash	3 (23%)	-	1 (13%)	-	-
Constipation	1 (8%)	-	-	-	-
Pruritis	1 (8%)	-	-	-	-
Chills	-	-	1 (13%)	-	-
Edema	-	-	1 (13%)	-	-
Enterocolitis	-	-	1 (13%)	-	-
Headache	-	-	1 (13%)	-	-
Pain	-	-	1 (13%)	-	-
Cough	-	-	1 (13%)	-	-
Oral pain	3 (23%)	-	-	-	-
Epistaxis	2 (15%)	-	-	-	-

B. Treatment-related grade 3 or 4 laboratory abnormalities observed, n; DLTs in bold

	DL 1 (N=13)		DL 2 (N=8)			Total (N=21)		
Laboratory test	Gr 3	Gr 4	Gr 3	Gr 4	Gr5	Gr 3	Gr 4	Gr5
Anemia	2	0	0	0	0	2	0	0
Neutropenia	0	2	0	0	0	0	2	0
Thrombocytopenia	0	0	0	1	0	0	1	0
Total bilirubin increase	0	0	0	0	**1**	0	1	**1***

+ Of the 2 cases with grade 3 fatigue, only one was dose-limiting; of the 2 cases with grade 3 muscle weakness, only one was dose-limiting. The dose-limiting toxicities were seen in a single patient.

*** All grade 5 toxicities were seen in a single patient**

Table 2, continued

C. Blood count recovery data - responding patients

Patient #	Time to ANC>500 (days)	Time to Platelets >50K (days)	Time to Platelets >100K (days)	Time to platelet transfusion independence (days)	Time to RBC transfusion independence (days)
1	29	32	34	25	26
6	42	44	-	42	38
8	33	29	30	25	20
14	36	33	47	32	48
19	32	33	43	30	31
20	30	30	32	28	28
23	69	60	76	47	60
mean	39	37	44	33	36
median	**33**	**33**	**43**	**30**	**31**
range	29-69	29-60	30-76	25-47	20-60

Table 3. Vorinostat + ara-C + etoposide: Response

A. Clinical response by dose level and age stratification (N=16)

Response	Dose level 1* (N=13)		Dose level 2** (N=8)		All Patients (N=21)	
	< 65 years	≥ 65 years	< 65 years	≥ 65 years	< 65 years	≥ 65 years
CR	3 (23%)	2 (15%)	0	0	3 (14%)	2 (10%)
CRp***	1 (8%)	0	1 (13%)	0	2 (10%)	0
PD	2 (15%)	4 (31%)	2 (25%)	1 (13%)	4 (19%)	5 (24%)
Vori only****	1	0	1	0	2	0
ED*****	0	0	2	0	2	0
Protocol violation	0	0	0	1	0	1
CR+CRp	**46%**		**13%**		**33%**	

* Dose level 1: 200 mg vorinostat BID x 7 days

** Dose level 2: 200 mg vorinostat TID x 7 days

***CRp: CR with incomplete platelet recovery

****Vori only, patient received only vorinostat, and no other protocol chemotherapy

*****ED, early death, i.e. while pancytopenic, hence unable to assess response to therapy

B. Treatments following remission for patients who achieved CR (N=7)

Pat-ient	Diagnosis	Response	Protocol consoli-dation given?	Post protocol treatment	CR Duration, months	Overall Survival, months
14	tAML-rel/ref	CRp	No	None	1	3
19	tMDS-> tAML	CR	No	One HDAC consolidation, Phase I trial upon relapse	4	9
20	AML-rel	CR	Yes	No further consolidation. HAM salvage, then Phase I trials upon relapse	6	18
8	AML-rel/ref	CR	Yes	Matched sibling BMT while in CR, palliative care upon relapse	7	12
1	AML-rel	CR	No	DMA, then palliative care	10	17
6	AML-rel	CRp	No	Allogeneic BMT while in CR, MEC salvage upon relapse	17	20
23	MDS-> AML	CR	No	Allogeneic BMT while in CR, remains in CR	19+	21+

Abbreviations used: HDAC, high dose ara-C; HAM, ara-C+mitoxantrone; BMT, bone marrow transplant; DMA, demethylating agent; MEC, mitoxantrone, etoposide, ara-C.

Patient 14 was treated at vorinostat DL2; all others were treated at DL1.

Table 4: Serum vorinostat pharmacokinetic parameters

Parameter	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}8h}$ (ng•h/mL)	$AUC_{0\text{-}inf}$** (ng•h/mL)	Cl/F (L)	Vd/F (L/h)
Mean	185	2.6	2.5	632	779	300	1003
Geometric mean	167	2.0	2.1	591	722	277	829
Median	161	2.0	1.7	623	757	265	689
CV%	44	77	73	36	40	42	80
N	21	21	20	21	20	20	20
Literature (mean)*	279	2.0	1.2	-	933	347	-

* Literature values as reported by Kelly et al. following a 200 mg dose of vorinostat, N=6 (26).

** The percentage of $AUC_{0\text{-}inf}$ extrapolated beyond the last time point was on average 16.4% (range 1.4-61.9%)

Table 5: Translational Studies

A: Vorinostat effects on cell cycle phase

BUCCAL MUCOSA CELLS. N = 17

	% G_0/G_1		% S		% G_2/M		% sub G_0/G_1	
	Pre	On	Pre	On	Pre	On	Pre	On
MEAN	77.99	79.85	11.49	11.30	2.14	3.26	11.74	7.79
SD	10.16	11.58	7.05	9.57	2.67	4.25	10.28	6.95
P*	0.5705		0.9168		0.2586		0.1942	

BLAST CELLS. N = 16

	% G_0/G_1		% S		% G_2/M		% sub G_0/G_1	
	Pre	On	Pre	On	Pre	On	Pre	On
MEAN	83.97	87.60	9.22	7.17	3.83	3.55	4.29	2.60
SD	10.38	6.18	8.12	4.81	3.74	3.09	2.65	1.62
P*	0.1519		0.2841		0.7556		0.0693	

***Two-sample paired t-test**

B: % of cells expressing DR4

	BUCCAL MUCOSA CELLS			LEUKEMIA BLAST CELLS		
Response	Mean (N)	Std	P-value*	Mean (N)	Std	P-value*
Pre	27.90 (4)	22.30	0.5873	12.21 (14)	22.45	0.5498
On	41.39 (4)	30.28		13.82 (14)	21.12	

***Two-sample paired t-test**

C: % of cells expressing DR5

	BUCCAL MUCOSA CELLS			LEUKEMIA BLAST CELLS		
Response	Mean (N)	Std	P-value*	Mean (N)	Std	P-value*
Pre	26.46 (4)	39.04	0.9316	14.75 (14)	17.95	0.3617
On	25.98 (4)	36.48		17.68 (14)	21.69	

***Two-sample paired t-test**

D: % of cells expressing DcR1 or DcR2

	DcR1			DcR2		
	LEUKEMIA BLAST CELLS			LEUKEMIA BLAST CELLS		
Response	Mean (N)	Std	P-value*	Mean (N)	Std	P-value*
Pre	17.59 (11)	12.36	0.1364	15.83 (11)	15.47	0.5279
On	25.97 (11)	19.42		17.56 (11)	11.19	

***Two-sample paired t-test**

E: Control studies for DR or DcR expression

	% positive cells			
Cell Line	DR4	DR5	DcR1	DcR2
HL-60/W	55.8±14	47.3±27	23±2	56.2±3
HL-60/W + V*	73.2±15	84.2±6.5	94.3±1.7	96.3±1.1

***** cells were treated with 1 µM vorinostat x 24 hours**

Table 5, continued. F: Multidrug resistance transporter data

Patient or Cell Line	mRNA[a]				Protein[b]				Functional Assay[c]							
									Pgp				BCRP			
	MDR1		BCRP		Pgp		BCRP		Accum		Retn		Accum		Retn	
	PRE	ON	PRE	ON	PRE	ON	PRE	ON	PRE	ON	PRE	ON	PRE	ON	PRE	ON
2	5.1	6.4	155	21.2	nd[d]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	4	**22.3**	11.9	1.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	0.7±0.1	**64.1±3.8**[e]	0.4±0.1	0.5±0.1	0.03	0.05	0.05	0.05	**274**	**1069**	**194**	**65**	8.3	-4.2	-2.7	5.7
13	1.6±0.3	2.75±0.26[f]	1.8±0.5	0.07±0.004[f]	nd	nd	nd	nd	2.4	24.5	**50**	19.7	-2.6	-3.9	-12.1	-12.3
17	7.5±1	**17±6.2**[h]	13.1±3.4	12.8±3.7	0.08	0.07	**0.24**	**0.15**	-52	**211**	**99**	**299**	-5.3	11.5	13.7	6.2
18	0.5±0.01	1.7±0.29[g]	11.1±0.4	8±0.9	nd	nd	nd	nd	**165**	14.1	11.8	21.1	2.3	-1.2	-21.2	29.3
22	**162±6**	**39.3±5.8**[e]	**105±71**	**429±726**	nd	nd	nd	nd	**716**	**861**	**174**	**147**	0.1	-2.1	-18.8	-23.7
24	**30±8**	**42±7**	**22±20**	**188±228**	0.03	0.08	**0.34**	**0.19**	**209**	**253**	**265**	**295**	1	-4.3	-2.4	12.5
HL-60/W	<.01	-	4.5±2.7	-	0.07	-	nd	-	1.1	-	-2.9	-	nd	-	nd	-
HL-60/Vinc	**7,022±1,560**	**-**	0.02±0.01	-	**1**	**-**	nd	-	**759**	**-**	**26**	**-**	nd	-	nd	-
K562/W	4.5±1.3	-	0.01±0.09	-	nd	-	0.01	-	nd	-	nd	-	-6.5	-	-4.8	-
K562/MX10	0.1±0.02	-	**9,560±1,534**	**-**	nd	-	**0.98**	**-**	nd	-	nd	-	**88**	**-**	**109**	-

a. Values for mRNA are the amount of MDR1 or BCRP mRNA expressed relative to expression of beta-actin x 10^{-4}. The values shown after the ± sign indicate standard deviation of 3 replicate RT-PCR reactions. Values shown in **bold** are more than 3 times the expression of MDR1 mRNA in K562/W cells or BCRP mRNA in HL-60/W cells.

b. Values shown for protein expression are the Kolmogorov-Smirnov D-values of the difference between histograms for isotype antibody control and anti-MDR1 antibody (UIC2) or anti-BCRP antibody (5E2). A D-value of <0.15 is considered to indicate no overexpression of the transporter.

c. Accumulation or Retention of DiOC2 (for Pgp function) or pheophorbide A (for BCRP function) is expressed as % change defined previously (31):

$$\% \text{ change} = 100 \times [(A_{mod} \text{ or } R_{mod}) - (A_{ctrl} \text{ or } R_{ctrl})]/(A_{ctrl} \text{ or } R_{ctrl}),$$

where A_{mod} = dye accumulation in the presence of the transporter modulator (PSC833 or Ko143), R_{mod} = dye retention in the presence of the transporter modulator, A_{ctrl} = dye accumulation in absence of modulator; R_{ctrl} = dye accumulation in absence of modulator.

d. nd, not determined

e. "Pre" and "on" vorinostat samples differ significantly by 2 sample t-test, $P < 0.001$.

f. "Pre" and "on" vorinostat samples differ significantly by 2 sample t-test, $P < 0.01$

g. "Pre" and "on" vorinostat samples differ significantly by 2 sample t-test, $P = 0.05$

h. Comparison of differences between "pre" and "on" vorinostat samples by 2 sample t-test, $P = 0.057$

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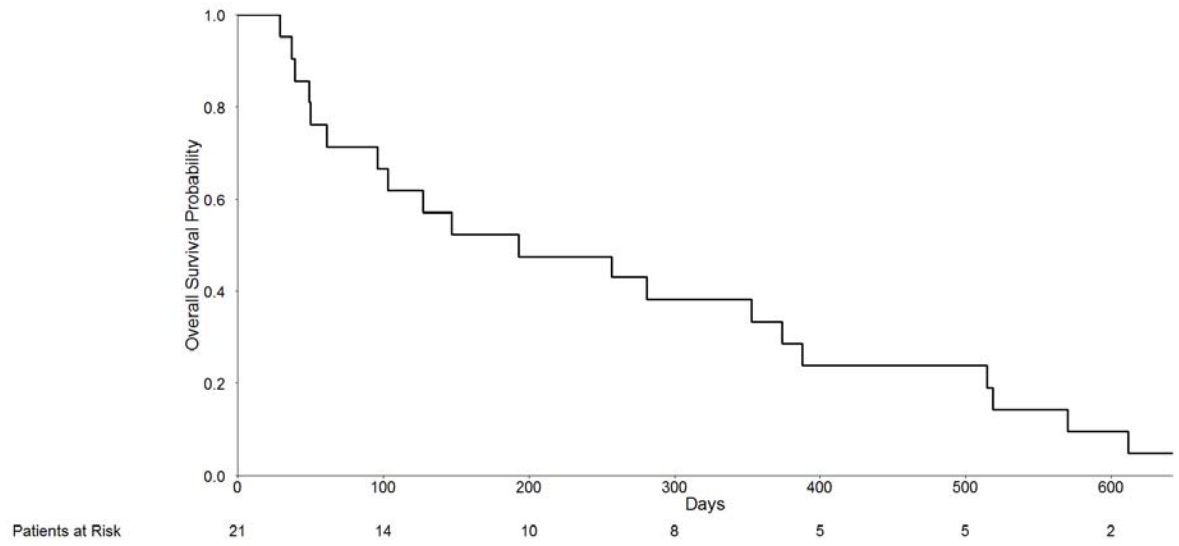
FIGURE LEGEND

Figure 1 Kaplan-Meier plots. A. Overall survival. The 'patients at risk' at the bottom margin shows overall survival of patients (N=21). The median survival in days (95% CI) is 193 (96-515). B. Progression-free survival. The 'patients at risk' at the bottom margin denotes progression-free survival of patients (N=16). The median survival in days (95% CI) is 45.5 (42-342).

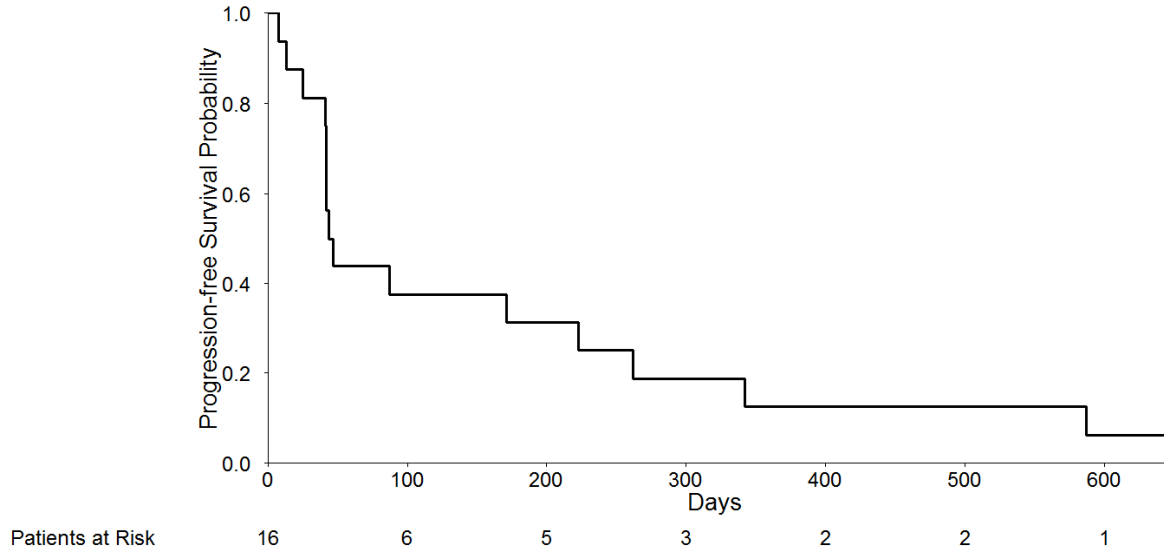
Figure 1. Primary Efficacy Results

A. Overall Survival



Kaplan-Meier curves with 'numbers at risk' at bottom margin showing overall survival of patients (N=21). The median survival is 193 days (95% CI 96-515).

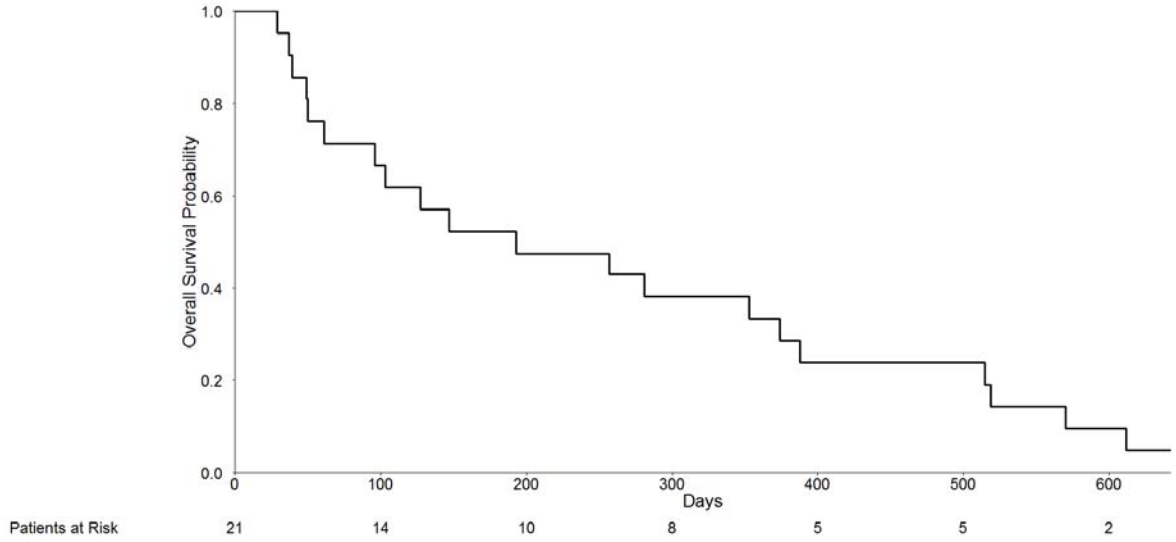
B. Progression-Free Survival



Kaplan-Meier curves with 'numbers at risk' at bottom margin showing progression-free survival of patients (N=16). The median progression-free survival is 45.5 days (95% CI 42-342).

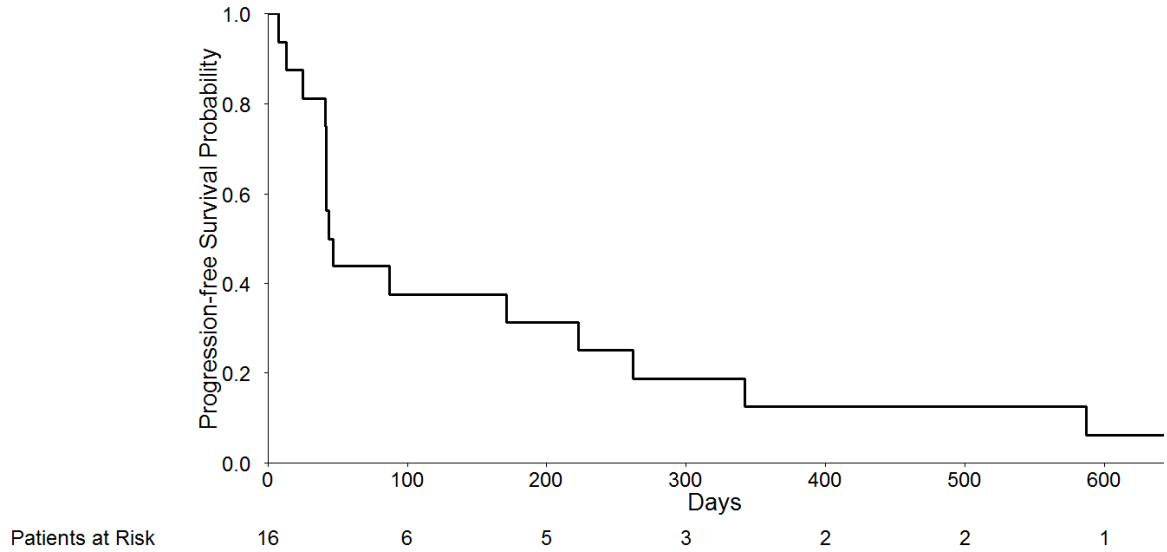
Figure 1. Primary Efficacy Results

A. Overall Survival



Kaplan-Meier curves with 'numbers at risk' at bottom margin showing overall survival of patients (N=21). The median survival is 193 days (95% CI 96-515).

B. Progression-Free Survival



Kaplan-Meier curves with 'numbers at risk' at bottom margin showing progression-free survival of patients (N=16). The median progression-free survival is 45.5 days (95% CI 42-342).

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Translational phase I trial of vorinostat combined with cytarabine and etoposide in patients with relapsed, refractory, or high-risk acute myeloid leukemia

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