Clinical Cancer Research

# Histone Deacetylase Inhibition with Panobinostat Combined with Intensive Induction Chemotherapy in Older Patients with Acute Myeloid Leukemia: Phase I Study Results

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## Abstract

**Purpose:** The histone deacetylase (HDAC) inhibitor panobinostat potentiates anthracycline and cytarabine cytotoxicity in acute myeloid leukemia (AML) cells. We hypothesized that panobinostat prior to and during induction chemotherapy would be tolerable and augment response in patients showing increased histone acetylation.

**Patients and Methods:** Patients received panobinostat 20–60 mg oral daily on days 1, 3, 5, and 8 with daunorubicin 60 mg/m$^2$/day intravenously on days 3 to 5 and cytarabine 100 mg/m$^2$/day intravenously by continuous infusion on days 3 to 9 ("7+3"). Peripheral blood mononuclear cells (PBMCs) were isolated for HDAC expression and histone acetylation changes.

**Results:** Twenty-five patients ages 60–85 years (median age, 69) were treated. Fifteen patients had *de novo* AML, six AML with myelodysplasia-related changes, two AML with prior myeloproliferative neoplasm, one therapy-related myeloid neoplasm, and one myelodysplastic syndrome with excess blasts-2. No dose-limiting toxicities occurred in dose escalation cohorts. In dose expansion, six patients received panobinostat at 60 mg and nine patients at 50 mg due to recurrent grade 1 bradycardia at the 60-mg dose. The complete response (CR)/incomplete count recovery (Cri) rate was 32%. Median overall survival was 10 months: 23 months with CR/CRi versus 7.8 months without CR/CRi (log-rank $P = 0.02$). Median relapse-free survival was 8.2 months. Increased histone acetylation 4 and 24 hours after panobinostat was significantly associated with CR/CRi.

**Conclusions:** Panobinostat with "7+3" for older patients with AML was well tolerated. Panobinostat 50 mg on days 1, 3, 5, and 8 starting 2 days prior to "7+3" is recommended for future studies. Panobinostat-induced increases in histone acetylation in PBMCs predicted CR/CRi.

## Introduction

Acute myeloid leukemia (AML) in patients over 60 years of age carries a dismal prognosis with low complete remission rates, high early mortality, and short survival with both intense and low-intensity induction regimens. In large part, this is due to poor-risk AML with adverse karyotypes, AML from antecedent hematologic disorders, and/or therapy-related disease, poor performance status, and possibly advanced age itself (1–3). For fit older patients with AML, intensive induction with 7 days of infusional cytarabine and 3 days of anthracycline ("7+3") yields superior remission rates to low-intensity therapies, although remission rates are still low at 45%–64% (1–3). Attempts to improve on "7+3" through anthracycline or cytarabine dose escalation have failed to improve outcomes for most older patients with AML (4, 5). Cladribine with "7+3" improves response rates and survival in 50- to 60-year-olds with AML but the safety of this regimen in older adults remains to be determined (6). For older patients with secondary or therapy-related AML, liposomal encapsulation of daunorubicin and cytarabine improves response rates and survival relative to "7+3" but with still inadequate complete response (CR) rates, prolonged cytopenias, and short median event-free and overall survival (7). Agents to enhance the anti-leukemic activity of anthracycline and cytarabine induction without significantly increasing toxicity are needed in this population.

Altered myeloblast DNA methylation and histone methylation and acetylation lead to gene expression patterns that contribute to AML pathogenesis by blocking cell differentiation and promoting cell proliferation, genetic instability, resistance to apoptosis, and resistance to chemotherapy (8). Histone deacetylase (HDAC) inhibition is highly active in laboratory models of AML. HDAC inhibitors have pleotropic effects including induction of myeloblast apoptosis, cell-cycle arrest, downregulation of DNA repair gene expression, and depletion of CXCR4 (9–12). The pan-histone deacetylase inhibitor panobinostat has limited single-agent activity against AML (13, 14), although it was shown to be safe and well-tolerated up to doses of 60 mg three days a

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## Translational Relevance

*In vitro* studies have shown synergy between histone deacetylase inhibitors (HDACis) and anthracyclines or cytarabine in killing of acute myeloid leukemia (AML) cells, although this has not yet translated to improvement in therapy for AML. We hypothesized that a brief course of treatment with the pan-histone deacetylase inhibitor panobinostat prior to and during anthracycline with cytarabine (7+3) induction chemotherapy would potentiate the effects of induction chemotherapy in those patients showing changes in histone acetylation *in vivo*. Consistent with our hypothesis, high levels of normalized histone acetylation in response to panobinostat were significantly correlated with complete remission providing evidence in patients with AML that on-target inhibition of histone deacetylation by HDACis may augment the activity of induction therapy. The novel sequencing of histone deacetylase inhibitor and induction chemotherapy in this study was well tolerated in older patients with AML and warrants further investigation in larger clinical trials.

week (14). Preclinical studies demonstrate synergistic anticancer activity of panobinostat in combination with anthracylines and cytarabine in AML *in vitro* (10, 11). Given single-agent safety in AML and preclinical synergy with chemotherapy, clinical studies have combined panobinostat with induction therapy. A phase 1b/2 PETHEMA study (Panobidara) reported a CR rate of 64% and median overall survival (OS) of 17 months in 38 older (median age, 71 years; range, 65–83) patients with *de novo* AML treated with "7+3" followed by panobinostat given for 3 weeks after completion of induction (15). With this design, panobinostat proved poorly tolerated and the MTD was only 10 mg. Notably, panobinostat was given after induction rather than prior to and during induction to potentiate the effect of chemotherapy.

We hypothesized that a brief course of panobinostat prior to and during "7+3" induction would be well tolerated and would sensitize AML blasts to daunorubicin and cytarabine thereby improving response rates for older patients with AML. We report the results of a phase I study to determine the MTD and recommended phase II dose of panobinostat in combination with standard-dose daunorubicin and cytarabine (7+3) for older patients 60 years of age or older with newly diagnosed AML or advanced myelodysplastic syndrome (MDS). Correlative studies evaluated the association between AML response and peripheral blood mononuclear cell (PBMC) HDAC expression and HDAC acetylation after panobinostat.

## Patients and Methods

### Eligibility

Eligible patients were $\geq$ 60 years old and had untreated AML, advanced MDS (IPSS INT-2 or high risk) or therapy-related myeloid neoplasm, an ECOG performance status $\leq$ 2, and adequate organ function [AST and ALT $\leq$ 2.5 times upper limit of normal (ULN), bilirubin $\leq$ 1.5 $\times$ ULN, and potassium, magnesium, and calcium $\geq$ lower limit of normal]. Prior treatment for myelodysplastic syndromes or myeloproliferative neoplasms was allowed as was leukapheresis and hydroxyurea for control of hyperleukocytosis. Excluded were patients with acute promyelocytic leukemia, known central nervous system involvement, myeloid sarcoma without bone marrow involvement, cumulative prior anthracycline exposure greater than 200 mg/m$^2$ doxorubicin isotoxic equivalents, prior allogeneic hematopoietic cell or solid organ transplant, known bleeding diathesis, significant cardiac disease (history of sustained ventricular tachycardia, ventricular fibrillation, or torsades de pointe, bradycardia, QTcF >450 msec, bifascicular block, myocardial infarction or unstable angina within 6 months, or left ventricular ejection fraction <50% or symptomatic heart failure), impaired gastrointestinal tract function (including uncontrolled vomiting, uncontrolled diarrhea, malabsorption, or bowel obstruction), known HIV, hepatitis B virus, or hepatitis C virus infection, active second malignancy, or concomitant use of drugs known to prolong the QT interval. The study was approved and overseen by the University of California San Francisco Institutional Review Board and required federal agencies. All patients provided informed consent in accordance with good clinical practice prior to screening.

### Study treatment

Dose escalation of panobinostat followed standard "3+3" design (16). Induction therapy consisted of panobinostat orally once a day at 20, 30, 40, or 60 mg based on cohort assignment administered on days 1, 3, 5, and 8 of induction with daunorubicin 60 mg/m$^2$/day intravenously on days 3 through 5 and cytarabine 100 mg/m$^2$/day intravenously by continuous infusion over 24 hours on days 3 through 9. A day 16–18 bone marrow biopsy was performed and patients with $\geq$ 5% myeloblasts and bone marrow cellularity > 20% were eligible for reinduction with panobinostat orally once daily on days 1, 3, and 5 with daunorubicin on days 3 and 4 and cytarabine on days 3 through 7 at the same doses given in induction. Patients in CR/incomplete count recovery (Cri) after induction/reinduction could receive optional consolidation with one cycle of the induction regimen or alternative consolidation including allogeneic hematopoietic cell transplantation at the treating physician's discretion. The MTD was defined as the maximum dose at which zero or one of six patients experienced a dose-limiting toxicity (DLT). DLTs were any grade 3 or higher nonhematologic toxicity as defined by NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v.4.0, NCI, CTC web site https://ctep.cancer.gov/) possibly, probably, or definitely related to the addition of panobinostat to daunorubicin plus cytarabine induction therapy. In addition, failure to achieve absolute neutrophil count (ANC) >500/μL or unsupported platelet count >20,000/μL by day 42 of induction/reinduction or ANC $\geq$1,000/μL or unsupported platelet count $\geq$100,000/μL by day 58 of induction/reinduction unless due to residual disease was considered a DLT. The DLT monitoring period was until count recovery (ANC $\geq$1,000/μL and platelets $\geq$100,000/μL) or day 42 without recovery to ANC $\geq$500/μL or unsupported platelet count $\geq$20,000/μL without residual/persistent disease or until day 58 without count recovery without residual/persistent disease, documentation of lack of significant cytoreduction on day 16–18 bone marrow biopsy, time of initiation of nonprotocol therapy, or documentation of persistent or recurrent disease after completion of all induction therapies. Once the MTD or maximum planned dose (60 mg) was reached, dose expansion was planned for a total of 10 patients at the MTD. Anthracycline exposure was limited to lifetime exposure $\leq$450 mg/m$^2$ doxorubucin isotoxic equivalents. All patients received hyperuricemia prophylaxis with allopurinol for at least

days 1–9 of therapy. Neutropenia prophylaxis at ANC <500/μL consisted of levofloxacin or ciprofloxacin and caspofungin or voriconazole. Patients with hypoplasia on a day 16–18 bone marrow biopsy received G-CSF until recovery of ANC.

### Treatment assessment

Baseline studies included medical history, physical exam, vital signs, ECOG performance status, medication review, complete blood count with differential, comprehensive metabolic panel, LDH, uric acid, PT, PTT, fibrinogen, TSH, free T4, urinalysis, hepatitis B and C serologies, bone marrow biopsy with karyotype and molecular studies as indicated, echocardiogram, and ECG. Patients were treated in the in-patient setting for administration and convalescence after induction and reinduction therapy. ECG was performed prior to each panobinostat dose to monitor for QTcF prolongation. Peripheral blood samples for correlative studies were drawn prior to and then at 4, 24, and 48 hours after the first and second doses of panobinostat. Bone marrow biopsies with complete blood counts were performed on day 16–18 and at count recovery defined as ANC >1,000/μL and platelets >100,000/μL or day 37, whichever came first, then as needed to confirm AML response, persistence, or relapse. Lumbar puncture was performed at confirmation of first complete remission for patients with FAB M4 or M5 morphology or presenting white blood cell count >100,000/μL.

### Histone expression and acetylation

Acetylated histone H4, HDAC1, HDAC2, and GAPDH antibodies were purchased from Millipore. Pan-histone H3 and HDAC3 antibodies were purchased from Cell Signaling Technology. HDAC6 antibody was purchased from Santa Cruz Biotechnology. Whole blood was collected on day 1 prior to and 4, 24, and 48 hours after panobinostat treatment. On day 3, whole blood was collected 4, 24, and 48 hours after panobinostat treatment. PBMCs were isolated from whole blood using Vacutainer CPT™ Cell Preparation Tubes (BD Diagnostics). Western blots were conducted as described previously (17). For each sample, acetyl-H4 signal was normalized to pan-histone H3 to control for loading variability. For each patient, sample acetyl-H4 signal is presented relative to that for pretreatment day 1. To account for interpatient variability, each patient's baseline HDAC expression was normalized first to GAPDH and then to the respective HDAC in MCF7 cells. HDAC, GAPDH, pan-histone H3, and acetyl-histone H4 protein were quantified using NIH image J software.

### Statistical analyses

The primary endpoint was the safety and tolerability of panobinostat with standard-dose cytarabine and daunorubicin (7+3). Secondary endpoints were the rate of CR, the rate of complete response with CRi, overall response rate (ORR, CR + CRi), relapse-free survival, overall survival, the effect of panobinostat on histone acetylation in PBMCs, and identification of biomarkers of response to the regimen. Descriptive statistics were used to summarize patient characteristics and adverse events. Genetic risk was assigned using NCCN AML Guidelines Version 1.2017 disease risk categories. Clinical responses were determined using the International Working Group criteria for CR, treatment failure, and relapse for AML (18) and MDS (19). For AML, CRi was defined as per CR but without recovery of ANC to >1,000/μL or platelets to >100,000/μL. Early death was defined as death within 30 days of enrollment. OS was defined as the time from start of therapy to death from any cause. Relapse-free survival (RFS) was defined as the time from CR or CRi until relapse or death from any cause. Survival was estimated using the method of Kaplan and Meier and survival comparisons made by log-rank testing (20). Correlation of histone expression and histone acetylation with response and survival was performed using Pearson's correlation coefficient.

**Table 1.** Baseline patient characteristics

| Characteristic ($n = 25$) | No. (%) |
|---|---|
| Age, y | |
| 60–69 | 14 (56) |
| 70–79 | 6 (24) |
| 80+ | 5 (20) |
| Median | 69 |
| Range | 60–85 |
| ECOG performance status | |
| 0 | 6 (24) |
| 1 | 17 (68) |
| 2 | 2 (8) |
| WBC at diagnosis $\times 10^9$/L | |
| Median | 8.1 |
| Range | 1.1–55 |
| Platelet count at diagnosis $\times 10^9$/L | |
| Median | 56 |
| Range | 13–360 |
| Diagnosis | |
| *De novo* AML | 15 (60) |
| Secondary AM[a] | 9 (36) |
| MDS, EB-2 | 1 (4) |
| Karyotype | |
| Favorable | 1 (4) |
| Intermediate | 11 (44) |
| Poor | 12 (48) |
| Complex | 7 (28) |
| Monosomy 7 | 3 (12) |
| NK, FLT3-ITD+ | 2 (8) |
| Not determined | 1 (4) |
| Prior hypomethylating agent | 2 (8) |

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EB, excess blasts; NK, normal karyotype; NPM1, nucleophosmin 1; FLT3, fms-like tyrosine kinase 3; ITD, internal tandem duplication.

[a]Includes AML with myelodysplasia-related changes ($n = 6$), therapy-related myeloid neoplasm (tAML, $n = 1$), and prior myeloproliferative neoplasm ($n = 2$).

## Results

### Patient characteristics

Twenty-five patients were treated from January 2012 to September 2015 (Table 1). The median age was 69 years (range, 60–85 years) with 11 patients 70 years of age or older. Fifteen patients had *de novo* AML, nine patients had secondary AML, and one had MDS, EB-2. Thirteen patients (62%) had intermediate-risk cytogenetics. Among 12 poor-risk patients, 10 patients had complex/monosomal karyotypes and two had normal karyotype with FLT3-ITD mutation. Two patients had progressed on prior hypomethylating agents for MDS. All 25 patients completed induction treatment. Two patients received protocol reinduction for persistent disease on day 14 marrow and one patient received a single cycle of protocol-defined consolidation.

### Tolerability and determination of MTD

Panobinostat dose escalation proceeded with three patients per dose group without any DLTs observed up to the maximum

**Table 2.** Grade 3/4 adverse events in more than one subject

| Event ($n = 25$) | Number (%) |
|---|---|
| Febrile neutropenia | 18 (72) |
| Hyponatremia | 8 (32) |
| Rash | 4 (16) |
| Hypophosphatemia | 4 (16) |
| Diarrhea | 4 (16) |
| Sepsis | 3 (12) |
| Atrial fibrillation | 3 (12) |
| Elevated AST | 2 (8) |
| Hypertension | 2 (8) |

planned dose of 60 mg. A total of 32% of patients (8/25) experienced asymptomatic grade 1 sinus bradycardia peaking on day 6 following panobinostat in setting of additional exposure to daunorubicin, and 5-HT3 inhibitor antiemetics. Four of six patients (67%) at the 60-mg dose developed asymptomatic (grade 1) bradycardia prompting exploration of an intermediate 50-mg dose level, even though this did not meet criteria for a DLT. At 50 mg, four of nine patients (44%) developed asymptomatic bradycardia. On the basis of the low-grade nature of the bradycardia, 50 mg was determined to be the MTD and recommended phase II dose. In addition, 16% of patients (4/25) experienced new-onset grade 2–3 atrial fibrillation. There was no evidence of myocardial dysfunction on echocardiogram in any patient following induction. Outside of bradycardia, treatment-emergent adverse events were as expected for intensive induction: occurring in greater than 10% of patients each, grade 3–4 febrile neutropenia, hyponatremia, rash, diarrhea, sepsis, hypophosphatemia, or atrial fibrillation (Table 2). There were two deaths (8%) during induction, one due to typhlitis and the other due to sepsis.

### Response and survival

Among all patients in this high-risk AML population, the cumulative CR/CRi rate to therapy was 32% ($n = 8/25$; Table 3). Two patients were not evaluable for response due to death in hypoplasia. Fifteen patients (60%) had resistant disease. By genetic risk stratification, CR/CRi was achieved in 100% (1/1) of favorable-risk, 33% (4/11) of intermediate-risk, and 25% (3/12) patients with poor-risk AML. Responses were seen across dose cohorts. The median and 1-year overall survival for the entire cohort was 10 months and 44%, respectively (Fig. 1A). For patients achieving a CR/CRi, the median and 1-year relapse-free survivals were 8.2 months and 37.5%, respectively (Fig. 1B). The median overall survival was 23 months with a CR/CRi versus 7.8 months without a CR/CRi ($P = 0.02$) with a 1-year OS of 75% for responders (Fig. 1C).

**Table 3.** Patient outcomes

| Response ($n = 25$) | Number (%) | |
|---|---|---|
| ORR (CR + CRi) | 8 (32) | |
| CR | 7 (28) | |
| Cri | 1 (4) | |
| Early death | 2 (8) | |
| Resistant disease | 15 (60) | |
| Median survival | Months | *P* (Log-rank) |
| OS | 10 | |
| OS, CR/CRi | 23 | 0.02 |
| OS, no CR/Cri | 7.8 | |
| Median RFS | 8.2 | |

### Histone expression and acetylation

Material was available for comprehensive histone expression and acetylation studies on 15 of the 25 study patients (median WBC 96,000/μL, range 13–277,000/μL). The level of expression of HDAC 1, 2, 3, and 6 in PBMCs showed no correlation with CR/CRi or overall survival. As a measure of the pharmacodynamic effect of panobinostat on HDAC inhibition, we assessed changes in global histone acetylation in PBMCs after dosing. The level of increase in normalized global histone acetylation increase at 4 and 24 hours after the first dose of panobinostat was significantly higher in complete responders versus refractory patients (normalized acetylation postpanobinostat, 4 hours 60.2 vs. 13.4, $P = 0.034$; 48 hours 26.3 vs. 8.2, $P = 0.019$; Fig. 2). No difference was observed in the percentage of patients showing increased acetylation in PBMCs after panobinostat between the five dose levels.

## Discussion

In this proof-of-concept phase I study, panobinostat was found to be safe and tolerable with no significant additional serious adverse events or DLTs when combined with standard induction with "7+3" chemotherapy. Asymptomatic grade 1 sinus bradycardia was an unexpected side effect that led to exploration of an intermediate dose of 50 mg that was found to be the MTD and recommended phase II dose of panobinostat in this regimen. With this change, reversible asymptomatic bradycardia was reduced but still noted and should be followed closely in future trials of HDAC inhibitors with induction chemotherapy. The mechanism of bradycardia is unclear, although it occurred in the setting of coadministration with daunorubicin, cytarabine, dexamethasone, and prophylactic 5-$HT_3$ antagonist antiemetics suggesting a combinatorial effect. Notably, bradycardia was not a safety finding in the approval of panobinostat for multiple myeloma suggesting that interaction with other medications may underlie the bradycardia (21).

A previous study found panobinostat to be intolerable when given after completion of "7+3." Ocio and colleagues reported results of a PETHEMA phase Ib/II study of panobinostat given 3 days a week for 2 weeks after completion of 7 + 3 in 38 older patients with *de novo* AML. Because of excessive DLTs at the starting dose of 20 mg (three of six related deaths and two of six significantly delayed count recovery), the dose of panobinostat was reduced 10 mg. Complete response rate was as expected for the *de novo* AML population at 64% with two of five patients with minimal residual disease at CR clearing MRD with ongoing panobinostat treatment (15). Given the limited activity of HDAC inhibition alone in AML (13, 14) and the known synergism of concurrent panobinostat and chemotherapy *in vitro* (10, 11), regimens that separate HDAC inhibition from the administration of chemotherapy are unlikely to show a significant efficacy improvement as the added benefit of HDAC inhibition in isolation is minimal. In addition, prolonged panobinostat exposure, except at very low doses, appears to be intolerable in the setting of intensive AML induction.

The small size of our study precludes conclusions about the efficacy of the combination. The complete response rate of 32% seen in this study is comparable with that seen in other studies of 7+3 in poor-risk AML age 60 years and older (1, 4, 7). Notably, our population tended to be older, have secondary AML, and have poor cytogenetic risk, factors known to reduce response rates and survival duration (1, 2). A recent randomized phase III study of

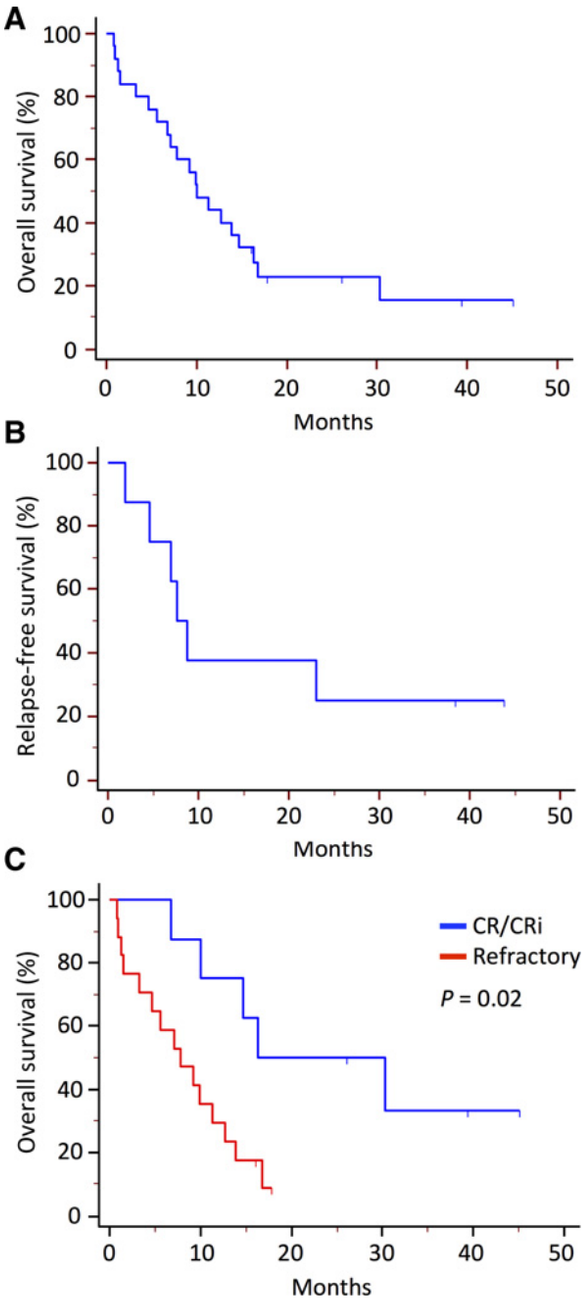


**Figure 1.**
Overall and relapse-free survival. **A,** Overall survival for all treated patients ($N = 25$). **B,** Relapse-free survival for patients achieving a CR/CRi to induction ($N = 8$). **C,** Overall survival by response to induction (CR/CRi $N = 8$, No CR/CRi $N = 17$).

CPX-351, a liposomal encapsulation of cytarabine and daunorubicin in a synergistic 5:1 molar ratio, versus standard 7+3 showed a significant complete response (47.7% vs. 33.3%, $P = 0.016$) and median overall survival (9.56 vs. 5.95 months, $P = 0.003$) advantage for CPX-351 in older patients with AML with myelodysplasia-related changes and therapy-related AML. Much of the survival benefit appears to be coming from superior survival in patients undergoing allogeneic hematopoietic cell transplantation after CPX-351 relative to 7+3 (7). In that population, combining CPX-351 with pan-HDAC inhibitors might further improve outcomes.

One caveat to the promise of HDAC inhibition with intensive chemotherapy in AML is the result of vorinostat in combination with idarubicin and intermediate dose cytarabine for younger patients with AML. Although a phase II study of the combination showed a promising response rate of 85%, a randomized phase III study led by SWOG in patients with AML ages 15–60 years showed no difference in response or survival with the addition of vorinostat to idarubicin and intermediate dose cytarabine (22, 23). Notably, in the phase II study vorinostat was administered prior to induction chemotherapy, whereas in the phase III study vorinostat was administered concurrent with chemotherapy leaving the question open as to the benefit of HDAC inhibition as priming to enhance DNA damage and myeloblast death when given prior to the administration of genotoxic chemotherapy. Inducing histone acetylation with opening of the chromatin and resultant changes in gene regulation may be important for synergy between HDAC inhibitors and induction chemotherapy and preclinical studies suggest a strong correlation of efficacy with a preexposure schedule of administration for the HDAC inhibitor in some tumor types (24). In addition, a pan-HDAC inhibitor such as panobinostat, in contrast to a selective HDAC inhibitor such as vorinostat, may have enhanced efficacy across broader subsets of AML.

Although the magnitude of benefit, if any, of the addition of panobinostat to standard induction chemotherapy is difficult to ascertain at this time in this small phase I study, the correlative studies support the hypothesis that HDAC inhibition, and resultant changes in histone acetylation, chromatin structure, and presumably gene expression, may potentiate the effects of daunorubicin and cytarabine induction chemotherapy leading to higher response rates in patients with induction of histone acetylation after panobinostat. *In vitro*, panobinostat potentiates the antileukemic and DNA-damaging effects of anthracyclines and cytarabine on myeloblasts due to increased DNA damage induced by the anthracycline in the setting of HDAC inhibition (10, 11). Consistent with these findings, higher levels of acetylation in response to panobinostat were significantly associated with complete response to induction in our study and suggest predictability of response and the possibility to enrich for patients with an ability to induce histone acetylation when adding panobinostat or other HDAC inhibitors to induction chemotherapy. Whether this is due to synergy of the panobinostat+7+3 combination or is a biomarker for leukemic response to chemotherapy is unclear at this time, although the *in vivo* findings here are most consistent with preclinical studies showing that HDAC inhibition increases myeloblasts DNA damage and death in response to anthracyclines and cytarabine.

Given the tolerability of panobinostat in this regimen relative to previous studies and panobinostat's possible potentiation of the antileukemic effects of daunorubicin and cytarabine *in vivo*, further research is warranted on the combination. Rapid biomarker-driven patient selection based on early histone acetylation response may help select patients most likely to derive a response benefit from panobinostat in combination with intensive induction chemotherapies without exposing patients unlikely to respond to additional panobinostat with chemotherapy. In addition, further research into mechanisms of resistance of AML cells

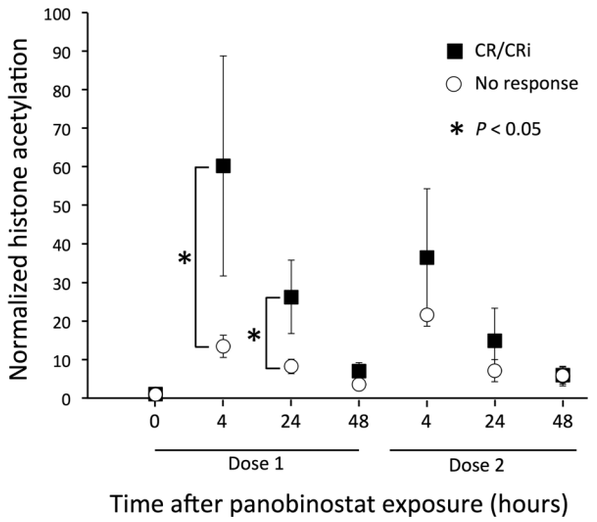


**Figure 2.**

Histone acetylation in PBMCs after panobinostat exposure and response to induction. Change in PBMC histone acetylation after first and second panobinostat doses normalized to baseline PBMC histone acetylation at time 0 prior to panobinostat exposure. No response, $N = 10$; CR/CRi, $N = 5$.

to the effects of histone deacetylase inhibitors may help better select patients for the therapy. Randomized study is ultimately needed to evaluate efficacy and if the association of increased acetylation and disease response represents chemo-sensitization by panobinostat or is a general marker of myeloblast chemosensitivity.

We have shown that panobinostat at biologically relevant doses is well tolerated when administered as a brief course prior to and during "7+3" induction for older patients with newly diagnosed AML. The small size of this phase I study, heterogeneous population, and high-risk older population precludes drawing conclusions about efficacy. We conclude that the addition of panobinostat to 7+3 is safe, well-tolerated, and may augment the antileukemic effect of 7+3 induction, especially in those patients with increased global histone acetylation in PBMCs. Panobinostat warrants further investigation in combination with standard induction with 7+3 or CPX-351.

## Disclosure of Potential Conflicts of Interest

N. Pawlowska is an employee of Alessa Therapeutics, Inc. R. Olin is a consultant/advisory board member for Jazz Pharmaceuticals and Genentech, and has immediate family members who are consultant/advisory board members for Jazz Pharmaceuticals and Revolution Medicine. C. Andreadis has immediate family members who are employed by and hold ownership interest in Genentech; and reports receiving commercial research grants from and is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other authors.

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