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## Histone Deacetylase Inhibitor Romidepsin Has Differential Activity in Core Binding Factor Acute Myeloid Leukemia

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### Abstract

**Purpose**—Recruitment of histone deacetylases (HDAC) is a mechanism of transcriptional repression implicated in the differentiation block in acute myeloid leukemia (AML). We hypothesized that the HDAC inhibitor romidepsin could cause transcriptional derepression, up-regulation of specific target genes in AML, and differentiation of the leukemic clone. The primary objectives of the study were to evaluate the safety and efficacy of romidepsin in advanced AML.

**Experimental Design**—Twenty patients were stratified into cohort A or B based on the absence or presence of chromosomal abnormalities known to recruit HDACs, including those involving core binding factor (CBF). Romidepsin was administered i.v. at 13 mg/m<sup>2</sup>/d on days 1, 8, and 15 of a 28-day cycle. Pharmacodynamic endpoints were evaluated at serial time points.

**Results**—Common adverse effects noted were grade 1 to 2 nausea, anorexia, and fatigue. No objective evidence of antileukemic activity was seen in cohort A. In cohort B, although there were no clinical responses by standard criteria, antileukemic activity was observed in 5 of 7 patients. Two patients had clearance of bone marrow blasts and 3 patients had a >50% decrease in bone marrow blasts. Furthermore, in cohort B, at 24 h, there was a significant increase in *MDR1* ( $P = 0.005$ ), *p15* ( $P = 0.01$ ), and *p14* ( $P < 0.0001$ ) expression. In cohort A, although there was a trend toward up-regulation of *MDR1*, *p15*, and *p14* expression, these changes were not statistically significant.

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#### Disclosure of Potential Conflicts of Interest

O. Odenike has received research support from MGI, Curagen, and TopoTarget, is on the advisory board for MGI, and has an ownership interest in Gloucester Pharmaceuticals. O. Odenike is an inventor on a patent that relates to the use of HDACIs for treating AML. The patent is owned by the University of Chicago.

**Conclusion**—Romidepsin has differential antileukemic and molecular activity in CBF AML. Development of this agent in CBF AML should focus on combinations that target related mechanisms of gene silencing such as DNA methylation.

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Recruitment of histone deacetylases (HDAC) is an important epigenetic mechanism of transcriptional dysregulation and gene silencing in acute myeloid leukemia (AML). Acetylation of the lysine tails of histones, a process mediated by histone acetyl transferases, results in loosening of DNA-histone interactions and facilitates binding of specific transcriptional coregulators, resulting in transcriptionally active chromatin. HDACs cleave acetyl groups from histones and block this DNA conformational change, resulting in transcriptional repression of specific genes, including those important for cellular differentiation, cell cycle regulation, and apoptosis (1).

AML is a biologically heterogeneous disease; both epigenetic and genetic alterations have been implicated in disease pathogenesis. Approximately 30% to 40% of AML is associated with recurring chromosomal translocations that result in the generation of chimeric fusion genes. We and others have shown that histone acetyl transferases such as CBP and p300 (2–4) are disrupted in chromosomal translocations in leukemia. In addition, transcriptional repression by several chimeric fusion proteins in AML occurs via recruitment of HDACs either directly or in cooperation with corepressors (5–8). These findings provide strong evidence for transcriptional dysregulation involving the histone acetylation/deacetylation pathway in leukemogenesis. For example, the fusion proteins AML1-ETO and core binding factor  $\beta$  (CBF $\beta$ )-MYH11 resulting from the t(8;21)(q22;q22) and (inv 16)(p13;q22) or t(16;16)(p13;q22), respectively, have been shown to act as transcriptional repressors. Either directly or through its hetero-dimeric partner CBF $\beta$ , AML1 is the target of all three chromosomal rearrangements and acts as a transcriptional activator, whereas the fusion proteins repress transcription of AML1 target genes such as *pl4ARF*, *MDR1*, *interleukin-3*, and *CSF1-R* (5, 9, 10).

Unlike gene deletions that are irreversible, transcriptional repression by epigenetic mechanisms can be reversed for therapeutic benefit by chromatin remodeling agents such as HDAC inhibitors (HDACI). Inhibitors of HDACs are now in clinical trials and have been shown to exert antitumor effects *in vitro* and *in vivo*. Romidepsin (depsipeptide) is a potent HDACI currently in development that inhibits class I HDACs. In addition, the agent has been shown to induce G<sub>1</sub> and G<sub>2</sub>-M cell cycle arrest, differentiation, and morphologic reversion of transformed cells in parallel with accumulation of highly acetylated histones within cells (11–15). Significant antileukemic effects, including cellular differentiation coupled with reexpression of *AML1* target genes such as interleukin-3, have been noted with HDACIs including romidepsin in cell lines and primary leukemia samples harboring the t(8;21) (7, 16–19). This raises the possibility that this cytogenetic subset of AML could be particularly amenable to therapy with HDACIs. The finding that myeloid leukemia cell lines that lack this specific chromosomal translocation are also susceptible *in vitro* to treatment with HDACIs, however, suggests that treatment with HDACIs could be more widely applicable in AML (17, 20).

We report here our results from a phase II trial of romidepsin in AML, including a cohort of patients with CBF AML. The primary objectives of the trial were to determine the efficacy and safety of romidepsin in AML. Secondary objectives included an evaluation of the effects of romidepsin *in vivo* on various biologic markers including global and promoter-specific histone acetylation and expression of specific target genes.

## Patients, Materials and Methods

### Patients and eligibility criteria

Patients were eligible for this study if they had relapsed or refractory AML. Patients with relapsed AML who were ages <60 years had to be in second relapse or greater, whereas patients who were ≥ 60 years could be in first relapse. Patients with previously untreated AML, ages ≥ 60 years and who were not candidates for or who refused conventional chemotherapy, were also eligible for this trial. Patients enrolled on this trial could not be candidates for allogeneic stem cell transplantation at the time of enrollment. Patients had to be aged at least 18 years, have a CALGB performance status of ≤ 2, and give informed consent. There was no limitation to the number or types of prior therapies received, including autologous or allogeneic stem cell transplantation. No cytotoxic therapy or radiation therapy was permitted within 2 weeks (6 weeks for mitomycin C and nitrosoureas and 4 weeks if prior therapy was with an investigational agent). The exception was hydroxyurea, which was permitted during the first cycle of therapy to control hyperleukocytosis (defined as WBC count over 20,000/ $\mu$ L). Patients could not have received prior therapy with romidepsin and could not be receiving concurrent therapy with drugs known to have HDACI activity such as sodium valproate (21).

Because of the potential for QTc prolongation, a class effect of HDACIs, patients had to have a left ventricular ejection fraction of ≥ 40% and QTc interval of <500 ms on electrocardiogram to be eligible. In addition, patients could not have had a myocardial infarct within the preceding 3 months or have symptomatic heart disease or left ventricular hypertrophy on electrocardiogram.

Patients were ineligible if the serum creatinine or serum bilirubin were greater than twice the upper limit of normal or the serum transaminases were greater than three times the upper limit of normal. Patients were also excluded if they had an uncontrolled intercurrent illness such as an active infection, symptomatic heart failure, unstable angina, cardiac arrhythmia, pregnancy, HIV infection, or any other medical condition that would make the administration of romidepsin hazardous. The protocol was reviewed and approved at each institution's institutional review board, and all subjects enrolled gave written informed consent.

### Treatment and response evaluation

This was an open-label, multicenter phase II study conducted through the University of Chicago Phase II Consortium. Patients were stratified into two cohorts based on the absence (cohort A) or presence (cohort B) of chromosomal aberrations such as the t(8;21), inv(16), and t(15;17) that encode proteins known to recruit HDACs. Treatment was identical for both cohorts. Romidepsin was administered i.v. over 4 h through a central or peripheral venous

catheter at a dose of 13 mg/m<sup>2</sup>/d on days 1, 8, and 15 with cycles repeated every 28 days. Prophylactic antiemetics (ondansetron and prochlorperazine or the equivalent) were administered before and 24 to 72 h after therapy. During the course of the study, the protocol was amended to require that potassium and magnesium levels be maintained at a minimum level of 4 mmol/L and 2 mg/dL, respectively, to minimize the likelihood of QTc prolongation and/or cardiac arrhythmias arising from romidepsin therapy. Cardiac monitoring was employed with serial electrocardiograms and multigated acquisition scans before and after therapy. Treatment could continue indefinitely in the absence of disease progression or unacceptable adverse events. Toxicity was graded using National Cancer Institute Common Toxicity Criteria version 3.0. Patients experiencing a drug-related grade 3 or 4 nonhematologic toxicity were eligible for retreatment at a reduced dose of 10 mg/m<sup>2</sup>/d provided the toxicity had resolved to a grade 2. Response was evaluated at day 21 of each cycle of therapy using previously described criteria for AML (22).

### Pharmacodynamic studies

Peripheral blood mononuclear cells were collected immediately before therapy (hour 0) and at 4 and 24 h following therapy with romidepsin on days 1 and 8 of the initial cycle of treatment for the pharmacodynamic assays described below.

**Gene expression analysis—*pl4ARF*, *MDR1*, and *pl5INK4B***—Total RNA was extracted from the purified cells using RNA STAT-60 (Tel-Test “B”). Total RNA (1-5 µg) was converted into cDNA according to the Transcriptor Reverse Transcriptase (Roche Diagnostics) manufacturer's instructions.

Real-time reverse transcription-PCR was done with individual primer and probe sets designed for detection of *pl4ARF*, *MDR1*, and *pl5INK4B* (*pl5*) transcripts. Patient specimens, serial 5-fold dilutions of cell line cDNA, and controls lacking template were amplified within a LightCycler instrument (Roche Diagnostics). For each assay, a standard curve was generated using cDNA derived from the appropriate cell line. Each reaction was done in a 20 µL reaction mix including cDNA, Amplitaq Gold DNA polymerase (PE Biosystems), primers, and TaqMan probe. Similarly, the *ABL* transcript was amplified to quantitate the target gene (*pl4ARF*, *MDR1*, and *pl5*) transcript copy number relative to an endogenous control. The *MDR1*, *pl5*, and *ABL* transcripts were amplified using previously published primer and probe sequences (23–25). The *pl4ARF* transcript was detected using the following primer and probe sequences: [*pl4ARF* 5′ TCGTGCTGATGC-TACTG, [*pl4ARF* 3′ CAGCGTGTCAGGAAG, and [*pl4ARF* probe) FAM-TTCTAGAAGACCAGGTCATGATGATGG-TAMRA.

All patient and negative control samples were evaluated in triplicate, and absolute transcript quantities were obtained by comparing C<sub>T</sub> (threshold cycle) values to respective standard curves following PCR amplification of *pl4ARF*, *MDR1*, and *pl5* transcripts. To adjust for differences in RNA integrity and cDNA synthesis efficiency, the absolute *pl4ARF*, *MDR1*, and *pl5* transcript copy number was normalized to the absolute transcript copy number of *ABL*. The normalized transcript copy number was expressed as the ratio of the absolute *pl4ARF*, *MDR1*, and *pl5* transcript copy number over the *ABL* transcript copy number.

**Chromatin immunoprecipitation/promoter-specific histone acetylation—**

Chromatin immunoprecipitation analysis was done using the Chromatin Immunoprecipitation Assay Kit (Upstate Biotechnology). Formaldehyde was added to cells to crosslink associated proteins to DNA and after sonicating extracted chromatin to an average length of 500 bp, protein-DNA complexes were immunoprecipitated with commercial (Upstate Biotechnology) antibodies to acetylated histone H3 and H4. After immunoprecipitation, DNA was extracted, purified, and analyzed by quantitative PCR using specific primer pairs for regions *p15*, *MDR1*, and *ABL* (control) promoters.

**Global histone acetylation—**Global histone H3 acetylation was analyzed by flow cytometry for detection of acetylated histone H3 as described previously (26). Briefly,  $0.5 \times 10^6$  cells were processed with permeabilization reagents according to the manufacturer's instructions (Caltag). The cells were then incubated with anti-acetylated H3 antibodies (Upstate Biotechnology), washed, and subsequently incubated with FITC-conjugated secondary antibody (Biosource International). Samples were analyzed (30,000 events) by flow cytometry (Coulter system II software). The assay was validated using Kasumi-1 cells, an AML1-ETO-expressing cell line.

**Statistical methods**

For patients in cohort A, patients without t(8:21), inv(16) or other chromosomal abnormality involving *AML1*, a Simon's optimal two-stage design was employed. Enrollment to this cohort would be stopped for lack of efficacy if no responses were observed in the first 12 evaluable patients. Otherwise, another 25 evaluable patients would be enrolled for a total of 37 patients, and if 4 responses were observed, the treatment would be considered sufficiently active to warrant further study. The sample size was calculated to test a null hypothesis of 5% response rate against an alternative hypothesis of 20% response rate, with 90% power at one-sided significance level of 0.10.

A second, smaller cohort of patients (cohort B, maximum number = 10) with t(8:21), inv16, or t(15;17) or other translocations encoding proteins known to recruit HDACs would also be recruited and their responses were analyzed separately.

A random-effects linear model was used to examine whether there was a significant change in gene expression over the time points analyzed within the first 24 h of treatment (hour effect: hour 0 versus hours 4 and 24) and whether there was a significant change in the kinetics of gene expression between days 1 and 8 of therapy (day effect: day 8 versus day 1). Logarithms of gene expression values were analyzed, because these made the values more normally distributed.

**Results****Patient characteristics**

Twenty patients were enrolled between June 2002 and October 2006, including 13 patients on cohort A and 7 patients on cohort B. Pretreatment patient characteristics for the study as a whole are summarized in Table 1. Baseline characteristics for patients enrolled on cohort B are summarized in Table 2. Despite the fact that these cytogenetic subgroups of AML have

been shown traditionally to have a good prognosis, all patients enrolled in cohort B had multiply relapsed and/or refractory disease. In addition, almost all patients within this cohort had additional chromosomal abnormalities or other features predictive of poor risk disease, in addition to translocations involving *AML1* or *CBFb*. One patient (patient 7) with a novel  $t(4;21)(q31.3;q22)$  was stratified into cohort B, because interphase cytogenetics (fluorescence in situ hybridization analysis) with a *TEL/AML1* probe showed involvement of *AML1* on chromosome band 21q22.

### Treatment outcome

**Antileukemic activity**—Of the 13 patients enrolled in cohort A 6 received 1 cycle of therapy, 1 patient received 1.66 cycles (two of the three planned doses during cycle 2), and 2 patients received 2 cycles. Four patients received <1 cycle of therapy (two of the three planned doses of therapy). All 13 patients were evaluable for toxicity, and 12 patients were evaluable for response. One patient with therapy-related leukemia and a background history of breast cancer failed to complete 1 cycle of therapy due to recurrence of a malignant pleural effusion from her breast cancer and was considered inevaluable for response. Treatment was discontinued in three other patients before completion of cycle 1 because of disease progression. There was no objective evidence of antileukemic activity in cohort A.

Of the 7 patients enrolled in group B, 1 patient received 3 cycles of therapy, 3 patients received 2 cycles of therapy, and 4 patients received 1 cycle of therapy. There were no objective responses by classic response criteria in part due to the failure of normal hematopoiesis. Antileukemic activity was observed with disappearance of bone marrow blasts after 1 and 2 cycles of therapy, respectively, in 2 patients, in the setting of a relatively cellular marrow (marrow cellularity of 20%). An additional 3 patients had a >50% decrease in bone marrow blasts (Table 2). None of these 5 patients had received hydroxyurea while being treated on this study. In addition, none of these 5 patients had any evidence of an intervening period of marrow aplasia, and in 3 of these patients, an increasing percentage of promyelocytes, myelocytes, and monocytes was observed in the marrow aspirates concurrent with the reduction in blast count. These findings suggest that the antileukemic effects of romidepsin in these patients were mediated through a differentiating, rather than a nonspecific cytotoxic, effect. The antileukemic response was short-lived, however, with all 5 patients developing disease progression within 30 days of achieving their best response to treatment with romidepsin.

**Toxicity**—The treatment was well tolerated. The most common treatment-related toxicities were mild (grade 1-2) nausea, anorexia, and fatigue occurring in 35%, 25%, and 20% of patients, respectively. Twenty-five percent of patients had grade 1 cardiac changes consisting mainly of asymptomatic nonspecific ST and T wave abnormalities (Table 3). Grade 1 and 2 liver function abnormalities occurred in 10% of patients.

### Pharmacodynamic studies

**Target gene expression**—Kasumi-1 cells (an *AML1-ETO*-expressing cell line) were used for assay validation. This cell line was treated with romidepsin for 4 h at concentrations ranging from 1 to 100 nmol/L. A dose-dependent up-regulation in the expression of the

*MDR1* gene, a known target of *AML1-ETO* mediated transcriptional repression, was observed. Similarly, up-regulation of *pl4ARF*, which is also a target of *AML1-ETO*-mediated repression, was shown. To determine whether the use of romidepsin in AML *in vivo* results in up-regulation of specific target genes at the doses evaluated in this study, quantitative real-time reverse transcription-PCR analysis for *MDR1*, *pl4ARF*, and *pl5* in patient-derived samples was done in 11 patients in cohort A and 6 patients in cohort B. These revealed a trend toward up-regulation of *MDR1*, *pl5*, and *pl4ARF* expression by hour 24 in cohort A (42%, 12%, and 36% mean increase in expression, respectively); however, these changes were not statistically significant (Fig. 1A-C). In contrast, in cohort B, at 24 h, there was a 75% mean increase in *MDR1* expression ( $P = 0.005$ ), a 162% mean increase in *pl5* ( $P = 0.01$ ), and a 106% mean increase in *pl4ARF* ( $P < 0.0001$ ; Fig. 1A-C). The kinetics of the changes in gene expression were similar on both days 1 and 8 of therapy in both cohorts of patients.

**Global histone protein acetylation**—This assay was optimized using Kasumi-1 cells treated with romidepsin at a dose of 10 nmol/L for 48 h. An increase in histone H3 acetylation was observed following romidepsin treatment (Fig. 2A). Similarly, an increase in histone H3 acetylation was seen in 6 of 7 (86%) patients in cohort B, including all 5 patients with evidence of antileukemic activity (Fig. 2B and C). Four of 13 (31%) patients in cohort A also showed an increase in H3 acetylation. These results confirm that romidepsin induces genome-wide histone acetylation in AML, as reported previously, and suggest that this effect may be more pronounced in patients with translocations involving *AML1*.

**Promoter-specific histone acetylation**—In 4 patients with sufficient material preserved, chromatin immunoprecipitation analysis was carried out for promoter-specific changes in histone H3 and H4 acetylation. An induction in H3 acetylation was observed at both *pl5* and *MDR1* promoters (Fig. 3A and B), in keeping with the gene expression data that showed an up-regulation of *pl5* and *MDR1* expression in the same 4 patients.

## Discussion

This phase II study of romidepsin in relapsed and refractory AML shows that this HDACI has a differential antileukemic activity in patients with CBF AML [predominantly t(8;21)] and that this was associated with an up-regulation of *AML1* target genes. These findings are in keeping with observations *in vitro* that have established that t(8;21) AML is sensitive to therapy with various HDACIs including romidepsin (7, 16–19, 27). Despite the *in vitro* evidence suggesting an inherent sensitivity of this cytogenetic subset of AML to this class of agents, this is the first study to actually show antileukemic activity *in vivo* in a cohort of patients with t(8;21) AML and other translocations involving the *AML1* gene.

Romidepsin has been investigated in a phase I trial (28) conducted at this dose and schedule in two groups of patients with chronic lymphocytic leukemia and AML, respectively. In that trial that involved 10 AML patients, there were no patients with CBF AML. There were no objective responses reported, nor was there evidence of significant antileukemic activity (such as clearance of bone marrow blasts). The phase I experience mirrors our experience in the 13 patients enrolled in cohort A of this study. The authors of the phase I trial also

observed an increase in global H3 and H4 acetylation as well as acetylation at the *p21* promoter in the patients studied. Another recently published pilot trial of romidepsin in myeloid neoplasms included 9 patients with AML and 3 with MDS, none of whom had CBF AML. Of these, 1 patient with previously untreated AML and a del (7q) achieved a complete remission after one infusion of romidepsin administered at a higher dose (18 mg/m<sup>2</sup>) than used in our study. The response duration was brief, however, with recurrent cytopenias being documented 29 days after the achievement of CR (29). Other clinical trials investigating HDACi in AML have recruited very few patients with CBF AML and have seldom enrolled patients with t(8;21) (30–36).

In our own trial, an antileukemic effect was seen in the majority of CBF AML patients studied, and global histone H3 acetylation was observed following romidepsin therapy in several patients, particularly those with CBF AML. In addition, up-regulation of *MDR1* and *pl4ARF* (both targets of AML1-ETO mediated transcriptional repression; refs. 9, 10) were observed, and these effects were most pronounced in the CBF AML subgroup as we had originally hypothesized. Interestingly, although *p15*, a well-described target of epigenetic silencing in AML, was also up-regulated in several patients in both subgroups, the increase in expression after romidepsin treatment was most striking, as a whole, in patients with CBF AML.

Although we observed an antileukemic effect of romidepsin in several patients with CBF AML, including a patient with a novel t(4;21) shown by fluorescence *in situ* hybridization analysis to involve *AML1*, these effects were short in duration. The reason for this finding remains to be determined. One likely explanation is that the patients enrolled on this study had advanced disease and the majority had evidence of clonal evolution with additional chromosomal aberrations present besides those involving *AML1* or *CBFb*, implicating other pathways. Because romidepsin up-regulates *MDR1* *in vitro* and *in vivo* (37, 38), a finding that has been described with several other HDACi and observed in our study in both subgroups of patients, and romidepsin is a known substrate for MDR1, (39) another possibility is that the drug may mediate its own resistance *in vivo*. *In vitro*, acquired resistance to romidepsin has been shown from reversible MDR1 induction caused by histone hyperacetylation at the MDR1 promoter (37), and MDR1-mediated drug efflux has also been shown in cell lines following romidepsin exposure (40). Although we were able to show for the first time that romidepsin induced histone acetylation at the *MDR1* promoter *in vivo*, insufficient material was available to assess the level and function of MDR1 and other MDR-related proteins. Future clinical trials investigating this agent should, therefore, evaluate the possibility of up-regulation of MDR1 and MDR1-mediated drug efflux as a potential mechanism of chemoresistance to romidepsin. If true, this may provide a potential rationale for therapeutic combinations with MDR inhibitors.

In summary, our experience in this trial provides *in vivo* evidence to support a proof of principle for further clinical investigation of HDACi in CBF AML and particularly t(8;21) AML. Although CBF AML has a better prognosis than other subtypes of AML, 50% of patients relapse, with the prognosis of patients with the t(8;21) apparently worse than those with the inv(16) (41). Recently, the AML1-ETO fusion protein has been shown to associate with DNA methyltransferase 1 (27) in addition to recruiting HDACs. Therefore, future



studies in this area should consider combining HDACIs with agents such as DNA methyltransferase inhibitors that have been shown to be synergistic with HDACIs *in vitro* in a variety of malignancies including t(8;21) AML (17, 27, 42, 43). Our pharmacodynamic studies raise the possibility of *MDR1* up-regulation as a potential mechanism of chemoresistance to romidepsin in AML and underscore the need for additional investigation of this issue in preclinical studies and clinical trials investigating romidepsin and similar agents for the treatment of AML.

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**Translational Relevance**

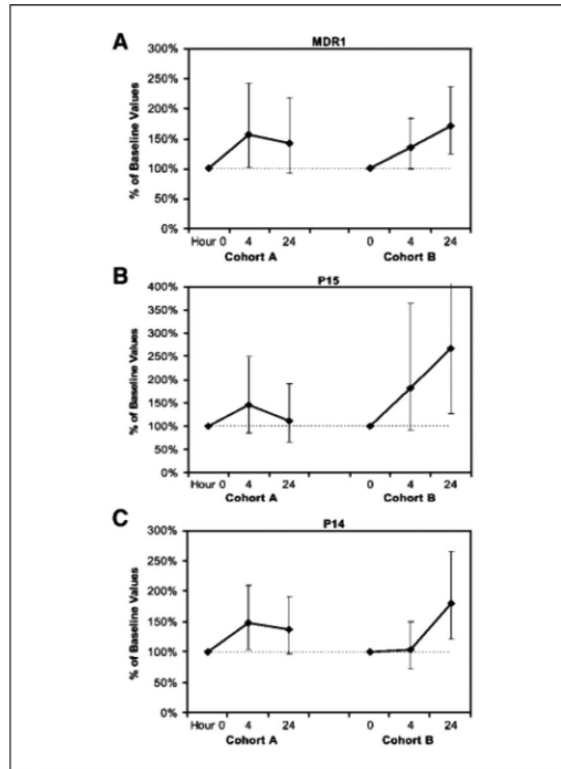
This phase II multicenter study was based on the hypothesis that the HDACI romidepsin could cause transcriptional derepression, up-regulation of specific target genes in AML, and differentiation of the leukemic clone. The study was designed to include a cohort of patients with CBF AML, a subgroup that we hypothesized may be particularly amenable to therapy with HDACI based on the presence of chromosome abnormalities such as t(8;21), known to recruit HDACs. The article provides the first *in vivo* evidence of clinical and molecular effects of a HDACI in a cohort with CBF AML. These results serve as a proof of principle for further clinical investigation of HDACIs in CBF AML, particularly t(8;21) AML.

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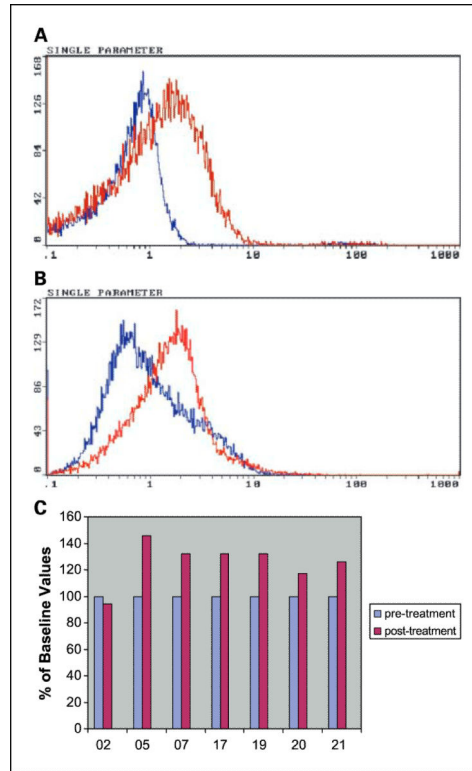
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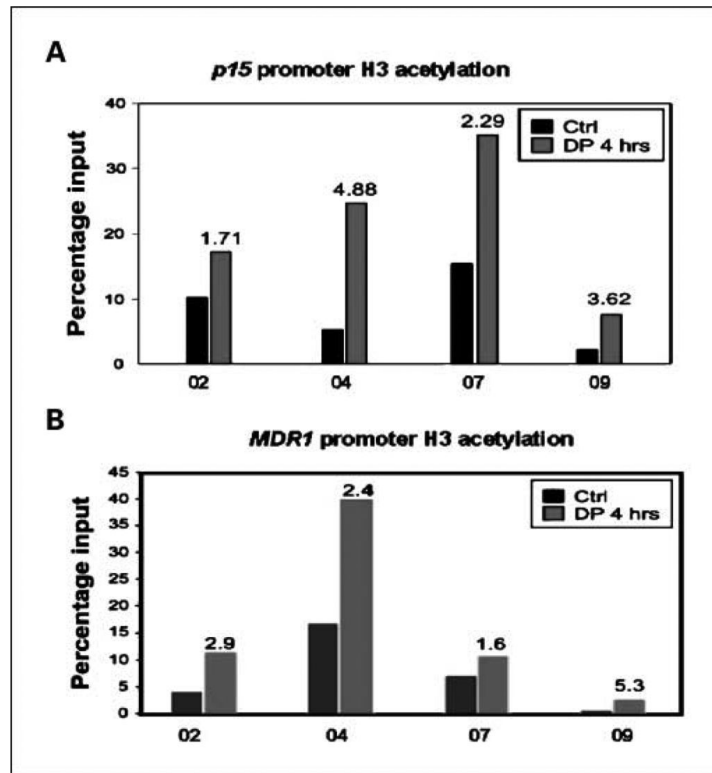
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**Fig. 1.** Romidepsin induced an up-regulation of *MDR1*, *p15*, and *p14ARF* genes. Quantitative PCR analysis of (A) *MDR1*, (B) *p15*, and (C) *p14ARF* at baseline, 4 h, and 24 h following romidepsin treatment in patients in cohort A revealed a trend toward increased gene expression, but these changes were not statistically significant. In cohort B, at 24 h, there was a 75% mean increase in (A) *MDR1* expression ( $P = 0.005$ ), a 162% mean increase in (B) *p15* ( $P = 0.01$ ), and a (C) 106% mean increase in *p14* ( $P < 0.0001$ ). Statistical significance was determined using a random-effects linear model. Geometric means and corresponding 95% confidence intervals are presented.

**Fig. 2.**

Romidepsin induced an increase in global histone (H3) acetylation. *A*, an increase in H3 acetylation is shown after incubation of Kasumi-1 cells with 10 nmol/L romidepsin for 48 h (*red curve*) compared with untreated sample (*blue curve*). *B*, representative M3 acetylation histogram on a patient (patient 17) in cohort B. *Blue curve*, hour 0 (pretreatment) sample. *Red curve*, 24 h post-therapy sample and demonstrates an increase in H3 acetylation. *C*, an increase in global histone H3 acetylation after therapy is shown in 6 of 7 (86%) patients in cohort B.



**Fig. 3.** Romidepsin-induced histone acetylation H3 acetylation at the (A) *p15* promoter and (B) *MDR1* promoter in 4 patients analyzed at 4 h after therapy compared with pre-therapy samples.

**Table 1**

## Baseline patient characteristics

Characteristics	No. patients (%)
Age (y)	
< 60	9 (45)
60	11 (55)
Sex	
Male	8 (40)
Female	12 (60)
Performance status	
0	7 (35)
1	11 (55)
2	2 (10)
Diagnosis	
AML	
De novo	14 (70)
Therapy related/antecedent hematologic disorder	6 (30)
Bone marrow cytogenetics	
t(8;21)/inv(16) or t(4;21)	7 (35)
Normal karyotype	3 (15)
11 q23, -5, -7, complex	6 (30)
Other	4 (20)
Stage of disease	
Primary refractory	6 (30)
First relapse	2 (10)
Beyond first relapse	10 (50)
Previously untreated (and age > 60 y)	2 (10)
Prior therapy	
Cytarabine	18 (90)
High-dose cytarabine	16 (80)
Autologous stem cell transplant	7 (35)
Duration of first complete remission	
12 mo	2 (10)



**Table 2**

Cohort B: baseline characteristics and response to therapy

Patient no.	Age	Stage of disease	Duration of first complete remission (mo)	Prior high-dose cytarabine	Prior autologous hematopoietic stem cell transplant	Bone marrow cytogenetics	No. cycles	Response
2	60	Relapse 3	2	Yes	Yes	46,XY,t(8;21)(q22;q22)/t(1;5)(q32;q13)	1	Progressive disease
5	39	Relapse 5, refractory	16	Yes	Yes	46,XX,t(8;21)(q22;q22),del(9)(q13q22)	2	Bone marrow blast clearance (from 68% to < 5%) after 1 cycle
7	75	Relapse 3	8	No	No	46,XX,t(4;21)(q31.3;q22)/46,XX	3	Bone marrow blast clearance (from 70% to < 5%) after 2 cycles
17	25	Relapse 1, refractory	5	Yes	Yes	45,X,-X,t(8;21)(q22;q22)/46,XX	1	Progressive disease
19	60	Relapse 2	12	Yes	No	45X,-Yadd(1)(p36.3),t(8;21)(q22;q22)/46XY	2	> 50% decrease in marrow blasts (from 63% to 10%) after 1 cycle
20	35	Relapse 2	7	Yes	Yes	47,XY,+8,inv(16)(p13.1q22)/46XY	2	> 50% decrease (from 74% to 31%) in marrow blasts after 1 cycle
21	51	Relapse 1, refractory twice	10	Yes	No	46,XX,add(7)(p22),t(8;21)(q22;q22);47,idem,+der(21)t(8;21)(q22;q22)/46XX	1	> 50% decrease (from 76% to 27% blasts) in marrow blasts

NOTE: HIDAC: high dose cytarabine. Auto HCT: autologous hematopoietic stem cell transplant. CR: complete remission.

**Table 3**

Incidence of commonly encountered toxicities (%)

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Vomiting	25	10	5	0
Anorexia	20	5	0	0
Cardiac	25	0	0	0
Fatigue	10	10	0	0
LFT	5	5	5	0

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