Phase I and pharmacological study of cytarabine and tanespimycin in relapsed and refractory acute leukemia

by Scott H. Kaufmann, Judith Karp, Mark Litzow, Ruben Mesa, William Hogan, David P. Steensma, Karen Flatten, David Loegering, Paula Schneider, Kevin Peterson, Matthew Maurer, B. Douglas Smith, Jacqueline Greer, Yuhong Chen, Joel Reid, S. Percy Ivy, Matthew Ames, Alex Adjei, Charles Erlichman, and Larry Karnitz

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ABSTRACT

Background. In preclinical studies the heat shock protein 90 (Hsp90) inhibitor tanespimycin induces downregulation of checkpoint kinase 1 (Chk1) and other client proteins as well as increased sensitivity of acute leukemia cells to cytarabine. We now report a phase I and pharmacological study of the cytarabine/tanespimycin combination in adults with recurrent or refractory acute leukemia.

Design and Methods. Patients received cytarabine 400 mg/m²/day continuously for 5 days and tanespimycin infusions at escalating doses on days 3 and 6. Marrow mononuclear cells harvested before therapy, immediately prior to tanespimycin, and 24 h later were examined by immunoblotting for Hsp70 and multiple Hsp90 clients.

Results. 26 patients were treated at 5 dose levels. The maximum tolerated dose was cytarabine 400 mg/m²/d x 5 days along with tanespimycin 300 mg/m² on days 3 and 6. Treatment-related adverse events included disseminated intravascular coagulation (grades 3 and 5), acute respiratory distress syndrome (grade 4), and myocardial infarction associated with prolonged exposure to tanespimycin and its active metabolite 17-aminogeldanamycin. Among 21 evaluable patients, there were 2 complete and 4 partial remissions. Elevations of Hsp70, a marker used to assess Hsp90 inhibition in other studies, were observed in >80% of samples harvested 24 hours after tanespimycin, but downregulation of Chk1 and other Hsp90 client proteins was modest.

Conclusions. Because exposure to potentially effective concentrations occurs only for a brief time in vivo, tanespimycin has little effect on resistance-mediating client proteins in relapsed leukemia at clinically tolerable doses and exhibits limited activity in combination with cytarabine (clinicaltrials.gov identifier: NCT00098423).
INTRODUCTION

Heat shock protein 90 (Hsp90) is an abundant and ubiquitous molecular chaperone that helps many key cellular polypeptides assume or maintain their active conformations (1-3). Extensive preclinical studies have examined this chaperone as a potential new drug target in hematological malignancies (3-7). Among the >80 currently identified Hsp90 client proteins are several kinases involved in myeloid neoplasms, including Bcr/abl (8, 9), Flt3 (4, 10, 11), and c-Kit (12), as well as the anti-apoptotic kinase Akt (13, 14). When Hsp90 is inhibited, these polypeptides and other Hsp90 clients fail to fold properly during synthesis and, as a consequence, decrease in abundance as mature molecules turn over (15, 16). This client protein downregulation raises the possibility that Hsp90 inhibitors such as tanespimycin and alvespimycin might be used to simultaneously target multiple potential resistance mechanisms (1, 3, 6). In addition, tanespimycin selectively inhibits growth of aneuploid cells (17). Accordingly, there has been substantial interest in testing this class of agents in acute leukemia.

Although a recent phase I study of the Hsp90 inhibitor alvespimycin in patients with advanced myeloid leukemia revealed only a modest 18% CR rate (18), the realization that pathways targeted by Hsp90 inhibitors contribute to drug resistance raises the question of whether Hsp90 inhibitors could be used as sensitizing agents in combination chemotherapy. Previous studies have also provided the preclinical rationale for combining tanespimycin with a number of other agents, including imatinib (19), Flt3 inhibitors (20), histone deacetylase inhibitors (21, 22), etoposide (23) and cytarabine (24). The present work focused on the cytarabine/tanespimycin doublet because of its potential broad applicability to myeloid malignancies. Cytarabine triggers sequential activation of Ataxia Telangiectasia Mutated/Rad3-related (ATR) kinase and its substrate checkpoint kinase 1 (Chk1) ex vivo (24-26), thereby activating the replication checkpoint (27). Conversely, Chk1 downregulation by RNA interference or treatment with tanespimycin abrogates this checkpoint (28) and sensitizes cells to cytarabine in vitro (24), suggesting that replication checkpoint signaling is protective.

We now report a phase I study of cytarabine and tanespimycin in patients with relapsed/refractory acute leukemia and accelerated phase (AP) or blast crisis (BC) CML. The goals of this study were to not only assess the toxicities and establish the maximum tolerated dose (MTD) of the cytarabine/tanespimycin combination, but also examine the effect of tanespimycin on levels of Hsp90 clients in leukemic blasts in situ.

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DESIGN AND METHODS

Patient Selection. Adults (age ≥ 18 years) with AML (except M3) or ALL were eligible if they
i) failed to respond to intensive induction chemotherapy or relapsed after a first CR of ≤1 year;
ii) had a history of a prior MDS or MPN; or iii) presented with cytogenetic abnormalities of
chromosome 5, 7, 8, or 11 or ≥3 karyotypic abnormalities. Patients with AP- and BC-CML were
eligible only if prior imatinib therapy and ≤1 other induction regimen had failed.

Additional eligibility criteria included ECOG performance status 0-2; total bilirubin <1.5 x
ULN; calculated creatinine clearance >60 ml/min; QTc <450 msec (men) or 470 msec (women)
on electrocardiogram (ECG); cardiac ejection fraction >40%; pulmonary CO diffusing capacity
>80% of normal; and recovery from toxicities of preceding therapy. Hydroxyurea or
glucocorticoids administered to prevent impending leukostasis were stopped ≥48 h before
initiation of cytarabine. Active central nervous system leukemia, uncontrolled infection,
symptomatic pulmonary disease, or a requirement for a medication known to cause QTc
prolongation precluded enrollment. Patients with a history of prior stem cell transplantation,
pulmonary irradiation, chemotherapy-induced pulmonary toxicity, or egg allergy were also
excluded. The study was approved by Institutional Review Boards of the participating
institutions. Written informed consent was obtained from all patients.

Patient Evaluation. Before treatment a history and physical examination, ECG, complete blood
count (CBC), prothrombin time, activated partial thromboplastin time, fibrinogen, serum
creatinine, sodium, potassium, uric acid, calcium, phosphate, magnesium, total protein, albumin,
total bilirubin, direct bilirubin, AST and alkaline phosphatase, echocardiogram or Multiple Gated
Acquisition scan, pulmonary function tests and baseline pulse oximetry were performed. The
physical examination and chemistry tests were repeated at least twice weekly during each cycle.
CBCs were performed daily until neutrophils and platelets were >500/mm³ and 20,000/mm³,
respectively.

Dose, Schedule and Escalation Scheme. A cytarabine regimen previously employed for
consolidation (29) was utilized because the cytarabine dose (400 mg/m²/d x 5 days) was higher
than patients typically received as part of their prior induction therapy. Because tanespimycin
induces a G1 and G2 arrest (24), which could potentially inhibit the replication required for
cytarabine-induced cytotoxicity (24, 30, 31), tanespimycin was administered for the first time
beginning 48 h after the start of the 5-d cytarabine infusion (Fig. 1). The starting dose of tanespimycin (150 mg/m\(^2\)/dose on days 3 and 6), ~50% of the MTD of single-agent tanespimycin administered to solid tumor patients on a weekly schedule (32), was chosen because of anticipated synergy of the agents (24). The tanespimycin dose was increased in 50 mg/m\(^2\) increments.

**Drug Formulation and Administration.** Cytarabine was reconstituted in sterile water at 50 mg/ml, diluted into D5W and administered as a continuous infusion at 400 mg/m\(^2\)/d (rounded to the nearest 25 mg) in 500 ml D5W/d for 5 days. Tanespimycin supplied by the Division of Cancer Treatment (NCI, Bethesda, MD) as a 25 mg/ml solution in dimethyl sulfoxide was diluted to 1 mg/ml in EPL egg phospholipid diluent and administered (rounded to the nearest 5 mg) as a 2-h infusion beginning 48 and 120 h after the start of cytarabine. Because of concern about possible tanespimycin-induced QTc prolongation, serum potassium, calcium and magnesium levels were checked and repleted to the normal range <24 h prior to tanespimycin administration. ECGs were checked prior to, during the second half and within 6 h after completion of the first tanespimycin infusion; and QTc intervals were measured manually and corrected for rate using Bazett’s and Fridericia’s methods.

**Ancillary Treatment.** All patients received allopurinol 300 mg/day on days 1-7 and oral valacyclovir 500 mg daily (or acyclovir 400 mg twice daily) beginning on day 9. Oral fluoroquinolones were administered prophylactically according to institutional practices. Patients with neutropenic fevers initially received empiric antibiotics with anti-Pseudomonal coverage.

**Retreatment and consolidation therapy.** Patients who did not achieve a CR after one course were eligible for a second course on day 21 or later if the blast index (% cellularity x % blasts) decreased >4-fold and all nonhematological toxicities had resolved to grade ≤1. Patients in CR were eligible for up to 4 courses of consolidation on the induction schedule beginning 30 ± 10 days from hospital discharge after the preceding cycle. Dose reductions of one dose level were permitted for DLT.

**Definition of DLT and MTD.** Adverse events were graded by Common Terminology Criteria for Adverse Events, version 3.0. DLT was defined as a) grade 4 hematological toxicity persisting beyond day 35 not attributable to persistent leukemia; b) grade ≥3 QTc prolongation;
c) grade ≥2 allergic, nonQTc cardiac, pulmonary, genitourinary or neurocortical toxicity; d) grade 4 diarrhea, nausea or emesis despite maximal medical treatment; e) grade ≥3 ALT, AST, alkaline phosphatase or bilirubin elevation lasting ≥15 days; or f) any other grade ≥3 nonhematological toxicity that did not resolve with routine medical management.

**Response evaluation.** Bone marrow aspirates and biopsies were obtained within 48 h prior to initiation of therapy, on day 10-15, and every 7-14 days thereafter until counts recovered. CR and PR were defined as previously reported (33), consistent with existing recommendations (34).

**Pharmacokinetic Analysis.** Blood samples were drawn on day 3 before tanespimycin infusion; 115 min into the 2-h infusion; and 5 min, 3 h, 9 h and 24 h after the end of infusion. Plasma concentrations of tanespimycin and its principal metabolite 17-aminogeldanamycin (17AG) were determined as described (32). Tanespimycin and 17AG plasma concentration-time data were analyzed by non-compartmental methods using WINNONLIN version 4.1 (Pharsight Corp., Mountainview, CA). Buffy coat DNA was genotyped as described (32) for CYP3A5 polymorphisms, which are known to affect tanespimycin clearance.

**Immunoblotting.** Marrow mononuclear cells were isolated (35) before treatment, on day 3 prior to tanespimycin, and on day 4 at 22 ± 2 h after the start of tanespimycin (Fig. 1). Whole cell lysates prepared in guanidine hydrochloride were processed for immunoblotting (35), which was performed using antibodies identified previously (24, 32, 36). Marrow mononuclear cells from pretreatment samples were also treated *ex vivo* as described (24).

**RESULTS**

**Patient characteristics.** Twenty-six adult leukemia patients (Table 1) received 30 courses of cytarabine + tanespimycin at five dose levels (Table 2). Of the 22 AML patients enrolled, 17 failed to enter remission with their preceding regimens; and 5 relapsed after ≤1 year in first CR, several while still receiving consolidation therapy. Among the remaining patients, 2 had ALL and 2 had AP- or BC-CML that had not responded to Bcr/abl inhibitor-containing therapy.

**Hematological toxicities.** All patients experienced prolonged grade 4 myelosuppression requiring platelet and red blood cell support. During induction, those patients with normal or elevated WBC typically experienced a WBC decrease before day 5. The ANC reached a nadir of <100/mm³ by median day 7 (range 3-10) and, in those who subsequently achieved a CR,
remained <500 until day 24-34. The hematological toxicities were less severe during second or later courses. Patients receiving the combination while in PR or CR did not become neutropenic until day 7-12 of therapy and had briefer periods of grade 4 neutropenia (generally 7-10 days), with recovery by day 21-28.

Nonhematological toxicities (Table 3). Consistent with the observed myelosuppression, neutropenic fever occurred in all patients and was treated with empiric antimicrobial regimens. There were three infectious deaths, one at dose level 3 (on day 7 due to disseminated Candida tropicalis) and two at dose level 5 (one on day 9 with fungal pneumonia and one on day 19 with E. coli sepsis). A third patient at level 5 developed acute respiratory distress syndrome (ARDS) on day 7 leading to multi-organ failure and >2 weeks of mechanical ventilation before recovery. Patients treated at dose level 5 were also noted by their physicians to appear sicker and less tolerant of stresses such as fever or transient hypotension. Based on these observations, as well as the knowledge that 350 mg/m²/dose exceeds the weekly tanespimycin MTD in solid tumor patients, dose level 4 (cytarabine 400 mg/m²/d x 5 days, tanespimycin 300 mg/m²/dose on days 3 and 6) was identified as the MTD and recommended phase 2 dose.

Other severe adverse events at least possibly related to the treatment included a myocardial infarction (MI) on day 6 in a patient without a prior cardiac history (dose level 2), an episode of grade 5 disseminated intravascular coagulopathy (DIC) leading to intracerebral hemorrhage on day 6 (dose level 3), and grade 3 DIC associated with tumor lysis on day 4 (dose level 4). As indicated in Table 3, both episodes of DIC occurred in patients with evidence of DIC earlier in their chemotherapy but were exacerbated by tanespimycin administration and, by definition, represent treatment-related adverse events. Notably, therapy-induced arrhythmias and QTc prolongations were not observed.

Responses. Among the 21 evaluable patients, there were 2 CRs. One occurred at dose level 4 in an AML patient who had experienced CRs of 10 months and 6 months after two prior cytarabine-containing regimens. That patient received two consolidation cycles before declining further therapy and relapsing after a 10-month CR. The second CR occurred at dose level 5 in a patient with refractory Ph⁺ ALL but lasted only one month. In addition there were two PRs in AML patients and two hematological PRs in CML patients, all lasting <60 days.

Pharmacokinetics. The pharmacokinetics of tanespimycin and its metabolite 17AG, which also
affects client protein stability (24), were evaluable for 20 patients (Table S1). Mean tanespimycin clearance was 17.8 ± 6.4 L/h/m². The half-lives for tanespimycin and 17AG were 4.5 ± 2.7 hours and 6.1 ± 1.9 hours, respectively. Moreover, tanespimycin and 17AG peak plasma concentrations and AUCs increased with dose over the 150-350 mg/m² range. Importantly, tanespimycin mean peak plasma concentrations were >3000 nM at all dose levels; and 17AG mean peak plasma concentrations exceeded 1500 nM.

Because client protein downregulation is known to require extended tanespimycin exposure (16, 24), we also examined the duration of tanespimycin and 17AG plasma concentrations above 300 nM, a concentration previously shown to induce substantial client protein downregulation after 24 h in leukemia cell lines in vitro (24), and 1000 nM, a concentration commonly used in preclinical studies. The sum of the tanespimycin and 17AG concentrations exceeded 300 nM for >10 h in all patients (Table 4). This sum exceeded 300 nM for >24 h in only three patients, none of whom had an obvious explanation based on cytochrome P450 polymorphisms. All three patients with prolonged drug exposure experienced severe adverse events, including an MI (dose level 2) and fatal sepsis (1 patient each at dose level 3 and dose level 5).

**Hsp90 client protein expression.** Correlative studies were designed to determine whether treatment affects Hsp90 function in situ. Bone marrow aspirates harvested prior to therapy, on day 3 before tanespimycin, and on day 4 at 22 ± 2 h after tanespimycin (Fig. 1) were blotted for several Hsp90 clients that are downregulated by tanespimycin in leukemia cell lines (Fig. 2A and ref. 24). Whenever possible, aliquots of pretreatment marrow were also exposed to cytarabine or tanespimycin ex vivo for comparison.

Based on preclinical results, decreased levels of the Hsp90 clients and increased levels of Hsp70 (due to transcription mediated by heat shock factor-1 (HSF-1) released from Hsp90—ref. 1) were anticipated on day 4. Contrary to this prediction, decreases of the Hsp90 clients ILK, Akt, c-Raf and IGF1R were difficult to discern, although there were modest increases in Hsp70 (Fig. 2B-2D, in vivo). Exposure of pretreatment blasts to tanespimycin for 24 h ex vivo confirmed that tanespimycin was able to downregulate Chk1, Akt and c-Raf at 300 and 1000 nM (Fig. 2B and D ex vivo), albeit less extensively than in AML lines (Fig. 2A, lane 4) or samples of previously untreated AML (24). In total, 17 sets of sequential samples were analyzed. Hsp70 showed a readily discernible increase between day 3 and day 4 in 10 of 12 (83%) samples that
yielded an Hsp70 signal. In contrast, only 3 of 13 (23%) and 1 of 13 (8%) day 4 samples had decreased c-Raf or ILK, respectively, relative to baseline, indicating that effects on client proteins in leukemic blasts \emph{in situ} were modest at the tanespimycin exposures achieved clinically. Moreover, there was no apparent relationship between these changes and clinical response.

**DISCUSSION**

In the present study, we report the first results of a tanespimycin-containing combination in patients with relapsed/refractory acute leukemias and the first analysis of effects of these agents on Hsp90 clients in leukemia specimens \emph{in situ}. Our results have potentially important implications for current efforts to modulate leukemia resistance mechanisms with Hsp90 inhibitors.

Several issues were considered in designing the present study. First, the trial built on an established cytarabine regimen with defined activity and toxicity (29). Second, a cytarabine regimen that incorporates more than the usual 100-200 mg/m²/d was chosen to avoid treating refractory leukemias with a cytarabine dose that previously failed to induce remission. While we recognize that an even higher dose of cytarabine might, as a single-agent, be more effective in the setting of relapsed and refractory leukemia (37), high-dose cytarabine might also have further diminished the amount of tanespimycin that could be administered without DLT. The present cytarabine dose represented a compromise between these two concerns. Finally, tanespimycin was delayed until day 3 to avoid ansamycin-induced inhibition of cell cycle progression (24, 28), which antagonizes the effects of cytarabine (24).

In addition to the typical hematological toxicities of cytarabine infusion, we observed a number of DLTs that were attributed to the combination, including grade 3 and grade 5 DIC during therapy-induced tumor lysis, ARDS progressing to multi-organ failure, and a possibly higher than expected frequency of septic deaths. The occurrence of two grade 5 toxicities and grade 4 ARDS in 3 patients at dose level 5, combined with the information that 350 mg tanespimycin/m²/dose exceeds the weekly single-agent MTD in solid tumor patients (32), led to identification of the MTD as tanespimycin 300 mg/m²/dose on days 3 and 6 in combination with cytarabine 400 mg/m²/day by 5-day infusion.

Among 22 AML patients, 1 CR and 2 PRs were observed, all in leukemias that relapsed soon after or were refractory to prior cytarabine-containing regimens. In addition, a CR was observed
in a patient with primary refractory Ph\(^+\) ALL; and two hematological PRs were observed in patients with AP-CML, for an overall response rate of 23%. Enthusiasm for this regimen, however, was tempered by the short duration of all but one response and the seriousness of the adverse events.

Correlative studies were performed to determine whether tanespimycin had the anticipated impact on Hsp90 clients. Comparison of samples harvested before and 22 ± 2 h after tanespimycin demonstrated discernible Chk1 decreases in 6 of 13 (46%) of cases. The magnitude of Chk1 downregulation, however, was relatively modest compared to tanespimycin-treated AML cell lines (Fig. 2A and refs. 24, 28). Moreover, it was unclear that Chk1 downregulation reflected client protein degradation, as the Hsp90 client proteins c-Raf and ILK were downregulated in only 1 and 3 of 13 serial samples, respectively, on day 4 (Fig. 2B-D). An equally plausible explanation is that Chk1, which is expressed in a cell cycle-dependent manner (38), decreased due to tanespimycin-induced changes in cell cycle progression (24).

A number of prior studies have examined Hsp70 upregulation, which reflects increased activity of the transcription factor HSF-1 after release from Hsp90 (1), as a marker of tanespimycin action (18, 32, 39). It is important to emphasize that Hsp70 upregulation results in resistance to Hsp90 inhibitors, not sensitization (40, 41). In our study Hsp70 was upregulated after tanespimycin administration in blasts from 83% of patients. However, this Hsp70 upregulation sometimes occurred before tanespimycin administration (Day 3, Figs. 2B and 2D), possibly reflecting cytarabine-induced stress. Moreover, Hsp70 upregulation did not predict client protein downregulation. In preclinical studies published after completion of our work, response to Hsp90 inhibitors in xenografts correlated best with prolonged Hsp90 active site occupancy, which in turn correlated with Hsp90 client downregulation (16). Accordingly, some marker other than Hsp70 upregulation might be needed to measure prolonged Hsp90 inhibition and sensitization in future combination studies.

Analysis of tanespimycin pharmacokinetics (Tables S1 and 4) provided a potential explanation for the limited downregulation of client proteins. The clearance of tanespimycin and 17AG in these leukemia patients (Table S1) was similar to that reported in earlier solid tumor trials (32, 39). At the MTD, tanespimycin + 17AG exceeded 1000 nM, a concentration that induces substantial client protein downregulation in some samples after 24 h (Fig. 2B), for only 5 h. In contrast, our previous studies demonstrated that a 6 h tanespimycin incubation is not
sufficient to induce client protein downregulation in vitro (24). Even though 350 mg/m² tanespimycin resulted in more prolonged exposure to potentially effective concentrations of tanespimycin + 17AG (Table 4), this dose was not tolerable in combination with cytarabine. Accordingly, it appears that a single 2-hour tanespimycin infusion at tolerable doses might not give sufficient exposure to induce substantial client protein downregulation in leukemic blasts in vivo. Examination of the pharmacokinetic data in Table S1 and a graph of pooled mean plasma-concentration data for each dose level suggests that levels of tranespimycin + 17AG above 300 nM could be sustained in most patients for >24 h by administration of 150 mg/m² tanespimycin every 12 hours x 2 doses on day 3 and again on day 6. Based on the adverse events observed in the present study, however, it is unclear whether such a schedule would be tolerable.

In summary, the present study has defined the MTD of the cytarabine + tanespimycin regimen as cytarabine 400 mg/m²/d x 5 days and tanespimycin 300 mg/m²/dose on days 3 and 6. Despite the ability of tanespimycin to modulate multiple resistance mechanisms in vitro, disruption of these same pathways in leukemic cells in situ was difficult to demonstrate, likely reflecting drug exposure that was insufficient to downregulate client proteins in leukemic blasts. It is possible that alternative Hsp90 inhibitors, especially those lacking a geldanamycin backbone (42), might exhibit a wider therapeutic index in this setting. Alternatively, in view of the recent development of highly effective and selective inhibitors of clients such as checkpoint kinases (43-45), Akt (42, 46, 47) and IGFR (47), it will also be interesting to see whether these agents offer any benefit when combined with cytarabine in AML patients.

AUTHORSHIP AND DISCLOSURES
SHK was the principal investigator and takes primary responsibility for the paper. JEK, RAM, SPI, JG, AA, CE and LMK participated in study design and conduct. SHK, JEK, MRL, RAM, WH, DPS, and BDS recruited patients. KSF, DAL, PAS, KLP, YC, JMR and MMA participated in laboratory studies. MJM performed statistical analysis. SHK and JEK wrote the paper with input from all authors. The authors reported no potential conflict of interest.
REFERENCES


# Table 1

**Characteristics of Treated Patients**

| Characteristic                                      | No. of patients/courses | Median number of courses/pt. (Range) | Gender          | Median age, years (Range) | Leukemia characteristics | Number of prior induction regimens | High risk karyotypes |
|-----------------------------------------------------|-------------------------|-------------------------------------|-----------------|--------------------------|--------------------------|-----------------------------------|---------------------|----------------------|
| No. of patients/courses                             | 26/30                   | 1 (1-3)                             | Male            | 11                       | ALL                      | 2                                 | Abnormalities of chr 5, 7 or 8 |
| Median number of courses/pt. (Range)                |                         |                                     | Female          | 15                       | CML-AP                   | 2                                 | Complex             |
| Gender                                              |                         |                                     |                 |                          | AML                      | 22                                |                     |
| Male                                                |                         |                                     | M0              | 7                        |                          | FAB                               |                     |
| Female                                              |                         |                                     | M1              | 3                        |                          | M0                                |                     |
|                                                     |                         |                                     | M2              | 3                        |                          | M1                                |                     |
|                                                     |                         |                                     | M4              | 4                        |                          | M2                                |                     |
|                                                     |                         |                                     | M5              | 2                        |                          | M4                                |                     |
|                                                     |                         |                                     | Unspecified     | 3                        |                          | Unspecified                      |                     |
| Prior disorder                                      |                         |                                     | MDS             | 4                        | 2                        | 0                                 |                     |
|                                                     |                         |                                     | MPN             | 2                        | 1                       | 1                                 |                     |
| Number of prior induction regimens                  |                         |                                     |                 |                          | 1                        | 1                                 |                     |
|                                                     |                         |                                     |                 |                          | 2                       | 2                                 |                     |
|                                                     |                         |                                     |                 |                          | 3 or more               | 3                                 |                     |
|                                                     |                         |                                     |                 |                          |                          | High risk karyotypes              |                     |
| High risk karyotypes                                |                         |                                     |                 |                          | 7                        |                                    |                     |
|                                                     |                         |                                     |                 |                          | 5                        |                                    |                     |
Table 2

Summary of Dose Escalation

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Cytarabine (mg/m²/d) days 1-5</th>
<th>Tanespimycin (mg/m²/dose) days 3 and 6</th>
<th># Pts. enrolled</th>
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<tr>
<td>1</td>
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<td>5</td>
<td>400</td>
<td>350</td>
<td>4</td>
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\(^a\)Includes one patient who was replaced due to septic death on day 7.
Table 3
Nonhematological adverse events ≥ grade 3 that were possibly, probably or definitely attributed to treatment

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Total Patients</th>
<th>Diarrhea</th>
<th>Tumor Lysis</th>
<th>Myocardial Infarction</th>
<th>Infection (Gr 5 only)</th>
<th>Fatigue</th>
<th>Hyperglycemia</th>
<th>Bilirubin</th>
<th>AST</th>
<th>DIC</th>
<th>Creatinine</th>
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<td>1*</td>
<td>1</td>
<td>3c</td>
<td>1d</td>
<td>1d</td>
<td></td>
<td></td>
<td>1f</td>
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<td>V</td>
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</tbody>
</table>

*Disseminated C. tropicalis on day 7.
*Progressive fungal pneumonia on day 9 and E. coli sepsis on day 19
*Easily managed with sliding scale insulin and not felt to be dose-limiting by physicians caring for patients.
*Grade 3 elevations lasting <15 days that did not meet the predefined definition of a DLT.
*Grade 5 DIC with intracranial hemorrhage on day 6. This patient had DIC on admission that resolved by day 5 with a normal fibrinogen prior to day 6 tanespimycin and a fibrinogen of 40 mg/dl 12 h after tanespimycin at the time of an intracranial bleed.
*Grade 3 DIC on days 3 and 6. This patient had hyperphosphatemia with normal coagulation beginning on day 2; hyperphosphatemia and hyperkalemia with DIC and hypofibrinogenemia on day 4 24 h after tanespimycin; and worsening hypofibrinogenemia through day 6 (fibrinogen nadir 81 mg/dl on day 6 despite replacement) that resolved on day 7.
Table 4

Duration of Plasma Concentrations above 300 nM or 1000 nM by Dose Level

<table>
<thead>
<tr>
<th>Level (N)</th>
<th>Tanespimycin</th>
<th>17AG</th>
<th>Tanespimycin + 17AG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 300 nM</td>
<td>&gt; 1000 nM</td>
<td>&gt; 300 nM</td>
</tr>
<tr>
<td>1 (1)</td>
<td>4.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.33</td>
<td>10.33</td>
</tr>
<tr>
<td>2 (4)</td>
<td>7.27</td>
<td>4.02</td>
<td>10.14</td>
</tr>
<tr>
<td>3 (4)</td>
<td>7.34</td>
<td>7.34</td>
<td>11.35</td>
</tr>
<tr>
<td>4 (6)</td>
<td>11.09</td>
<td>5.04</td>
<td>10.10</td>
</tr>
<tr>
<td>5 (2)</td>
<td>18.50</td>
<td>8.17</td>
<td>11.09</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values represent the median number of hours that tanespimycin, 17AG or the total of tanespimycin + 17AG exceeded the indicated level.
FIGURE LEGENDS

Figure 1. Schematic representation of trial events. After pretreatment bone marrow aspirates were obtained, cytarabine was administered at 400 mg/m^2/d by continuous infusion for 5 days. Approximately 48 h into this infusion, the day 3 bone marrow aspirate was obtained. Patients then received escalating doses of tanespimycin (repeated again on day 6 at the very end of the cytarabine infusion), followed 22 ± 2 h by the day 4 bone marrow aspirate to assess the impact on client protein expression.

Figure 2. Effects of treatment on client protein levels. A, after U937 cells were treated for 24 h with diluent (0.1% DMSO, lane 1) or tanespimycin at 30, 100, 300 or 1000 nM (lanes 2-5), whole cell lysates were subjected to SDS-polyacrylamide gel electrophoresis followed by immunoblotting. Numbers at left, molecular weight markers in kDa. B-D, marrow mononuclear cells from patients exposed to tanespimycin in vivo at (B) dose level 3, (C) dose level 4 or (D) dose level 1. In each case whole cell lysates were isolated from marrows harvested prior to treatment (day 0), after 48 h of cytarabine (day 3) and 22 ± 2 h after tanespimycin administration (day 4). Polypeptides from 0.5-5 x 10^5 HL-60 cells were included on each gel to provide a standard curve for quantitating signals (35). Hsp90B and Histone H1 represent two loading controls. After immunoblotting, digitized signals were normalized for histone H1 content and compared to HL-60 cell dilutions. A value of 1.0 indicates the same antigen signal as an equal number of HL-60 cells. In panels B and D, pretreatment marrow mononuclear cells from the same patients were also prospectively exposed ex vivo to diluent (0.1% DMSO), 300 nM cytarabine, 1000 nM cytarabine, 300 nM tanespimycin, or 1000 nM tanespimycin (lanes 1-5, respectively) for 24 h and subjected to immunoblotting. Cells for ex vivo exposure were not available from the patient in panel C.
Figure 1
Figure 2
Table S1

Tanespimycin and 17AG Pharmacokinetic Parameters by Dose Level

<table>
<thead>
<tr>
<th>Level (N)</th>
<th>Dose (mg/m²)</th>
<th>Tanespimycin</th>
<th>17AG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t₁/₂ (hr)</td>
<td>Cₘₐₓ (nM)</td>
</tr>
<tr>
<td>1 (3)</td>
<td>150</td>
<td>Mean 3.20</td>
<td>7344</td>
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<tr>
<td></td>
<td></td>
<td>SD 1.60</td>
<td>3760</td>
</tr>
<tr>
<td>2 (4)</td>
<td>200</td>
<td>Mean 3.02</td>
<td>5633</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 1.39</td>
<td>794</td>
</tr>
<tr>
<td>3 (4)</td>
<td>250</td>
<td>Mean 4.35</td>
<td>8261</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 1.81</td>
<td>7242</td>
</tr>
<tr>
<td>4 (6)</td>
<td>300</td>
<td>Mean 5.53</td>
<td>6077</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 4.37</td>
<td>2524</td>
</tr>
<tr>
<td>5 (3)</td>
<td>350</td>
<td>Mean 5.73</td>
<td>8082</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD 1.00</td>
<td>849</td>
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</table>

ªTerminal elimination rate constants (k₂) were determined by linear least-squares regression through the 9-25 h plasma-concentration time points. Elimination half-life (t₁/₂) was calculated as 0.693/k₂. Areas under the plasma concentration-time curves (AUC) were determined using the linear trapezoidal rule from time zero to the time of the last detectable sample (Cₙₐₛₖ). Areas under the plasma concentration-time curves through infinite time (AUC₀₋∞) were calculated by adding the value Cₙₐₛₖ/k₂ to AUCₙₐₛₖ. The clearance (CL) of tanespimycin was calculated as dose/AUC₀₋∞.

ªSix patients were not included in the pharmacokinetic analysis, including three (one at dose level 2 and two at dose level 3) who had missing samples that would have made analysis inaccurate and three (one each at dose levels 2, 4 and 5) who had extremely high plasma concentrations during infusion, suggesting that blood draws were inadvertently removed from the infusion line.