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Phase I study of the ribonucleotide reductase inhibitor 3-aminopyridine-2-carboxaldehyde-thiosemicarbazone (3-AP) in combination with high dose cytarabine in patients with advanced myeloid leukemia

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Summary

Purpose—This Phase I dose escalation study was based on the hypothesis that the addition of 3-aminopyridine-2-carboxaldehyde-thiosemicarbazone (3-AP) to cytarabine would enhance cytarabine cytotoxicity. The primary objective of the study was to establish the maximum tolerated dose of 3-AP when given in combination with a fixed dose of cytarabine.

Experimental design—Twenty-five patients with relapsed or refractory myeloid leukemia were enrolled to three dose levels of 3-AP. Cytarabine was administered as a 2 h infusion at a fixed dose of 1,000 mg/m²/day for 5 consecutive days. Escalating doses of 3-AP as a 2 h infusion were administered on days 2 through 5. The 3-AP infusion preceded the start of the cytarabine infusion by 4 h.

Results—In general, the toxicities observed with the combination were similar to the expected toxicity profile for cytarabine when utilized as a single agent at this dose and schedule. However, two of three patients developed dose-limiting methemoglobinemia at the highest 3-AP dose studied (100 mg/m²). Transient reversible methemoglobinemia was documented in 11 of 15 patients enrolled at the 75 mg/m² dose level. Objective evidence of clinical activity was observed in four patients.

Conclusions—The combination of 3-AP and cytarabine given on this schedule is feasible in advanced myeloid leukemia. The recommended Phase II dose is 75 mg/m²/day of 3-AP on days 2–5 given prior to cytarabine administered at a dose of 1,000 mg/m²/day over 5 consecutive days. Methemoglobinemia is a common toxicity of this combination and requires close monitoring.

Keywords

3-Aminopyridine-2-carboxaldehyde-thiosemicarbazone; 3-AP; Myeloid leukemia; Triapine; Cytarabine; Methemoglobinemia

Introduction

Cytarabine is one of the most active antileukemic agents and remains the mainstay of treatment for patients with acute myeloid leukemia (AML). Cytarabine's cytotoxicity is dependent on its conversion to the active metabolite cytarabine triphosphate (ara-CTP), a process which occurs intracellularly. The degree of cytarabine cytotoxicity correlates linearly with ara-CTP incorporation into DNA. In patients with AML, efficacy correlates with intracellular exposure to ara-CTP [1]. The initial and rate limiting step in cytarabine phosphorylation is catalyzed by deoxycytidine kinase (dCydK), which is saturable (in human leukemia blasts) at a plasma concentration of 7 to 10 μ M. This concentration is exceeded at dose rates of cytarabine greater than 0.25 or 0.5 g/m²/h; therefore, merely increasing the cytarabine dose rate will not result in an increase in intracellular ara-CTP [2]. dCydK is subject to negative feedback inhibition by deoxycytidine triphosphate (dCTP).

Therefore, a decrease in intracellular deoxynucleotide pools would be predicted to augment the activity of dCydK, and enhance the conversion of cytarabine to ara-CTP.

3-AP (3-aminopyridine-2-carboxaldehyde-thiosemicarbazone; Triapine[®], Vion Pharmaceuticals) is a potent inhibitor of the M2 subunit of ribonucleotide reductase (RR), an enzyme which catalyzes the conversion of ribonucleotides to deoxynucleotides, a rate limiting step in DNA synthesis and repair [3]. Mammalian RR has two distinct subunits-the M1 regulatory subunit and the M2 catalytic subunit. Various nucleoside analogues including fludarabine and gemcitabine inhibit the M1 regulatory subunit [4]. The M2 subunit activity depends on the generation of a tyrosyl free radical and the presence of iron in the active site. The only marketed inhibitor of the M2 catalytic subunit is the reversible inhibitor hydroxyurea. Its activity depends on reduction of the tyrosyl free radical. Regeneration of the tyrosyl free radical and overexpression of the M2 subunit, however abrogates hydroxyurea's effects [5]. In contrast, 3-AP is a small molecule inhibitor of RR that inhibits regeneration of the tyrosyl free radical and is also a strong iron chelator. 3-AP is estimated to be approximately 100–1,000 times as potent as hydroxyurea based on enzyme and tumor cell growth inhibition assays [3]. As a single agent, antileukemic activity has been seen in vitro and in vivo against leukemic cell lines, as well as the L1210 murine leukemia model [6]. In phase I single agent trials in hematologic malignancies, reductions in peripheral and/or bone marrow blasts were described in patients with advanced leukemia [7, 8], and in vivo depletion of deoxynucleotides has been observed in primary leukemia cells [7]. In addition, sequential exposure of tumor cell lines (e.g., HL-60) to 3-AP and then cytarabine in cell culture studies has been demonstrated to be synergistic, and to enhance cellular uptake and incorporation of ara-CTP into DNA [9].

Therefore, we conducted a Phase I trial of 3-AP in combination with cytarabine. We hypothesized that inhibition of the M2 subunit of RR by 3-AP would lead to an increase in the enzymatic activity of dCydK and increased intracellular levels of ara-CTP with a resultant potentiation of the antitumor effects of cytarabine.

The primary objective of this clinical trial was to establish the maximum tolerated dose (MTD) of 3-AP when given in combination with a fixed dose of cytarabine.

Patients and methods

Patient selection

Patients were eligible for this study if they had histologically confirmed relapsed or refractory AML, or chronic myeloid leukemia (CML) in accelerated or blast phase. Patients with secondary AML, including AML arising from an antecedent hematologic disease such as myelodysplastic syndrome (MDS) or myeloproliferative disorder (MPD) or therapy-related AML (t-AML) were also eligible for enrollment. All patients had disease refractory to standard therapy or for which no standard therapy existed. Patients were 18 years or older and had a performance status of 0, 1 or 2 (Cancer and Leukemia Group B [CALGB] criteria). Patients were ineligible if they had a serum creatinine >1.5 times the institutional upper limit of normal, serum total bilirubin 2.0 mg/dl or greater (unless due to Gilbert's syndrome), or if the serum transaminases were >2.5 times institutional upper limit of

normal. Patients were also excluded if they had significant local or systemic infection at the onset of the study, symptomatic central nervous system (CNS) disease, congestive heart failure, cardiac arrhythmia, pulmonary disease requiring oxygen or any other significant underlying medical condition that would make the administration of either cytarabine or 3-AP unusually hazardous. In addition, no cytotoxic therapy or radiotherapy was permitted within 2 weeks (6 weeks for mitomycin C and nitrosoureas) of starting therapy with the exception of hydroxyurea, which could be administered up to 72 h prior to the proposed treatment. Patients with known glucose 6 phosphate dehydrogenase (G6PD) deficiency were excluded in view of the potential for methemoglobinemia following treatment with 3-AP. The protocol was reviewed and approved by the University of Chicago Institutional Review Board and the Cancer Therapy Evaluation Program (CTEP), National Cancer Institute (NCI). All patients gave written informed consent.

Study design

This was an open label, single center, dose-escalating phase I study. Cytarabine was administered at a fixed dose of 1,000 mg/m²/day given as a 2-h infusion on days 1–5, while escalating doses of 3-AP were given as a 2-h infusion for 4 consecutive days on days 2–5 of a 28-day cycle. Based on preclinical studies that supported a sequence dependency for successful biochemical modulation of both cytarabine and gemcitabine when given in combination with 3-AP, the 3-AP infusion was initiated 4 h prior to the cytarabine infusion [9].

The 3-AP starting dose and schedule were chosen based on prior phase I single agent experience with this drug in solid tumors and hematologic malignancies [7, 10–12]. In these trials, a 96-h continuous infusion caused more renal and hepatic toxicity compared with a 2-h infusion given daily for 5 days and repeated every 2 to 4 weeks. The major dose limiting toxicity (DLT) with this latter schedule was transient neutropenia which was substantially less when the drug was administered for 4 days, and the MTD was not reached at dose levels of up to 96 mg/m²/day [11].

Drug supply and administration—Cytarabine was obtained commercially. 3-AP was supplied by Vion Pharmaceuticals, Inc. and distributed by the CTEP, DCTD, NCI. 3-AP was supplied in 10 ml amber vials containing 50 mg of 3-AP for IV infusion. 3-AP was diluted in 0.9% sodium chloride or 5% dextrose in water to a final concentration of 0.01 to 2 mg/ml. At this concentration, 3-AP has been found to be stable for 8 h at room temperature or at 2–8°C. Dilutions of 3-AP were performed in glass bottles, or in plastic IV bags that do not contain di(ethylhexyl)phthalate (DEHP), since the nonaqueous solvents in 3-AP for injection have been shown to extract DEHP. DEHP-free administration sets were used for the administration of 3-AP.

Dose escalation and definition of study endpoints

DLT was defined as any grade 3 or greater nonhematologic toxicity (except transient liver function abnormalities, transient nausea and vomiting, diarrhea and culture-negative neutropenic fever), or any grade 2 or greater neurologic toxicity which was assessed as being probably or definitely related to the combination of 3-AP and cytarabine. Grade 3

liver function test (LFT) abnormalities, nausea and vomiting and diarrhea that did not resolve to grade 1 by day 28 were also considered dose-limiting. For patients who had previously been treated with high dose cytarabine and had experienced a grade 3 nonhematologic toxicity that was attributed to cytarabine therapy, the recurrence of such a toxicity was not considered a DLT, unless it did not resolve to grade 1 or lower by day 28. The severity of hematologic toxicity was assessed, but not used to define DLT, since the study population had marrow failure at entry due to advanced disease, and/or extensive prior therapy. Similarly, the incidence and severity of infectious complications was assessed, but was not used to define DLT since infections arise commonly in the course of induction or salvage therapy for acute leukemias, and also commonly arise independent of therapeutic intervention in this patient population as a consequence of pre-existing marrow failure.

A minimum of three patients were evaluated at each dose level, if no DLT was observed. If one of the first three patients at a dose level experienced DLT, then at least three additional patients were treated at that dose level to determine if the MTD had been exceeded. Fewer than three additional patients would be enrolled if the MTD was clearly exceeded before 6 patients had been treated. If none of the first three patients at a dose level experienced DLT, then dose escalation could proceed to the next level. The MTD was defined as the dose level at which fewer than 2 of six patients experienced first-course DLT. At least 12 patients would be enrolled at this dose level to confirm that it was the MTD. Toxicity was graded according to the NCI-Common Terminology Criteria for Adverse Events version 3.0. Treatment could continue for a maximum of four cycles in the absence of unacceptable adverse events or disease progression.

Pretreatment and follow-up studies

Within 1 week prior to starting therapy, each patient underwent a detailed evaluation which included the following: history and physical examination, complete blood cell count with differential and platelets (CBC) and serum chemistries including liver and renal function tests and an electrocardiogram (ECG). A pre-treatment bone marrow aspiration and biopsy was performed on all patients within 2 weeks of starting therapy. CBC and serum chemistries were performed on the first day of each cycle and monitored at least once a week. Symptom assessment and physical examination were performed at least once a week. During the course of the clinical trial, following a report of severe hemolysis and methemoglobinemia occurring in a patient with G6PD deficiency treated with 3-AP [13], the protocol was amended to require baseline G6PD analysis in the appropriate clinical context (i.e., patients of Mediterranean, Asian or African ancestry). The protocol was also amended to require a methemoglobin (met-Hg) level to be measured after the completion of the 3-AP infusion on days 2 and 3 of cycle 1 (i.e., at the end of the first two infusions of 3-AP), in all subsequent patients enrolled. Met-Hg levels were measured by co-oximetry in an arterial blood gas analyzer. In addition, monitoring of vital signs and pulse oximetry was instituted prior to each infusion of 3-AP, every 30 min during the infusion, and every 60 min thereafter for up to 2 h following the completion of the infusion. An ECG was performed if the patient became dyspneic or hypotensive.

A follow-up bone marrow examination was performed between days 12 to 14 of the first course of treatment to assess the degree of cytoreduction, and subsequently as needed for response evaluation. Response was assessed after every one or two cycles of therapy, according to standard criteria for acute leukemia [14]. Briefly, complete remission (CR) required absolute neutrophil count (ANC) above $1.5 \times 10^9/l$, platelet count above $100 \times 10^9/l$, absence of circulating blasts, and bone marrow cellularity over 20% with less than 5% blasts and maturation of all cell lines. Patients who fulfilled the criteria for CR in the peripheral blood but still had 5–25% blasts in the marrow were considered to have a partial remission (PR). Hematologic improvement (HI) was defined as 50% decrease in bone marrow blasts associated with a significant improvement in blood counts (ANC above $1 \times 10^9/l$ and platelets above $100 \times 10^9/l$).

Results

Patient characteristics

The characteristics of the 25 patients studied are summarized in Table 1. Patients were enrolled on the study between January 2004 and December 2005. All had previously received cytarabine therapy, and 80% had received high dose cytarabine (HiDAC). Five patients had undergone allogeneic stem cell transplantation and had a relapse of their disease.

Nonhematologic toxicities

The most commonly encountered toxicities included fever, mild to moderate nausea, diarrhea, and rash (Table 2). Febrile reactions (grade 1–2) were commonly associated with the infusion of one or both drugs, occurring in 13 patients (52%) within 12 to 24 h after cytarabine and/or 3-AP. These reactions were transient and generally resolved promptly within 24 h following the completion of the drug infusions.

Severe adverse reactions were infrequent, and included grade 3 rash in two patients at dose level 1. One of these patients had been treated in the past with HiDAC, and had previously developed a rash of similar appearance and severity with HiDAC as a single agent. The other patient was on broad spectrum antimicrobial prophylaxis, which could have been the cause of the rash. Three patients (one at each of the dose levels studied) developed grade 3 ($n=1$) or grade 4 ($n=2$) LFT abnormalities, which were assessed as being related to concomitant medications, predominantly azole prophylactic antifungal agents that these patients received. These LFT abnormalities resolved or improved significantly after the offending agents were discontinued. DLTs observed at dose level 2 were grade 3 cerebellar toxicity occurring in 2 (of 15) patients after the third dose of 3-AP. Neither patient received any additional therapy on the study; both recovered without sequelae. Other non-hematologic toxicities encountered include methemoglobinemia and infectious complications, both of which are described in more detail below.

Methemoglobinemia

The occurrence of methemoglobinemia was routinely monitored starting with patients enrolled at dose level 3 ($100 \text{ mg/m}^2/\text{day}$), as outlined in the Patients and methods section. At

this dose level, two of three patients experienced met-Hg levels >17%, (Table 3) associated with a decline in arterial oxygen saturation (SaO₂) as measured by pulse oximetry (88% and 89% respectively). In one of these patients the hypoxemia was also confirmed by arterial blood gases (pO₂=145, SaO₂=83%). This toxicity was transient in both patients, occurring after the initial 3-AP infusion and resolving completely within several hours after completion of the infusion. Both patients were retreated at dose level 2 (75 mg/m² of 3-AP) and received three doses of 3-AP at this dose level and had recurrence of transient reversible methemoglobinemia.

Prior to this occurrence, four patients had been treated at the preceding dose level (75 mg/m²), and had tolerated the therapy without dyspnea or documented hypotension. However, these patients had not had routine measurement of methemoglobin levels. Therefore, 11 additional patients were subsequently treated at dose level 2 with serial analysis of met-Hg levels. We observed transient methemoglobinemia in all 11 additional patients on whom met-Hg levels were obtained at dose level 2. The median peak met-Hg level was 15.2% (range 10.1–17.9%). Met-Hg levels typically peaked within 2 h after completion of the 3-AP infusion, and were associated with mild to moderate hypoxemia as measured by pulse oximetry (median SaO₂, 91%; range, 86–94%). All patients had pre-treatment oxygen saturations of 95%. Methemoglobin levels declined to trough levels of <5% within 24 h, and patients were therefore able to receive subsequent scheduled infusions without sequelae.

Myelosuppression

As expected, grade 3 or 4 neutropenia and thrombocytopenia occurred in all patients treated with this combination. The kinetics of the recovery of normal hematopoiesis was difficult to assess, since the majority of patients (84%) had grade 3 and 4 cytopenias at baseline and many had persistent disease throughout the course of therapy. However in the four patients who achieved a response or hematologic improvement with this combination, nadir ANC and platelet counts occurred at day 11 (range, 10–17), and recovery to ANC >1×10⁹/l and platelets >100×10⁹/l occurred at a median of 25 (range, 25–32) and 29 (range, 20–32) days, respectively.

Infections

Of the 25 patients enrolled, 6 did not have any evidence of infection while on study. Eleven patients had documented bacteremia, including 4 patients with gram-negative organisms. Four patients had radiographic evidence of fungal pneumonia. Cellulitis, wound infection, sinus infection and urinary tract infection were noted in one patient each. *Clostridium difficile* colitis occurred in two patients.

Clinical activity

All patients enrolled were considered evaluable for response assessment (Table 4). Objective responses were observed in three patients with AML (two complete and one partial remission). All three were in their first relapse, had normal bone marrow cytogenetics and previous HiDAC exposure. All three responders proceeded on to a stem cell transplant.

In addition, an elderly patient with primary refractory AML and poor risk cytogenetics had hematologic improvement.

Discussion

This Phase I dose-escalation trial demonstrates that the combination of 3-AP and cytarabine is feasible in patients with advanced myeloid leukemias. The recommended Phase II dose is 75 mg/m² of 3-AP administered for 4 consecutive days given in combination with cytarabine administered at a dose of 1,000 mg/m²/day over 5 consecutive days. Methemoglobinemia is the dose limiting toxicity of this combination, when given at the dose range and schedule employed in this trial. The spectrum of toxicities (both hematologic and nonhematologic) observed with the combination of 3-AP and cytarabine was otherwise not significantly different than that expected for cytarabine alone given at this dose and schedule [15]. There was no evidence that 3-AP significantly altered the toxicity profile of cytarabine.

Methemoglobinemia is a recognized complication of 3-AP administration, and has been linked to the generation of reactive oxygen species [16, 17]. The development of clinically significant met-Hg with 3-AP use was initially reported in patients with G6PD deficiency [13]. G6PD is required for the generation of NADPH for the NADPH-dependent methemoglobin reductase which converts met-hemoglobin back to oxyhemoglobin. Therefore, patients with G6PD deficiency are susceptible to 3-AP induced methemoglobinemia. It is clear, however, from our experience and that of others [18, 19], that 3-AP can induce methemoglobinemia even in the absence of G6PD deficiency. In a recent report in which 3-AP was combined with gemcitabine, 5 of 18 patients treated at the 105 mg/m² dose level of 3-AP (given as a 2 to 4 h infusion), had documented hypoxia in association with the 3-AP infusion [18]. Three of these patients had met-Hg levels checked and had modest elevation in levels (10.8% to 12%) [18]. We observed dose-limiting methemoglobinemia at the 100 mg/m² dose level of 3-AP, and transient reversible methemoglobinemia in the majority of patients studied at the 75 mg/m² dose level.

Methemoglobinemia has not been reported as a common toxicity in other studies investigating 3-AP in advanced leukemia [7, 8, 20]. In a prior phase I trial of 3-AP and cytarabine, DLTs included grade 4 sensorimotor peripheral neuropathy, grade 4 mucositis and grade 3 hyperbilirubinemia occurring at the 800 mg/m² dose level of cytarabine. The dose and schedule of both drugs in that trial was different from that utilized in our trial. In that study, 3-AP was administered at a fixed dose of 105 mg/m², given as a 6 h infusion, daily for 5 days, while cytarabine was administered in a dose range of 100 to 800 mg/m², given over 18 h, daily for 5 days [20]. It is possible that this difference in dose and schedule may have contributed to the differences in the spectrum of toxicities observed. In another recent phase I study of 3-AP in leukemia, only 1 of 25 patients had documented methemoglobinemia [8]. None of these trials [7, 8, 20] incorporated routine monitoring of met-Hg levels; therefore it is possible that the incidence of this toxicity may have been underestimated.

We did observe several responses in patients treated with this combination. Prior experience with 3-AP [7, 8, 11] has shown that at the dose levels explored in this trial, peak serum concentrations in vivo should exceed levels that are required for antitumor activity and synergism as previously demonstrated in cell lines [6, 9]. However, although the majority of patients enrolled on this study had primary refractory or multiply relapsed leukemia, the patients who had a CR or PR were in their first relapse and had had an initial remission duration that exceeded 12 months. Given the relatively long initial remission duration, this group of patients would likely be considered relatively sensitive to HiDAC. Therefore, although all of the responders had been exposed to HiDAC in the setting of consolidation therapy, it is plausible that the responses observed in this trial could have occurred in the setting of HiDAC as a single agent. A randomized study would be required to answer the question of whether the response rate with this combination is significantly higher than with HiDAC alone. The decision to pursue such a study, however, needs to be weighed against the additional risk of methemoglobinemia and hypoxemia observed with the combination. In addition, it would be useful to formally evaluate the effect of 3-AP on intracellular ara-CTP levels, potentially as part of a randomized phase II study. In the future, we would recommend that patients who are receiving 3-AP should have careful monitoring of met-Hg levels, oxygen saturation and clinical status to define better the clinical sequelae of this toxicity.

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References

1. Estey E, Plunkett W, Dixon D, Keating M, McCredie K, Freireich EJ. Variables predicting response to high dose cytosine arabinoside therapy in patients with refractory acute leukemia. *Leukemia*. 1987; 1:580–583. [PubMed: 3669771]
2. Plunkett W, Liliemark JO, Adams TM, Nowak B, Estey E, Kantarjian H, Keating MJ. Saturation of 1-beta-D-arabino-furanosylcytosine 5'-triphosphate accumulation in leukemia cells during high-dose 1-beta-D-arabinofuranosylcytosine therapy. *Cancer Res*. 1987; 47:3005–3011. [PubMed: 3471322]
3. Cory JG, Cory AH, Rappa G, Lorico A, Liu MC, Lin TS, Sartorelli AC. Inhibitors of ribonucleotide reductase. Comparative effects of amino- and hydroxy-substituted pyridine-2-carboxaldehyde thiosemicarbazones. *Biochem Pharmacol*. 1994; 48:335–344. [PubMed: 8053929]
4. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, Grindey GB, Plunkett W. Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. *Mol Pharmacol*. 1990; 38:567–572. [PubMed: 2233693]
5. Wright JA, Chan AK, Choy BK, Hurta RA, McClarty GA, Tagger AY. Regulation and drug resistance mechanisms of mammalian ribonucleotide reductase, and the significance to DNA synthesis. *Biochem Cell Biol*. 1990; 68:1364–1371. [PubMed: 2085432]
6. Finch RA, Liu M, Grill SP, Rose WC, Loomis R, Vasquez KM, Cheng Y, Sartorelli AC. Triapine (3-aminopyridine-2-carboxaldehyde-thiosemicarbazone): a potent inhibitor of ribonucleotide reductase activity with broad spectrum antitumor activity. *Biochem Pharmacol*. 2000; 59:983–991. [PubMed: 10692563]
7. Giles FJ, Fracasso PM, Kantarjian HM, Cortes JE, Brown RA, Verstovsek S, Alvarado Y, Thomas DA, Faderl S, Garcia-Manero G, Wright LP, Samson T, Cahill A, Lambert P, Plunkett W, Sznol M, DiPersio JF, Gandhi V. Phase I and pharmacodynamic study of Triapine, a novel ribonucleotide

- reductase inhibitor, in patients with advanced leukemia. *Leuk Res.* 2003; 27:1077–1083. [PubMed: 12921943]
8. Gojo I, Tidwell ML, Greer J, Takebe N, Seiter K, Pochron MF, Johnson B, Sznol M, Karp JE. Phase I and pharmacokinetic study of Triapine, a potent ribonucleotide reductase inhibitor, in adults with advanced hematologic malignancies. *Leuk Res.* 2007; 31:1165–1173. [PubMed: 17324462]
 9. Sigmond J, Kamphuis JA, Laan AC, Hoebe EK, Bergman AM, Peters GJ. The synergistic interaction of gemcitabine and cytosine arabinoside with the ribonucleotide reductase inhibitor triapine is schedule dependent. *Biochem Pharmacol.* 2007; 73:1548–1557. [PubMed: 17324380]
 10. Feun L, Modiano M, Lee K, Mao J, Marini A, Savaraj N, Plezia P, Almassian B, Colacino E, Fischer J, MacDonald S. Phase I and pharmacokinetic study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) using a single intravenous dose schedule. *Cancer Chemother Pharmacol.* 2002; 50:223–229. [PubMed: 12203104]
 11. Murren J, Modiano M, Clairmont C, Lambert P, Savaraj N, Doyle T, Sznol M. Phase I and pharmacokinetic study of triapine, a potent ribonucleotide reductase inhibitor, administered daily for five days in patients with advanced solid tumors. *Clin Cancer Res.* 2003; 9:4092–4100. [PubMed: 14519631]
 12. Wadler S, Makower D, Clairmont C, Lambert P, Fehn K, Sznol M. Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-hour intravenous continuous infusion. *J Clin Oncol.* 2004; 22:1553–1563. [PubMed: 15117978]
 13. Dalal BI, Kollmannsberger C. Drug-induced haemolysis and methaemoglobinaemia in glucose 6-phosphate dehydrogenase deficiency. *Br J Haematol.* 2005; 129:291. [PubMed: 15842651]
 14. Cheson BD, Cassileth PA, Head DR, Schiffer CA, Bennett JM, Bloomfield CD, Brunning R, Gale RP, Grever MR, Keating MJ, et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *J Clin Oncol.* 1990; 8:813–819. [PubMed: 2185339]
 15. Bolwell BJ, Cassileth PA, Gale RP. High dose cytarabine: a review. *Leukemia.* 1988; 2:253–260. [PubMed: 3287015]
 16. Kalinowski DS, Richardson DR. Future of toxicology—iron chelators and differing modes of action and toxicity: the changing face of iron chelation therapy. *Chem Res Toxicol.* 2007; 20:715–720. [PubMed: 17402750]
 17. Shao J, Zhou B, Di Bilio AJ, Zhu L, Wang T, Qi C, Shih J, Yen Y. A ferrous-triapine complex mediates formation of reactive oxygen species that inactivate human ribonucleotide reductase. *Mol Cancer Ther.* 2006; 5:586–592. [PubMed: 16546972]
 18. Yen Y, Margolin K, Doroshow J, Fishman M, Johnson B, Clairmont C, Sullivan D, Sznol M. A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer. *Cancer Chemother Pharmacol.* 2004; 54:331–342. [PubMed: 15148626]
 19. Knox JJ, Hotte SJ, Kollmannsberger C, Winquist E, Fisher B, Eisenhauer E. Phase II study of Triapine(R) in patients with metastatic renal cell carcinoma: a trial of the National Cancer Institute of Canada Clinical Trials Group (NCIC IND.161). *Invest New Drugs.* 2007; 25:471–477. [PubMed: 17393073]
 20. Yee KW, Cortes J, Ferrajoli A, Garcia-Manero G, Verstovsek S, Wierda W, Thomas D, Faderl S, King I, O'Brien SM, Jeha S, Andreeff M, Cahill A, Sznol M, Giles FJ. Triapine and cytarabine is an active combination in patients with acute leukemia or myelodysplastic syndrome. *Leuk Res.* 2006; 30:813–822. [PubMed: 16478631]

Table 1

Baseline patient characteristics

Characteristic	No. of patients	Percentage
Age (years)		
<60	9	36
60	16	64
Sex		
Male	13	52
Female	12	48
Performance status		
0	14	56
1	9	36
2	2	8
Diagnosis		
AML		
De novo	16	64
Therapy related/AHD	8	32
CML-AP	1	4
Bone marrow cytogenetics		
t(8;21)	2	8
Normal karyotype	5	20
11q23, -5, -7, complex	16	64
t(9;22)/other	2	8
Stage of disease		
Primary refractory	10	40
First relapse	5	20
Beyond first relapse	10	40
Prior therapy		
Prior cytarabine	25	100
Prior HiDAC	20	80
Allogeneic stem cell transplant	5	25
Autologous stem cell transplant	1	4
Duration of first CR		
12 months	5	33
<12 months	10	67

AHD: antecedent hematologic disorder, CML-AP: chronic myeloid leukemia in the accelerated phase, HiDAC: high dose cytarabine

Table 2

Incidence of commonly encountered toxicities

3-AP dose cohorts: no. of patients (%)				
	50 mg/m², N=7	75 mg/m², N=15	100 mg/m², N=3	All patients, N=25
Toxicity				
Nausea/vomiting				
Grade 1/2	7	8	3	18 (72)
Grade 3/4	0	0	0	0
Fever				
Grade 1/2	5	8	0	13 (52)
Grade 3/4	0	0	0	0
Diarrhea				
Grade 1/2	4	6	2	12(48)
Grade 3/4	0	0	0	0
Rash				
Grade 1/2	2	4	1	7 (28)
Grade 3/4	2	0	0	2 (8)
Mucositis				
Grade 1/2	3	5	1	9 (36)
Grade 3/4	0	0	0	0
LFTs				
Grade 1/2	0	0	0	0
Grade 3/4	1	1	1	3 (12)
Cerebellar				
Grade 1/2	0	0	0	0
Grade 3/4	0	2 ^a	0	2 (8)

^aDenotes dose-limiting cerebellar toxicity occurring in two patients

Table 3

3-AP dose and methemoglobinemia

Pt #	3-AP dose (mg/m ²)	Baseline SaO ₂ (%)	Post 3-AP SaO ₂ (%)	Peak Met-Hg (%)	Trough Met-Hg (%)
12	100	94	88	17.6	0.8
12	75	96	90	13	1.1
13	100	96	92	ND	ND
14	100	96	89	17.8	1.4
14	75	98	92	14.1	1.8
15	75	96	88	15.7	1.5
16	75	96	90	13.5	1.6
17	75	98	94	15.7	1.4
18	75	97	89	10.5	1.7
19	75	97	90	10.3	1.4
20	75	95	92	15.2	1.7
21	75	99	91	15.4	0.9
22 ^a	75	98	91	10.1	1.5
23	75	96	92	17.9	3.2
24	75	96	86	12.9	1.0
25 ^a	75	95	88	15.3	1.3

ND: not done; Baseline O₂ sat-pulse oximeter reading prior to initial 3-AP dose, *Peak met-Hg*: met-Hg recorded within 2 h of completion of initial 3-AP infusion, *Post 3-AP SaO₂*: pulse oximeter reading that coincides with peak met-Hg level, *Trough met-Hg*: met-Hg level 24 h post initial 3-AP infusion

^aDeveloped symptoms of ataxia, agitation, tremors, dysmetria

Table 4

Responses by dose level

Dose level	Triapine (mg/m ²)	Ara-C (mg/m ²)	No. of patients	Response
1	50	1,000	7	1 CR, 1 HI
2	75	1,000	15	1 CR, 1 PR
3	100	1,000	3	0

CR: complete remission, *PR*: partial remission, *HI*: hematologic improvement