Published in final edited form as:

Leuk Res. 2008 January ; 32(1): 71–77. doi:10.1016/j.leukres.2007.05.003.

A PHASE I STUDY OF THE NOVEL RIBONUCLEOTIDE REDUCTASE INHIBITOR 3-AMINOPYRIDINE-2-CARBOXALDEHYDE THIOSEMICARBAZONE (3-AP, TRIAPINE®) IN COMBINATION WITH THE NUCLEOSIDE ANALOG FLUDARABINE FOR PATIENTS WITH REFRACTORY ACUTE LEUKEMIAS AND AGGRESSIVE MYELOPROLIFERATIVE DISORDERS

Judith E. Karp¹, Francis J. Giles², Ivana Gojo³, Lawrence Morris⁴, Jacqueline Greer¹, Bonny Johnson¹, Mya Thein⁵, Mario Sznol^{1,6}, and Jennifer Low⁷

¹ Leukemia Program, Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

² Department of Leukemia, MD Anderson Cancer Center, Houston, Texas

³ University of Maryland Greenebaum Cancer Center, Baltimore, MD

⁴ Blood and Marrow Transplant Group of Georgia, Atlanta, GA

⁵ Vion Pharmaceuticals, New Haven, CT

⁶ Vion Pharmaceuticals, New Haven, CT

⁷ Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD

Abstract

Triapine® is a potent ribonucleotide reductase (RR) inhibitor that depletes intracellular deoxyribonucleotide pools, especially dATP. We designed a Phase I trial of Triapine followed by the adenosine analog fludarabine in adults with refractory acute leukemias and aggressive myeloproliferative disorders (MPD). Two schedules were examined: **A.** Triapine 105mg/m²/day over 4 hours followed by fludarabine daily × 5 (24 patients, fludarabine 15–30 mg/m²/dose); **B.**

To whom correspondence should be addressed: Judith E. Karp, MD, Sidney Kimmel Cancer Center at Johns Hopkins, 1650 Orleans Street, CRB Room 289, Baltimore, Maryland 21231-1000, Phone: 410-502-7726, Fax: 410-614-1005, e-mail: jkarp2@jhmi.edu.
Current affiliation: Yale University School of Medicine, New Haven, CT
Current affiliation: Genentech, South San Francisco, CA

Contributions: J.E. Karp contributed to concept and design; performed research; collected, interpreted and analyzed data; wrote the paper; provided intellectual content and provided study material/patients. F.J. Giles, I. Gojo, and L.E. Morris performed research; collected, interpreted and analyzed data; provided intellectual content; and provided study material/patients. J. Greer and M. Thein collected and/or assembled data. M. Sznol contributed to concept and design and provided intellectual content. J. Low contributed to data analysis and provided intellectual content.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Triapine 200mg/m² over 24 hours followed by 5 days of fludarabine 30 mg/m²/day (9 patients). Complete and partial responses (CR,PR) occurred in Schedule A (5/24, 21%), with CR occurring at the 2 highest fludarabine doses (2/12, 17%). In contrast, no CR or PR occurred in Schedule B. Four of the 5 responses occurred in patients with underlying MPD (4/14, 29%). Drug-related toxicities included fever and metabolic acidosis. Triapine 105 mg/m² followed by fludarabine 30mg/m² daily $\times$ 5 is active in refractory myeloid malignancies and warrants continuing study for patients with aggressive MPD.

Keywords

refractory acute leukemia; myeloproliferative disorders; ribonucleotide reductase; triapine; fludarabine

INTRODUCTION

Refractory and relapsed acute and chronic leukemias and high-risk myelodysplastic syndromes (MDS) are characterized by dysregulation of hematopoietic cell proliferation, differentiation and the balance between cell survival and death pathways, with the balance shifter toward survival of the malignant clone. This interplay of multiple cellular survival mechanisms, in turn, confers drug resistance. As such, one goal of any new therapeutic approach must be the ability to overcome drug resistance and promote drug-induced cell death.

Ribonucleotide reductase (RR) converts ribonucleotides into deoxyribonucleotides and is therefore a pivotal enzyme in the processes of DNA synthesis, repair of DNA damage, and cellular growth and survival. Inhibition of RR depletes the intracellular pools of deoxyribonucleotides and their triphosphorylated forms (dNTPs). The depletion enhances the intracellular accumulation of nucleoside analogs and the incorporation of triphosphorylated nucleoside analogs into DNA, thereby heightening the cytotoxic effects of those agents.

RR is composed of 2 subunits: a regulatory (M1) subunit which is inhibited by nucleoside analogues such as gemcitabine, fludarabine, and clofarabine; and a catalytic (M2) subunit which requires iron and generation of a tyrosyl free radical for activity.¹ The M2 subunit is inhibited by hydroxyurea (HU)² and 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine®).^{3–5} HU has been used clinically for three decades, mainly in the settings of acute and chronic myelogenous leukemias including chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML) and other myeloproliferative disorders (MPDs).^{6–10}

Triapine is a potent inhibitor of the RR M2 subunit (100–1000-fold more potent than HU).^{3–5} The heightened ability of Triapine to inhibit the M2 subunit relates in part to Triapine chelation and sequestration of iron as well as to quenching the tyrosyl free radical, thereby preventing regeneration of the radical and exerting a less reversible inhibitory effect.^{11,12} Two Phase I studies of Triapine in adults with relapsed and refractory hematologic malignancies have demonstrated that the drug decreases peripheral blood blasts by $\geq$ 50% in roughly 80% of patients, with a limited spectrum of non-hematologic toxicities.^{13,14} While no true complete or partial remissions (CR, PR) were observed in either study, Triapine induced clinical activity with hematologic improvement, reduction of splenomegaly, and/or clearance of marrow blasts in 20–25% of patients with highly refractory AML, CML-BC and MPDs.¹⁴

In a number of experimental systems, inhibition of the M1 or M2 subunit of RR can enhance the anti-tumor activity of subsequently administered nucleoside analogues.^{15–22} The basis for this activity relates at least in part to the dNTP depletion that results from RR inhibition.²³ In

this regard, pharmacodynamic studies of circulating leukemic blasts obtained longitudinally throughout the clinical trial conducted by Giles, et al¹³ demonstrated that Triapine administration was associated with decreases in intracellular circulating leukemic blast dNTP pools (especially dATP but also dGTP and dCTP) with resultant short-lived inhibition of DNA synthesis. These data were used as a springboard for development of a “second generation” Phase I trial of Triapine 105 mg/m² followed immediately by escalating doses of an 18-hr ara-C infusion daily $\times$ 5 days in patients with refractory or relapsed, ara-C-resistant AML, and high-risk myelodysplasia (MDS).²⁴ This sequential combination yielded CRs in 4/31 (13%) patients, with 2 CRs exceeding 1 year.

Fludarabine is an analog of adenosine that is resistant to the deaminating effects of adenosine deaminase and has demonstrated remarkable activity in lymphoid malignancies.²⁵ Like the prototype nucleoside analog ara-C, fludarabine is triphosphorylated and is incorporated into DNA with termination of chain elongation and resultant DNA strand breakage.²⁶ Moreover, fludarabine inhibits several enzymes critical to DNA synthesis and repair, including DNA polymerases and the M1 subunit of RR.^{26,27} While fludarabine exhibits potent single agent activity against chronic lymphoid malignancies, its activity as a single agent against myeloid leukemias including AML is seen only at very high doses (≥ 100 mg/m² daily $\times$ 5) and is limited by delayed onset of severe, progressive demyelination.^{25,28,29} Nonetheless, fludarabine can interact synergistically with multiple cytotoxic agents including alkylators, ara-C, etoposide and hydroxyurea.³⁰ At least part of this interaction relates to fludarabine inhibiting RR and thereby decreasing dNTP levels.^{17,26,30} Clinical trials in refractory AML combining fludarabine and ara-C (with or without anthracycline) in a sequential fashion have demonstrated that the initial administration of fludarabine augments the rate of ara-CTP synthesis and accumulation, with resultant potentiation of ara-C cytotoxicity *ex vivo*^{17,27,31} and positive clinical results in adults and children with poor-risk leukemias.^{32,33}

On the basis of the data delineated above, we designed a Phase I clinical trial to test the combination of Triapine followed by escalating doses of fludarabine in adults with relapsed and refractory acute leukemias or transformed MPDs including CMML. Fludarabine was of particular interest because of its complementary and mechanistically non-cross resistant inhibition of RR and because of the apparent selective effect of Triapine on intracellular dATP levels.

PATIENTS AND METHODS

Patient Eligibility and Selection

Between January 2004 and July 2005, 33 adults (age ≥ 18 years) were entered on study at the Johns Hopkins Sidney Kimmel Cancer Center (JH-SKCCC, 20 patients), MD Anderson (5 patients), Bone Marrow Transplant Group of Georgia (BMT-GA, 5 patients), and University of Maryland Greenebaum Cancer Center (UMGCC, 3 patients). Patients were required to have confirmed diagnoses of relapsed and/or refractory AML including chronic myelomonocytic leukemia (CMML)/AML and MPD/AML, acute lymphoid leukemia (ALL), high-risk MDS (IPSS score >1.5 based on adverse cytogenetics, $>10\%$ marrow blasts, and cytopenias in at least 2 lineages), or CML in accelerated phase (AP) or blast crisis (BC) of either myeloid or lymphoid origin that was resistant to imatinib.

Other eligibility criteria included an ECOG performance status of ≤ 2 , serum creatinine within institutional normal limits or a calculated creatinine clearance ≥ 60 ml/min using a modified Cockcroft-Gault calculation based on actual body weight, hepatic transaminases $\leq 2.5 \times$ normal, serum total bilirubin ≤ 2.0 mg/dl, negative pregnancy test within 48 hrs prior to study, willingness to practice contraception, no more than 4 prior induction regimens (3 cytotoxic chemotherapy regimens), ineligible for established curative regimens including stem cell

transplantation (SCT), and able to provide informed consent. Patients who were unresponsive to or had relapsed after allogeneic or autologous SCT were eligible. The interval from prior treatment was at least 3 weeks for myelosuppressive cytotoxic agents (6 weeks for mitomycin or nitroureas), 2 weeks for radiotherapy, and 1 week for either non-myelosuppressive treatments or treatment with hematopoietic growth factors. HU and/or imatinib were permitted for control of blast counts prior to Triapine-fludarabine therapy, but had to be discontinued ≥ 72 hours before initiation of Triapine-fludarabine.

Triapine-Fludarabine Treatment Schema

Two schedules of Triapine-fludarabine were examined. In **Schedule A**, 24 patients (16 refractory AML including 2 MPD/AML, 3 CMML/AML, 2 refractory ALL, and 3 CML-AP/BC) received Triapine 105mg/m² over 4 hours followed immediately by fludarabine given as a 30 min infusion beginning within 1 hr of completion of Triapine infusion daily for 5 consecutive days. Fludarabine dose was escalated in successive cohorts of 3–6 patients as follows: 15, 20, 25, and 30 mg/m²/day. In **Schedule B**, 9 patients received Triapine 200mg/m² over 24 hours followed by fludarabine 30mg/m²/day for 5 days. Of these 9, 6 had refractory AML including 3 CMML/AML, and 1 each had ALL, MDS and CML-BC. Schedule B was designed to ameliorate any triapine-related infusional toxicities and concomitantly decrease the duration of any hospitalization or patient observation time over the treatment period. For both schedules, patients could receive a second or subsequent cycle no less than 3 weeks from initiation of the previous course if CR, partial response (PR), hematologic improvement (HI) or disease stabilization was observed, if the previous cycle was not associated with drug-related grade ≥ 3 neurologic toxicity or $\geq$ grade 2 autoimmune hemolytic anemia, and drug-related non-hematologic toxicity had resolved to baseline or $\leq$ grade 1.

Supportive Care

To prevent the occurrence and complications of tumor lysis-induced hyperuricemia, all patients without known allergy received Allopurinol 300 mg qd orally 24 hours prior to beginning Triapine and continuing through the period of maximal tumor lysis, the 5 days of chemotherapy.

Management of Triapine Infusion-Related Toxicities—Prior to the infusion, all patients received Tylenol 650 mg, repeated every 4 hours as needed for fever. Acute reactions to Triapine, included transient hypoxia (with or without dyspnea or cough) and hypotension, related at least in part to a transient and self-limited increase in methemoglobin. Isolated hypoxia without dyspnea was managed with supplemental oxygen. Triapine infusion was discontinued for patients developing moderate or severe dyspnea at rest or hypotension (systolic blood pressure < 85 mm Hg). If the event resolved within 6 hours, Triapine was restarted at a slower rate (decreased by 25% from initial rate), fludarabine was administered, and subsequent Triapine doses were reduced by 20%. Two Triapine reductions were allowed, to 80 mg/m² and 60 mg/m².

Antibiotic Prophylaxis—All patients received prophylaxis against pneumocystis carinii pneumonia (PCP) according to patient tolerance and institutional practice. Patients received prophylaxis directed against gram-negative gastrointestinal (GI) infections, candidiasis, and/or herpes simplex virus at the discretion of the principal investigator.

Response and Toxicity Evaluations

To assess response to therapy, bone marrow aspiration and biopsy were performed prior to treatment, on day 14, and at the time of hematologic recovery or when leukemia regrowth was suspected. Hematologic recovery was defined as absolute neutrophil count (ANC) ≥ 500 /

mm³ and a transfusion-independent platelet count $\geq 50,000/\text{mm}^3$. CR required a normal bone marrow aspirate with absence of identifiable leukemia, absence of peripheral blood blasts, ANC $\geq 1000/\text{mm}^3$, platelet count $\geq 100,000/\text{mm}^3$ and clearance of extramedullary disease including splenomegaly. Clearance of cytogenetic abnormalities was not required for CR, but was noted and described separately. PR was defined as trilineage hematopoiesis in the marrow with normalization of peripheral blood counts but with 5–25% blasts in the marrow, $\geq 50\%$ decrease in marrow blasts, or persistent splenomegaly. Response criteria for acute leukemias are consistent with those published by Cheson, et al.³⁶ Response criteria for MDS are consistent with those published by Cheson, et al.³⁷ Response criteria for MPDs are consistent with those published by Giles, et al.³⁸

The NCI Common Toxicity Criteria, version 3.0, was the basis upon which all adverse events were described and graded, based on the treating physician's assessment. DLT was based on toxicities incurred during the first treatment cycle.

RESULTS

Patient Characteristics

Characteristics of the 33 patients treated with Triapine-fludarabine are shown in Table 1. Ages ranged from 23–79, with median 60 years. ECOG performance status was 0 in 9 patients (28%), 1 in 20 patients (60%) and 2 in 4 patients (12%). The majority of patients (23/33, 70%) had refractory AML including 2 MPD/AML, 6 CMML/AML and 3 therapy-related AML. In addition, there were 3 (9%) patients with high-risk, previously treated MDS/CMML, 4 (12%) with aggressive CML, and 3 (9%) with refractory ALL, with similar disease distributions between Schedule A and Schedule B. Adverse cytogenetics were detected in 82%, and all patients had received prior therapy, including HU in 12 (36%), ara-C in 18 (55%), imatinib in 5 (15%), and SCT in 5 (15%). Median pretreatment WBC was $19,600/\text{mm}^3$ (range 300 – 112,000) for Schedule A and $12,100/\text{mm}^3$ (range 500 – 71,600) for Schedule B.

Toxicity

As detailed in Table 2, Triapine-related fever was relatively common, occurring in roughly 1/3 of patients receiving either Schedule A or B, but methemoglobinemia occurred in only 4 (12%) of patients, with modest ($\leq$ grade 2) severity and in association with brisk cytoreduction (tumor lysis) in 2 patients. Similarly, infusion-related bone pain occurred in 4 (12%) patients, commonly associated with fever and tumor lysis. Triapine-fludarabine resulted in a $\geq 50\%$ decrease in WBC by median day 3 (range 1–7) in 67% of patients on both schedules, and was accompanied by transient hyperphosphatemia (median 5.2 mg/dl, range 4.8–9.6) that peaked on median day 2 (range 1–6) and persisted ≤ 48 hrs in the vast majority of patients. Full-blown tumor lysis syndrome with cytokine release, hypoxia and renal failure requiring hemodialysis occurred in only one patient with CML-BC (Schedule A, dose level 2), with eventual full recovery of all organ function.

Development of metabolic acidosis (median serum CO₂ 15, range 10–18) without elevated lactate or specific organ dysfunction occurred in 9 (38%) of patients receiving Schedule A and 2 (22%) of patients receiving Schedule B. After peaking on median day 3 (range 2–8), the acidosis resolved without intervention. Mucosal toxicities as manifested by oral mucositis and diarrhea were $\leq$ grade 2 in intensity. One patient experienced a grade 3 decrease in LVEF accompanied by atrial fibrillation beginning Day 10 of therapy. Drug-induced myelosuppression occurred on median day 10 (range 5–22) and median day 8 (range 4–15) with median WBC nadirs of 300 (range 20–9500) and 800 (200–9000) for Schedules A and B respectively. Infections were documented in 8 (24%) of the 33 patient cohort, and neutropenic fever occurred in an additional 8 (24%) patients. No patient died of infectious complications.

One patient with refractory CMML/AML and one patient with refractory ALL died during cycle 1 of therapy, both due to progressive disease.

Clinical Outcome

Table 3 summarizes the clinical outcome of patients who received Triapine-fludarabine therapy. Of 24 patients who received Schedule A, 2 patients achieved CR (1 AML with complex cytogenetics, 1 CML-AP) and 3 (1 MPD/AML, 1 AML, 1 CML-BC) achieved PR, for an overall response rate of 21%. Three of the 5 responding patients had received prior therapy with HU. Of the 9 patients receiving Schedule B, however, none achieved CR or PR. Responses occurred across all doses of fludarabine, although both CRs occurred at the 2 highest fludarabine doses (2/12, 17%). Interestingly, 4 (1 CR, 3 PR) of the 5 responses occurred in patients with aggressive MPDs or MPD/AML, yielding a response rate of 29% for the 14 patients with underlying MPD as their primary disease. In contrast, only 1 of 16 with AML that was not preceded by MPD and none of 3 ALL patients enjoyed any type of response. Response durations were short and ranged from 1.5 to 7 months, with the exception of one patient with CML-AP treated on Schedule A dose level 3 who achieved CR, went on to allogeneic SCT and remains in CR now 2 years after beginning Triapine-fludarabine.

DISCUSSION

In this Phase I study, Triapine and fludarabine given in sequential combination had clinical activity in a group of patients with poor-risk, refractory myeloid malignancies, with an emphasis on aggressive or transformed MPDs including CMML and CML-AP/BC. Phase I trials of Triapine as a single agent demonstrated antileukemic activity against refractory myeloid leukemias but without achievement of complete or partial remissions.^{13,14} In contrast, the combination of Triapine with moderate doses of Fludarabine (an agent that has no demonstrable activity as a single agent against myeloid leukemias at doses ≤ 100 mg/m² daily $\times$ 5 days) yielded an overall complete plus partial remission rate of 15% (5/33). The overall response rate and, in particular, the 17% CR rate at the two highest doses of fludarabine tested in this study stand in distinction to the absence of CRs with either single agent Triapine^{13,14} or single agent fludarabine at doses of <100 mg/m² daily $\times$ 5.^{25,28,29} Interestingly, patients treated on Schedule A enjoyed a CR + PR rate of 21% (5/24), while there were no CRs or PRs for the 9 patients treated on Schedule B. This difference in response is consistent with the transient nature of RR inhibition once cells are no longer exposed to therapeutic concentrations of Triapine, as demonstrated *in vitro*. The sequential combination of Triapine plus fludarabine is active in patients who had received HU previously and in whom disease progression had ensued despite HU therapy. Our data are similar to those achieved with the combination of Triapine and ara-C,²⁴ which was tested in patients with refractory acute leukemia and high-risk MDS. Our patient population differs somewhat from the Triapine-ara-C study, in that we included patients with aggressive and transformed MPD and (perhaps as a result) more than one-third of our patients had received prior HU treatment. Overall, our findings support the hypothesis that the addition of fludarabine to Triapine may result in enhanced clinical activity relative to the use of either drug as a single agent, a notion that requires further testing for validation in those subpopulations exhibiting possible preferential sensitivity.

The toxicity profile for both Triapine-fludarabine schedules is manageable with a low occurrence of severe tumor lysis syndrome (1 CML-BC, Schedule A, Dose Level 2), modest degrees of oral and/or lower GI mucositis, and rapidly reversible metabolic and electrolyte disturbances. Overall, in these small subgroups of patients, the toxicity profile encountered in Schedule B appeared to be somewhat attenuated relative to Schedule A with regard to presence and magnitude of treatment-induced tumor lysis and metabolic acidosis, but without major differences in overall serious toxicities (including DLTs and death on study). We discontinued

fludarabine dose escalation at 30 mg/m²/day × 5 because of concern for potential central neurotoxicity.^{25,28,29} Given the overall modest toxicity profile of the Triapine-fludarabine regimen, the absence of toxicities related directly to fludarabine (including neurotoxicity), and the finding that both of our CRs occurred at the two highest dose levels of fludarabine, it may be reasonable to consider cautious additional escalation of the fludarabine dose in subsequent studies.

MPDs as a group may have protracted chronic phases but they eventually transform to an acute leukemia that is ultimately fatal and for which there is no effective therapy at this time. Interestingly, patients with underlying MPD, including CMML and CML, may exhibit preferential sensitivity to this combination, since 3/14 achieved CR or PR and an additional 3/14 exhibited HI and improvement in extramedullary tumor burden as evidenced by reductions in organomegaly and reversal of leukemia-related bone pain. On the basis of these data, Triapine 105 mg/m² over 4 hours followed within 1 hour by fludarabine 30mg/m² over 30 minutes given daily × 5 days every 21 days warrants continuing study, particularly for patients with MPDs that exhibit aggressive behavior and/or have transformed to full-blown acute leukemia.

Acknowledgments

This work was supported in part by National Cancer Institute Cooperative Agreement U01 CA70095 and the Johns Hopkins University School of Medicine General Clinical Research Center Grant M01-RR0052 from the National Center for Research Resources, National Institutes of Health.

References

1. Eklund H, Uhlin U, Farnegardh M, Logan DT, Nordlund P. Structure and function of the radical enzyme ribonucleotide reductase. *Prog Biophys Mol Biol* 2001;77:177–268. [PubMed: 11796141]
2. Wright JA, Chan AK, Choy BK, Hurta RAR, McClarty GA, Tagger AY. Regulation and drug resistance mechanisms of mammalian ribonucleotide reductase, and the significant to DNA synthesis. *Biochem Cell Biol* 1990;68:1364–1371. [PubMed: 2085432]
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. Liu MC, Lin TS, Cory JG, Cory AH, Sartorelli AC. Synthesis and biologic activity of 3- and 5-amino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone. *J Med Chem* 1996;39:2586–2593. [PubMed: 8691457]
5. Moore EC, Booth BA, Sartorelli AC. Inhibition of deoxyribonucleotide synthesis by pyridine carboxaldehyde thiosemicarbazones. *Cancer Res* 1971;31:235–238. [PubMed: 5102115]
6. Cortelazzo S, Finazzi G, Ruggeri M, Vestri O, Galli M, Rodeghiero F, Barbui T. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. *N Engl J Med* 1995;332:1132–1136. [PubMed: 7700286]
7. Hehlmann R, Heimpel H, Hasford J, et al. Randomized comparison of busulfan and hydroxyurea in chronic myelogenous leukemia: prolongation of survival by hydroxyurea. *Blood* 1993;82:398–407. [PubMed: 8329700]
8. Schwartz JH, Canellos GP. Hydroxyurea in the management of the hematologic complications of chronic granulocytic leukemia. *Blood* 1975;46:11–16. [PubMed: 1055610]
9. Spivak JL. Polycythemia vera: myths, mechanisms, and management. *Blood* 2002;100:243–252.
10. Wattel E, Guerci A, Hecquet B, et al. A randomized trial of hydroxyurea versus VP16 in adult chronic myelomonocytic leukemia. *Blood* 1996;88:2480–2487. [PubMed: 8839839]
11. Finch RA, Liu MC, Grill SP, et al. 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]

12. Nyholm S, Mann GJ, Johannson AG, et al. Role of ribonucleotide reductase in inhibition of mammalian cell growth by potent iron chelators. *J Biol Chem* 1993;268:26200–26205. [PubMed: 8253740]
13. Giles FJ, Fracasso PM, Kantarjian HM, et al. 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]
14. Gojo I, Tidwell ML, Greer J, et al. Phase I and pharmacokinetic study of Triapine®, a potent ribonucleotide reductase inhibitor, I adults with advanced hematologic malignancies. *Leuk Res* 2007in press
15. Bhalla K, Swerdlow P, Grant S. Effects of thymidine and hydroxyurea on the metabolism and cytotoxicity of 1-B-D-arabinofuranosylcytosine in highly resistant human leukemic cells. *Blood* 1991;78:2937–2944. [PubMed: 1954383]
16. Colly LP, Richel DJ, et al. Increase in Ara-C sensitivity in Ara-C sensitive and -resistant leukemia by stimulation of the salvage and inhibition of the de novo pathway. *Ann Hematol* 1992;65:26–32. [PubMed: 1643157]
17. Gandhi V, Plunkett W. Modulation of arabinosyl nucleoside metabolism by arabinosyl nucleotides in human leukemic cells. *Cancer Res* 1988;48:329–334. [PubMed: 3335008]
18. Iwasaki H, Huang Peng, Keating MJ, Plunkett W. Differential incorporation of Ara-C, gemcitabine, and fludarabine into replicating and repairing DNA in proliferating leukemia cells. *Blood* 1997;90:270–278. [PubMed: 9207462]
19. Rauscher F III, Cadman E. Biochemical and cytotoxic modulation of L1210 and HL-60 cells by hydroxyurea and effect of 1-b-d-arabinofuranosylcytosine metabolism and cytotoxicity. *Cancer Res* 1983;43:2688–2693. [PubMed: 6189585]
20. Tanaka M, Kimura K, Yoshida S. Mechanism of synergistic cell killing by hydroxyurea and cytosine arabinoside. *Jpn J Cancer Res* 1985;76:7290735.
21. Walsh CT, Craig RW, et al. Increased activation of 1-beta-D-arabinofuranosylcytosine by hydroxyurea in L1210 cells. *Cancer Res* 1980;40:3286–92. [PubMed: 7427943]
22. Zhou B, Mi S, Mo X, Shih J, Tsai J, Hu E, Hsu M, Kay K, Yen Y. Time and sequence dependence of hydroxyurea in combination with gemcitabine in human KB cells. *Anticancer Res* 2002;22:1369–1377. [PubMed: 12168813]
23. Gandhi V, Plunkett W, Kantarjian H, Talpaz M, Robertson LE, O'Brien S. Cellular pharmacodynamics of parenterally infused hydroxyurea during a phase I clinical trial in chronic myelogenous leukemia. *J Clin Oncol* 1998;16:2321–2331. [PubMed: 9667246]
24. Yee WLK, Garcia-Manero G, Verstovsek S, et al. Triapine and cytarabine is an active combination in patients with acute leukemia or myelodysplastic syndrome. *Leuk Res* 2006;30:813–822. [PubMed: 16478631]
25. Chun HG, Leyland-Jones B, Cheson BD. Fludarabine phosphate: a synthetic purine antimetabolite with significant activity against lymphoid malignancies. *J Clin Oncol* 1991;9:175–188. [PubMed: 1702143]
26. Huang P, Plunkett W. Fludarabine- and gemcitabine-induced apoptosis: incorporation of analogs into DNA is a critical event. *Cancer Chemother Pharmacol* 1995;3:181–188. [PubMed: 7781136]
27. Gandhi V, Estey E, Keating MJ, Plunkett W. Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. *J Clin Oncol* 1993;11:116–124. [PubMed: 8418222]
28. Chun HG, Leyland-Jones BR, Caryk SM, Hoth D. Central nervous system toxicity of fludarabine phosphate. *Cancer Treat Rep* 1986;70:1225–1228. [PubMed: 2428492]
29. Warrell RP Jr, Berman E. Phase I and II study of fludarabine phosphate in leukemia: therapeutic efficacy with delayed central nervous system toxicity. *J Clin Oncol* 1986;4:74–79. [PubMed: 2416889]
30. Kano Y, Akutsu M, Tsunoda S, Suzuki K, Ichikawa A, Furukawa Y, Bai L, Kon K. In vitro cytotoxic effects of fludarabine (2-F-ara-A) in combination with commonly used antileukemic agents by isobologram analysis. *Leukemia* 2000;14:3379–388.
31. Estey E, Thall P, Andreeff M, et al. Use of granulocyte colony-stimulating factor before, during and after fludarabine plus cytarabine induction therapy of newly diagnosed acute myelogenous leukemia

- or myelodysplastic syndromes: comparison with fludarabine plus cytarabine without granulocyte colony stimulating factor. *J Clin Oncol* 1994;12:671–678. [PubMed: 7512125]
32. Dinndorf PA, Avramis VI, Wiersma S, et al. Phase I/II study of idarubicin given with continuous infusion fludarabine followed by continuous infusion cytarabine in children with acute leukemia: a report from the Clindren's Cancer Group. *J Clin Oncol* 1997;15:2780–2785. [PubMed: 9256119]
 33. Fleischhack G, Hasan C, Graf N, Mann G, Bode U. IDA-FLAG (idarubicin, fludarabine, cytarabine, G-CSF), an effective remission-induction therapy for poor-prognosis AML of childhood prior to allogeneic or autologous bone marrow transplantation: experiences of a phase II trial. *British J Haem* 1998;102:647–655.
 34. Sherman PA, Fyfe JA. Enzymatic assay for deoxyribonucleoside triphosphates using synthetic oligonucleotides as template primers. *Anal Biochem* 1989;180:222–226. [PubMed: 2554751]
 35. Gao WY, Johns DG, Mitsuya H. Enzymatic quantification of deoxynucleoside triphosphates in human cells exposed to antiretroviral 2',3'-dideoxynucleosides. *Anal Biochem* 1994;222:116–122. [PubMed: 7856836]
 36. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol* 2003;21:4642–4649. [PubMed: 14673054]
 37. Cheson BD, Bennett JM, Kantarjian HM, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood* 2000;96:3671–3674. [PubMed: 11090046]
 38. Giles FJ, Karp JE, Silverman L, et al. Phase II study of SU5416, a small molecule vascular endothelial growth factor (VEGF) tyrosine kinase receptor inhibitor, in patients with refractory myeloproliferative diseases. *Cancer* 2003;97:1920–1928. [PubMed: 12673719]

TABLE 1
CHARACTERISTICS OF 33 PATIENTS UNDERGOING THERAPY WITH TRIAPINE-FLUDARABINE

	Schedule A (n = 24)	Schedule B (n = 9)	Total
Male/Female	9/15	6/3	15/18
Median Age (Range)	56 (23–74)	67 (54–79)	60 (23–79)
<u>Diagnosis/Stage of Disease</u>			
AML	17	6	23
High-Risk CMML	2	1	2
CML	3	1	4
Accelerated Phase	1	0	1
Blast Crisis	2	1	3
ALL	2	1	3
<u>Biologic Features</u>			
Adverse Cytogenetics *	21 (88%)	6/9 (67%)	27 (82%)
Secondary AML	7	4	11
MPD/AML	2	0	2
CMML/AML	3	3	6
Therapy-related	2	1	3
<u>Prior Drug Exposure</u>			
Hydroxyurea			12 (36%)
ara-C			18 (55%)
Gleevec			5 (15%)

* Adverse cytogenetics include -5/5q, -7/7q, +8, +19, +21, t(4;11), t(9;22) including Ph+ ALL and Ph+ duplication, complex.

TABLE 2
NON-HEMATOLOGIC TOXICITIES DURING TRIAPINE-FLUDARABINE

Toxicity	Schedule A (n=24)	Schedule B (n=9)
<u>Triapine Infusion-Related</u>	<u>12 (50%)</u>	<u>7 (78%)</u>
Fever	7 (1 gr 3)	3 (all ≤ gr 2)
Methemoglobinemia	2 (gr 1)	2 (gr 2)
Tumor Lysis Syndrome/Cytokine Release	1 (gr 3, dialysis)	0
Bone Pain	2 (gr 1)	2 (gr 2)
<u>Cardiac</u>	<u>1 (4%)</u>	<u>0 (0%)</u>
Decreased LVEF	1 (gr 3)	0
<u>Renal/Metabolic</u>	<u>14 (58%)</u>	<u>2 (22%)</u>
Metabolic Acidosis	9 (5 gr 1, 1 gr 2, 3 gr 3)	2 (gr 1)
Renal Dysfunction	5 (3 gr 1, 2 gr 3)	0
<u>Mucosal</u>	<u>15 (62.5%)</u>	<u>2 (22%)</u>
Oral Mucositis	6 (all ≤ gr 2)	0
Gastrointestinal	9 (all ≤ gr 2)	2 (1 gr 3)
<u>Infection</u>	<u>10 (42%)</u>	<u>6 (67%)</u>
Documented Infection	4	4
Bacteremia	1	2
Viral Gastroenteritis	1	0
Fungal Pneumonia/Sinusitis	2	2
Febrile Neutropenia (no site)	6	2
<u>Death within 30 days of Study</u>	<u>2 (8%, progressive disease)</u>	<u>0 (0%)</u>

TABLE 3
CLINICAL OUTCOME FOR PATIENTS TREATED WITH TRIAPINE®
FOLLOWED BY FLUDARABINE

Fludarabine Dose Level/No. pts.	CR	PR	Overall Response
<u>Schedule A/24 patients</u>			
<u>1:</u> 15 mg/m ² /6	0	2	2
<u>2:</u> 20 mg/m ² /6	0	1	1
<u>3:</u> 25 mg/m ² /6	1	0	1
<u>4:</u> 30 mg/m ² /6	1	0	1
SUBTOTAL:	2 (8%)	3 (12.5%)	5 (21%)
Schedule B/9 patients	0	0	0
TOTAL:	2 (6%)	2 (6%)	5(15%)