# Phase I and Pharmacokinetic Study of Flavopiridol followed by 1-β-D-Arabinofuranosylcytosine and Mitoxantrone in Relapsed and Refractory Adult Acute Leukemias

Judith E. Karp,1,2 Antonino Passaniti,2 Ivana Gojo,2 Scott Kaufmann,4 Keith Bible,4 Tushar S. Garimella,2 Jacqueline Greer,1,2 Janet Briel,1 B. Douglas Smith,1 Steven D. Gore,1 Michael L. Tidwell,2 Douglas D. Ross,2,3 John J. Wright,5 A. Dimitrios Colevas,6 and Kenneth S. Bauer2

## Abstract

**Purpose:** The serine/threonine kinase inhibitor flavopiridol targets multiple cyclin-dependent kinases, induces checkpoint arrest, and interrupts transcriptional elongation. We designed a phase I clinical trial using a timed sequential therapy approach where flavopiridol was given for the purpose of initial cytoreduction and enhancing cell cycle progression of the remaining leukemia cell cohort followed by cycle-dependent drugs 1-β-D-arabinofuranosylcytosine (ara-C) and mitoxantrone.

**Experimental Design:** Flavopiridol was given by 1-hour infusion daily for 3 days beginning day 1 followed by 2 g/m²/72 h ara-C beginning day 6 and 40 mg/m² mitoxantrone beginning day 9. In vivo correlates included pharmacokinetics, modulation of blast cycle regulators, and serum and marrow supernatant vascular endothelial growth factor levels.

**Results:** Of 34 adults receiving induction therapy, 16 (47%) evinced direct leukemia cytotoxicity with ≥50% drop in peripheral blast counts and tumor lysis in 9 (26%). Four (12%) died during therapy (two fungal infections and two sudden death). Dose-limiting toxicity occurred at 60 mg/m²/d with profound neutropenia >40 days duration, and maximal tolerated dose was 50 mg/m²/d. Overall response rate was 31% in 26 acute myelogenous leukemia and 12.5% in acute lymphoblastic leukemia. Pharmacokinetics showed that a linear two-compartment model with first-order elimination provided the best fit of the observed concentration versus time data. Flavopiridol down-regulated one or more target proteins in marrow blasts in vivo. Vascular endothelial growth factor was detected in sera and marrow supernatant pretreatment, and sera obtained on day 3 inhibited bovine aortic endothelial cell proliferation by a mean of 32% (range, 10–80%).

**Conclusions:** Our data suggest that flavopiridol is cytotoxic to leukemic cells and, when followed by ara-C and mitoxantrone, exerts biological and clinical effects in patients with relapsed and refractory acute leukemias. These findings warrant continuing development of flavopiridol at 50 mg/m²/d × 3 days in combination with cytotoxic and biological agents for acute leukemias.

---

**Authors' Affiliations:**

1Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; 2University of Maryland Greenebaum Cancer Center; and 3Baltimore Veterans Affairs Medical Center, Baltimore, Maryland; 4Mayo Clinic, Rochester, Minnesota; and 5Investigational Drug Branch, Clinical Trials Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland

Received 6/2/05; revised 9/6/05; accepted 9/13/05.

**Grant support:** National Cancer Institute cooperative agreements U01 CA69854 (J.E. Karp and K.S. Bauer) and CA70095 (J.E. Karp), NIH grant CA97129 (K. Bible), and American Cancer Society grant CCE-98842 (K. Bible).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Judith E. Karp, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, CRB Room 289, Baltimore, MD 21231-1000. Phone: 410-503-5399; Fax: 410-614-1005; E-mail: jkarp2@jhmi.edu. © 2005 American Association for Cancer Research. doi:10.1158/1078-0432.CCR-05-1201

---

Flavopiridol (L86-8275), a synthetic flavone derivative that was initially isolated from the stem bark of the Indian tree *Dysoxylum binectariferum* (1, 2), is a potent growth inhibitor of diverse human tumor cell lines and induces apoptosis in hematopoietic cell lines derived from acute myelogenous leukemia (AML), B-cell and T-cell lymphomas, and multiple myeloma (3–5). Flavopiridol-induced apoptosis results at least in part from inhibition of multiple serine/threonine cyclin-dependent kinases (2). Whereas inhibition of cyclin-dependent kinases 2 and 4 contributes to cell cycle arrest in G1 and G2 (6–8), flavopiridol-triggered inactivation of the cyclin-dependent kinase 9/cyclin T complex (also known as PTEF-b) inhibits the activating phosphorylation of RNA polymerase II and diminishes mRNA synthesis (9, 10). Consequently, flavopiridol-treated cells are unable to synthesize transcripts encoding polypeptides, such as cyclin D1 (11), which is expressed in a cell cycle-dependent manner.

Several cellular changes induced by flavopiridol could contribute to its cytotoxicity. Mcl-1 is a short-lived antiapoptotic Bcl-2 family member (12) that is highly expressed in hematopoietic stem cells and essential to promoting stem cell survival (13). Mcl-1 is up-regulated in ~50% of relapsed and refractory leukemias (14) and rapidly down-regulated by flavopiridol (5, 10, 15, 16). Studies in myeloma cells showed that forced Mcl-1 overexpression protects cells from flavopiridol-induced...
apoptosis (5), suggesting a critical role for Mcl-1 down-regulation in flavopiridol-induced cell death. More recent observations that flavopiridol-induced apoptosis involves caspase-8-independent release of cytochrome c from mitochondria (4) and is enhanced by inhibition of phosphatidylinositol-3 kinase in U937 and primary AML cells (6) are consistent with this model. Additional studies have shown that flavopiridol can decrease production of vascular endothelial growth factor (VEGF; ref. 17), a growth and survival factor for diverse tumor types, including certain acute leukemias (18).

1-[α-D-Arabinofuranosyl]cytosine (ara-C) and mitoxantrone exhibit significant clinical activity against AML and acute lymphoblastic leukemia (ALL; refs. 19–21). Both drugs induce double-strand breaks in DNA, ara-C by inhibiting DNA replication and repair and mitoxantrone by poisoning topoisomerase II. These insults not only lead to cell cycle arrest in S phase during ara-C exposure (22) and late S/G2 after mitoxantrone (23) but also subsequently trigger apoptosis in susceptible leukemic cells (22–25). These drugs have been combined in adults with AML and ALL (19–21) to induce complete remission (CR) in both older patients and patients with relapsed and refractory disease.

Previous studies from our laboratories have examined the effect of combining flavopiridol with a variety of antineoplastic agents (26, 27). Because flavopiridol induces cell cycle arrest, it antagonizes the effects of S-phase-dependent agents, such as ara-C and topotecan, when administered concomitantly (26). In contrast, when flavopiridol is administered first and then withdrawn in vitro, the surviving cells reenter the cell cycle and are sensitized to S-phase poisons (26). These observations, coupled with the ability of flavopiridol to kill noncycling cells (3), suggested that flavopiridol might be particularly effective when administered first and then followed several days later by ara-C. Consistent with these results, we showed recently that therapeutically achievable flavopiridol concentrations induced apoptotic cell death in bone marrow leukemic blasts in vitro and that flavopiridol-treated blast cultures exhibited increased sensitivity to the subsequent proapoptotic effects of ara-C relative to either agent alone (27).

This approach to maximizing the cytotoxic effects of flavopiridol-containing combinations is reminiscent of timed sequential therapy (TST), a therapeutic strategy that attempts to exploit drug-induced changes in residual leukemia cell growth kinetics to increase the sensitivity of surviving leukemic cells to cycle-dependent antileukemic agents (28, 29). TST has been shown to induce prolonged disease-free survival in certain groups of adults and children with AML (30–32), although there remains a significant proportion for whom TST alone is not curative.

Accordingly, we designed a phase I clinical trial in which flavopiridol, given for the dual purpose of initial cytodestruction and enhancing the cell cycle progression of the remaining leukemic cell cohort, was followed by ara-C and mitoxantrone. The purposes of this trial were to define the dose-limiting toxicities (DLT) of the combination, establish the maximum tolerated dose, evaluate in a preliminary manner the antileukemic effects of flavopiridol and the combination, and determine whether the levels of flavopiridol achieved in vivo were sufficient to inhibit RNA polymerase II phosphorylation and down-regulate cyclin D1, Mcl-1, and VEGF.

**Patients, Materials, and Methods**

**Patient eligibility and selection**

Adults ages ≥18 years with pathologically confirmed acute leukemia that was unlikely to be cured by existing therapies, including primary refractory (induction failure) or multirefractory (refractory or relapsed after less than three prior induction regimens) AML and ALL: newly diagnosed AML in adults with antecedent hematologic disorder, including myelodysplasia, treatment-related AML, and/or known adverse cytogenetics; and chronic myelogenous leukemia in lymphoid blast crisis (CML-LBC) of either myeloid or lymphoid origin that was resistant to imatinib, were eligible provided they had Zubrod performance status 0 to 2, normal bilirubin, hepatic enzymes less than two times normal, serum creatinine less than 1.5 times the normal, and left ventricular ejection fraction ≥45%. Patients who had undergone allogeneic or autologous stem cell transplantation and had relapsed or were refractory thereafter were eligible for this study. Complete history, physical examination, laboratory, imaging, and cardiac evaluations (electrocardiogram and left ventricular ejection fraction) were done within 3 days of study entry. Recovery from toxicities of previous treatment and intervals of ≥3 weeks from prior chemotherapy and ≥1 week from any growth factor therapy were required before beginning ara-C and mitoxantrone. Patients were ineligible if they had a peripheral blast count ≥50,000/mm3; disseminated intravascular coagulation; active uncontrolled infection; active central nervous system leukemia; history of ara-C-related neurotoxicity; prior radiation of >25% of bone marrow; concomitant radiotherapy, chemotherapy, or immunotherapy; or coexisting medical or psychiatric conditions that could interfere with study procedures. Pregnant or lactating women were ineligible. All patients provided written informed consent according to the University of Maryland at Baltimore and the Johns Hopkins medical institutional review boards and guidelines.

**Treatment schema.** Flavopiridol was administered over 1 hour daily × 3 beginning day 1 (33, 34). Using a modified dose escalation schema, flavopiridol was dose escalated, with the first four patients receiving 40 mg/m2/d × 3 days. Dose escalation could proceed if no more than one of three patients experienced DLT defined as grade ≥3 nonhematologic toxicity according to the National Cancer Institute Common Toxicity Criteria version 2, bone marrow transplantation criteria, or grade 4 marrow aplasia lasting >40 days. Administration of 2 g/m2 ara-C as a 72-hour continuous infusion (667 mg/m2/24 h; refs. 28, 31, 35, 36) began on day 6. Mitoxantrone (40 mg/m2) was administered as a single i.v. bolus over 30 to 60 minutes on day 9, 12 hours after completion of the ara-C infusion (36). Following completion of the first dose level, cohorts of four to six patients were treated with flavopiridol that was dose escalated by 10 mg/m2/d × 3 days per cohort. The occurrence of any DLT in 33% of patient cohort defined the maximal tolerated dose of flavopiridol. Once maximal tolerated dose was reached, an expanded cohort was treated at the dose level below maximal tolerated dose and observed for DLT. Patients who achieved CR or partial remission after cycle 1 were eligible to receive a second cycle of flavopiridol, ara-C, and mitoxantrone at the identical dose and schedule beginning 30 ± 7 days following hospital discharge from the first cycle.

**Supportive care.** To decrease the severity of the expected flavopiridol-induced secretory diarrhea (33, 37, 38) without inducing changes in gastrointestinal motility, all patients received 100 μg octreotide (somatostatin analogue) every 8 hours beginning 2 to 4 hours before the first dose of flavopiridol and continuing through day 4. All patients received daily oral allopurinol (300 mg) and aluminum hydroxide (30 mL) every 6 hours until 24 hours after completion of ara-C and mitoxantrone (days 1-9). Corticosteroid eye drops were used on days 6 to 12 to prevent ara-C-related conjunctivitis. Antiemetics were used according to standard practices. Premenopausal women were placed on hormonal therapy to suppress menstrual bleeding. Norfloxacin (400 mg b.i.d.) for gastrointestinal decontamination and acyclovir or
Famvir prophylaxis against herpes simplex virus activation began on day 1 and continued until achievement of an absolute neutrophil count >100/mm$^3$.

**Response and toxicity evaluations.** To assess response to therapy, bone marrow aspiration and biopsy were done before treatment and on day 3 following completion of the last dose of flavopiridol, day 6 before beginning the ara-C infusion, day 14, and at the time of hematologic recovery or when leukemia regrowth was suspected. Hematologic recovery was defined as absolute neutrophil count $\geq$500/mm$^3$ and transfusion-independent platelet count 50,000/mm$^3$. CR required a normal bone marrow aspirate with absence of identifiable leukemia, absolute neutrophil count $\geq$1,000/mm$^3$, platelet count $\geq$100,000/mm$^3$, and absence of blasts in peripheral blood (39). Clearance of cytogenetic abnormalities was not required for CR but was noted and described separately. Partial remission was defined as the presence of trilineage hematopoiesis in the marrow with normalization of peripheral counts but with 5% to 25% blasts in the marrow (39). NR was defined as persistent leukemia in marrow and/or blood without significant decrease from pretreatment levels. The National Cancer Institute Common Toxicity Criteria version 2.0 was the basis on which all adverse events were described and graded based on the treating physician’s assessment. DLT was based on the toxicities incurred during cycle 1.

**Pharmacokinetics**

**Sampling.** Plasma samples were obtained from the first 18 patients on day 0 before flavopiridol administration and at 0.5, 1, 1.5, 2, 4, 6, 10, 12, 24, 36, 48, and 72 hours, with the 24- and 48-hour samples being drawn before beginning on days 2 and 3 administration of flavopiridol. Blood was immediately centrifuged at 2,500 x g for 10 minutes at 4°C, and plasma was removed and stored at −70°C until the time of assay. Flavopiridol concentrations were determined using a reverse-phase high-performance liquid chromatographic assay as described previously (33, 38). Briefly, 100 μL plasma was precipitated using 500 μL acetonitrile containing the internal standard. The mixture was vortexed and centrifuged at 10,000 x g for 10 minutes at 4°C and the supernatant was evaporated to dryness under a stream of nitrogen. The residue was reconstituted using 100 μL mobile phase and injected into a Waters (Milford, MA) 2690 high-performance liquid chromatography. Separation was achieved by a Waters Xterra RP18 column preceded by a Phenomenex C18 guard column (Torrance, CA) and a mobile phase consisting of a linear gradient of 10 mmol/L ammonium acetate buffer (pH 4) and acetonitrile at a flow rate of 1 mL/min for UV and 0.150 mL/min for mass spectrometry assays. For UV detection, the detector was used as a dual-wavelength detector and flavopiridol was detected at 264 nm. For samples in which flavopiridol was undetectable, the UV detector, the flavopiridol was detected by a QuatroMicro liquid chromatography/tandem mass spectrometer detector (Micromass, Manchester, United Kingdom) using a positive electrospray mode with a transition of 401.85 > 221.9 for flavopiridol. The total run time was 25 minutes and the typical retention times were 9.1 minutes for drug and 12.3 minutes for internal standard.

**Pharmacokinetic data analysis.** Pharmacokinetic models were fit to flavopiridol plasma concentration data using the Nonlinear Mixed Effects Modeling Program version V (Globomax Service Group, Hanover, MD). Two- and three-compartment pharmacokinetic models with first-order elimination were specified using the Nonlinear Mixed Effects Modeling Program PREDP subroutines ADVAN3/TRANS4 and ADVAN11/TRANS4, respectively. The flavopiridol pharmacokinetic variables estimated were the volume of distribution of the central compartment ($V_c$), clearance from the central compartment (CL), volume of distribution of the peripheral compartment ($V_p$), and intercompartmental clearance (Q). Interpatient variability in pharmacokinetic variables was estimated by the exponential error model:

$$P_i = \theta \times \exp(\eta_i)$$

where $\theta$ is the population mean value for variable $P$, $P_i$ is the individual variable estimate, and $\eta_i$ is a random variable with a mean of 0 and a variance of $\Omega^2$, which describes the deviation of $P_i$ from $P$. Residual variability for flavopiridol pharmacokinetics was modeled by a proportional error model:

$$C_{\text{obs}} = C_{\text{pred}} 	imes (1 + \varepsilon_i)$$

where $C_{\text{obs}}$ is the observed concentration, $C_{\text{pred}}$ is the model-predicted concentration, and $\varepsilon_i$ is the proportional error component. The pharmacokinetic data were fit first. Once a satisfactory model was obtained, the empirical Bayesian estimates of the individual pharmacokinetic variables were plotted against demographic and physiologic variables to study the effect of patient characteristics on CL and $V_c$. This was carried out by plotting the individual partial residuals of each variable resulting from the base model against each potential covariate. The covariates tested were bovine serum albumin, sex, height, weight, serum albumin, and serum creatinine. Patterns in these residual plots suggest the addition of the covariate to the model for the variable. Identification of the best structural model was based on the value of the objective function from Nonlinear Mixed Effects Modeling Program output and the diagnostic plots. The maximum concentration after the first dose of flavopiridol ($C_{\text{peak}}$) was the observed concentration and the area under the plasma concentration curve (AUC) was calculated using noncompartmental modeling.

**Laboratory correlates**

**Flavopiridol target proteins.** In preparation for immunoblotting, mononuclear cells were isolated from bone marrow aspirates obtained on day 0 and again on day 3 immediately following completion of flavopiridol by Ficoll-Hypaque density gradient centrifugation. Mononuclear cells were washed twice with PBS and then solubilized in alkalylation buffer [6 mol/L guanidine hydrochloride, 250 mmol/L Tris-HCl (pH 8.5 at 21°C), and 10 mmol/L EDTA supplemented immediately before use with 150 mmol/L 2-mercaptopoethanol and 1 mmol/L α-phenylmethylsulfonyl fluorid] after confirmation that isolated cells contained at least 70% leukemic cells. Resulting lysates were then processed for SDS-PAGE and subsequent immunoblotting using techniques described previously in detail (14, 40). Membranes were probed using the following antibodies: Mc-l1 (BD PhatMing, San Diego, CA), Bcl-2 (DAKO, Glostrup, Denmark), cyclin D1 (Calbiochem, San Diego, CA), phospho-RNA polymerase II (Covance, Cumberland, VA), phospho-Tyr705-STAT3 (Cell Signaling, Beverly, MA), and actin (Santa Cruz Biotechnology, Santa Cruz, CA).

**Vascular endothelial growth factor and endothelial cell proliferation.** The effects of flavopiridol on VEGF expression and secretion were determined initially in U937 monoblastic leukemia cells and bovine aortic endothelial cells (BAEC). U937 cells (1 x 10^5 per well, six-well plates) were cultured for 24 hours in DMEM plus 2% fetal bovine serum with escalating doses of flavopiridol. Supernatants (0.5 mL) were collected following cell centrifugation and stored at −80°C. BAEC obtained from the Coriell Institute (Camden, NJ) were cultured in 96-well plates (1 x 10^4 per well) for 16 hours in DMEM plus 2% fetal bovine serum to allow cell attachment and recovery, after which graded doses of flavopiridol were added and cells were incubated for a further 72 hours at 37°C. BAEC proliferation was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Chemical, St. Louis, MO) reduction assay. BAEC monolayers were washed with medium and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye was added. After 4 hours, 10% SDS and 0.01 N HCl were added to solubilize the crystalline formazan product and absorbance at 595 nm was determined after 16 hours.

**VEGF levels in conditioned medium, sera, bone marrow cell lysates, and supernatants were measured using the Quantikine human VEGF immunoassay method (R&D, Inc., Minneapolis, MN). All reagents and working standards were prepared at room temperature, microplate strips were removed from the plate frame, and assay diluent (100 μL)
was added to each well. After VEGF standard or sample (100 μL/well) was added, wells were covered with adhesive strips and incubated at room temperature for 2 hours. After multiple washings and removal of liquid, the plate was inverted and blotted against a clean paper towel. VEGF conjugate (200 μL) was added to each well for 2-hour incubation at room temperature followed by multiple washings and addition of substrate solution (200 μL) for 25 minutes. The reaction was stopped by 50 μL stop solution and the color change was determined by measuring absorbance of 450 nm using a Bio-Rad (Hercules, CA) ELISA microplate reader. Wavelength correction at 595 nm was used to correct for optical imperfections in the plate.

Pretreatment bone marrow VEGF levels were determined on marrow cells obtained by routine heparinized needle aspiration on day 0 before treatment with flavopiridol and processed immediately. Bone marrow supernatants were obtained by centrifugation of the marrow cell suspension to separate cellular debris. Bone marrow cell lysates were obtained by Ficoll-Hypaque density gradient centrifugation of the marrow cell suspension followed by washing with HBSS and freeze/thaw lysis. Aliquots were stored at −80°C for subsequent ELISA determinations of VEGF levels.

The effects of in vivo flavopiridol administration on net VEGF production were determined in sera obtained longitudinally from nine patients on day 0 before flavopiridol, day 3 at the end of the flavopiridol bolus, and day 6 before the ara-C infusion. The effects of flavopiridol-containing sera on in vitro endothelial cell proliferation were measured using BAEC cultured for 72 hours with plasma samples obtained from six patients on days 0, 3, and 6 as above (10%, v/v). The relationship between inhibition of BAEC proliferation and flavopiridol exposure in vivo was assessed using linear regression analysis with Sigma Plot 2000. The percent of control growth versus the Cₘₐₓ and the AUC of flavopiridol were used for the regression analysis.

**Results**

**Patient characteristics.** Between March 2001 and November 2003, 34 adults with poor-risk, refractory or relapsed acute leukemia were entered in this phase I study of flavopiridol, ara-C, and mitoxantrone. Detailed clinical demographics are presented in Table 1. Four had newly diagnosed secondary AML (myelodysplasia/AML, treatment-related AML), 9 had acute leukemia in first (3 AML and 2 ALL) or second (4 AML) relapse, and 21 had refractory acute leukemia. Of the six patients with primary refractory leukemia, four AML patients failed to achieve CR following a single cycle of TST induction with ara-C, daunorubicin, and etoposide; one AML patient was refractory to standard ara-C plus idarubicin ("3 + 7") followed by one cycle of high-dose ara-C plus etoposide; and one ALL patient failed to achieve CR after two cycles of hyperfractionated cytoxan, vincristine, Adriamycin, and dexamethasone. The vast majority (91%) had received anthracycline or mitoxantrone therapy for acute leukemia or a prior malignancy. Similarly, 91% had received dose-intensive infusional ara-C and/or high-dose ara-C during initial antileukemia induction and/or consolidation regimens. Eleven (32%) had received previous TST with ara-C and anthracycline or mitoxantrone, including 4 with primary refractory AML and 3 in first relapse with CR 1 duration < 6 months.

**Toxicities.** Sixteen (47%) of the 34 patients evinced direct leukemia cell cytotoxicity as manifested by a ≥50% drop in peripheral blood blast counts. This effect was seen in AML (12 of 26) and ALL (4 of 8, including the patient with CML-LBC) at median day 4 (range, 2-6) following the initiation of flavopiridol and occurred at all dose levels. Evidence for flavopiridol-induced tumor lysis preceding blast clearance occurred in 9 (26%) patients on median day 2 (range, 1-4) of flavopiridol as manifested primarily as transient hyperphosphatemia (14 patients, peak, 4.9-7.5 mg/dL, median, 6.2) with or without hyperuricemia (3 patients, peak, 7.5-10.2 mg/dL), with resolution within 72 hours. Lactate dehydrogenase elevations occurred before and/or concomitant with blast clearance on median day 3 (range, 2-5) in 15 patients, with peak lactate dehydrogenase being 1.2- to 8.1-fold (median, 1.6-fold) higher than pretreatment levels. Hyperkalemia was not detected, no patient required hemodialysis, and no patient developed an associated coagulopathy.

The predominant nonhematologic toxicities (Table 2) were mucosal, with 3 (9%) patients experiencing grade 3 diarrhea within 48 hours of ending flavopiridol (day 5 or earlier) and 4 (12%) patients experiencing grade ≥2 oral mucositis (days 4,

**Table 1. Characteristics of 34 adults undergoing TST with flavopiridol**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>22 (65%)/12 (35%)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>54 (20-75)</td>
</tr>
<tr>
<td>Prior drug exposure</td>
<td></td>
</tr>
<tr>
<td>Ara-C</td>
<td>31 (91%)</td>
</tr>
<tr>
<td>HiDAC</td>
<td>15</td>
</tr>
<tr>
<td>2 g/m²/72 h</td>
<td>12</td>
</tr>
<tr>
<td>Anthracyclines</td>
<td>31 (91%)</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>9</td>
</tr>
<tr>
<td>Prior stem cell transplantation</td>
<td>5 (15%)</td>
</tr>
<tr>
<td>Allogeneic/autologous</td>
<td>2/3</td>
</tr>
<tr>
<td>Disease type</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>26 (76%)</td>
</tr>
<tr>
<td>ALL</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>CML-LBC</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Stage of disease</td>
<td></td>
</tr>
<tr>
<td>New diagnosis (poor-risk AML)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Relapse</td>
<td>9 (26%)</td>
</tr>
<tr>
<td>CR 1</td>
<td>5 (median, 8; range, 3.5-20 mo)</td>
</tr>
<tr>
<td>CR ≥2</td>
<td>4 (1.5, 3, 5.5, and 9 mo)</td>
</tr>
<tr>
<td>Refractory</td>
<td>21 (62%)</td>
</tr>
<tr>
<td>Primary refractory</td>
<td>6</td>
</tr>
<tr>
<td>Multirefractory</td>
<td>15</td>
</tr>
<tr>
<td>Biological features</td>
<td></td>
</tr>
<tr>
<td>Secondary AML</td>
<td>9 (26%)</td>
</tr>
<tr>
<td>Myelodysplasia/AML</td>
<td>7</td>
</tr>
<tr>
<td>Treatment-related AML</td>
<td>2</td>
</tr>
<tr>
<td>Adverse cytogenetics</td>
<td>21 (62%)</td>
</tr>
<tr>
<td>AML</td>
<td>17 (65%)</td>
</tr>
<tr>
<td>ALL</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>CML-LBC</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

1Ara-C (2-3 g/m²) every 2 hours over 12 hours × 4 to 12 doses.  
2Continuous infusion ara-C given over 72 hours.  
3Myelodysplasia/AML, 46XY; myelodysplasia/AML, 47XX, +8; treatment-related M5 AML (postmultiples therapies for ALL), 46XY, −6p21, inv(12p); treatment-related M2 AML (post-methotrexate for rheumatoid arthritis), 45XY, −7.  
4Refractory to two or more induction regimens or to reinduction after first relapse.  
5/5q-; 7/7q, +8.1q23 translocations, 20q-, complex (including duplicated Philadelphia chromosome plus multiple additional trisomies).
11, 12, and 24). No patient exhibited the full-blown proinflammatory syndrome that can accompany continuous infusion (2) or bolus (33) administrations of flavopiridol. Drug-induced myelosuppression occurred in all but one patient treated at dose level 1, with the depth and duration of suppression being comparable with that seen in other TST regimens using cytotoxic drugs (30–32). Median day of absolute neutrophil count recovery to >100/mm³ was day 30 (range, 24-55) and recovery to >500/mm³ was day 36 (range, 27-70). Platelet recovery to ≥50,000/mm³ occurred on median day 38 (range, 29-66).

There were no nonhematologic toxicities exceeding grade 1 in the four patients treated at dose level 1 (flavopiridol dose 40 mg/m²/d x 3). One episode of cardiac toxicity (grade 5; discussed below) occurred in the six patients treated at dose level 2 (50 mg/m²/d x 3). DLT was reached at dose level 3 (60 mg/m²/d x 3), where two of the five patients entered at this dose level experienced profound neutropenia >40 days in the absence of detectable residual leukemia in two patients (treatment-related M5 AML following multiple therapies for T-ALL, multirefractory ALL). An additional patient treated on this dose level experienced profound neutropenia >40 days in the absence of detectable residual leukemia in two patients (treatment-related M5 AML following multiple therapies for T-ALL, multirefractory ALL). An additional patient treated on dose level 3 suffered grade 5 cardiac toxicity as described in detail below. After 4 patients entered at dose level 2 (flavopiridol 50 mg/m²/d x 3) as part of an expanded cohort, it was decided in conjunction with Cancer Therapy Evaluation Program investigators to expand this cohort by an additional 15 patients for purposes of cardiac monitoring throughout the first 21 days of therapy. No arrhythmias and no episodes of congestive heart failure were detected in the subsequent 15 patients.

Four (12%) patients died during therapy with flavopiridol, ara-C, and mitoxantrone, two from disseminated fungal infections (days 30 and 37) and two with sudden death from probable cardiac arrhythmias (days 12 and 20). One episode of sudden death occurred on day 20 of therapy (flavopiridol dose level 2) in a 64-year-old male with refractory L3 ALL and with preexisting hypertensive coronary artery disease, fatty liver, diabetes, and a history of deep venous thromboses with left-sided congestive heart failure and mitral regurgitation) that resolved within 4 weeks (left ventricular ejection fraction 60%) and did not recur with consolidation therapy containing mitoxantrone. Her left ventricular ejection fraction before flavopiridol, ara-C, and mitoxantrone was 60%.

Clinical outcome. Table 2 summarizes the clinical outcome of one cycle of flavopiridol, ara-C, and mitoxantrone. Responses occurred in the first two dose levels, with clear evidence for dose response. For the entire group of 34 patients, 7 (21%) achieved CR and an additional 2 (6%) achieved partial remission for an overall response rate of 27%. Responses occurred in 8 of 26 (31%) AML patients, with the CR rate being 23% (6 of 26) and partial remission rate being 8% (2 of 26). For the ALL cohort (including one with CML-LBC), 1 of 8 (12.5%) achieved CR and there were no partial remissions. When examined according to disease status and prior therapy, 2 of 4 (50%) patients with newly diagnosed AML with poor-risk features achieved CR as did 2 of 7 (29%) with relapsed AML and only 2 of 15 (13%) with refractory AML.

Pharmacokinetics. The observed concentration versus time data were best fit by a linear two-compartment model with first-order elimination compared with a three-compartment model. There was no significant drop in the value of the minimum objective function and the AUC by fitting a three-compartment model to the data. Thus, variables for the two-compartment model are presented and 95% confidence intervals were calculated for each variable based on the asymptotic SEs reported by Nonlinear Mixed Effects Modeling Program. On evaluation of the effect of demographic and physiologic variables on CL and Vf, there was no significant effect of any of the variables.

Table 3 summarizes the results of the final pharmacokinetic model. Mean CL was estimated to be 27.7 L/h with an intersubject variability of 64%. The mean estimate was 57.4 L for Vf, 25.3 L/h for Q, and 392 L for Vf. The intersubject variabilities for Vf, Q, and Vf were 44% and 33.8%, respectively. Residual error was estimated at a modest 33%. Fig. 1 shows the population-predicted and individual-predicted concentrations obtained from the final model plotted against the observed concentrations. An example of a representative plasma concentration versus time plot for flavopiridol is depicted in Fig. 2. The Cmax and AUC were calculated using noncompartmental methods. There was a dose-related increase in the dose-normalized Cmax (2.4 ± 1, 2.9 ± 1.8, and 3.2 ± 2 μmol/L) with relatively similar AUC (8.1 ± 1.3, 6.3 ± 4, and 8.3 ± 4.7 μmol/L h) across the three flavopiridol dose levels (40, 50, and 60 mg/m²/d, respectively).

Flavopiridol target proteins. Previous in vitro studies in cells from diverse hematologic malignancies have shown that Mcl-1, cyclin D, Bcl-2, and phospho-RNA polymerase II are downregulated in response to flavopiridol treatment, whereas p53 and phospho-Tyr705-STAT3 are up-regulated (5, 15, 40, 41). To determine if flavopiridol could exert similar effects in vivo, we examined changes in selected cellular proteins derived from paired samples of leukemic marrow cells from 11 patients (8 AML and 3 ALL, including 1 CML-LBC) obtained on days 0 and 3 after flavopiridol. Figure 3 shows that flavopiridol administration yielded decreases in one or more proteins in 5 of the 11 day 3 marrow blast populations relative to pretreatment levels (obtained from patients P1, P2, P15, P19, and P33). Marrow

Table 2. Nonhematologic toxicities of TST with flavopiridol, ara-C, and mitoxantrone

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Post-flavopiridol</th>
<th>Post-ara-C/ mitoxantrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucositis</td>
<td>6 (18%)</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>1 grade 2</td>
<td>3 (1 grade 3, 2 grade 2)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>5 (3 grade 3, 2 grade 2)</td>
<td>4 (3 grade 3, 2 grade 1)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhythmia/sudden death</td>
<td>0</td>
<td>2 grade 5</td>
</tr>
<tr>
<td>Death</td>
<td>4 (12%)</td>
<td></td>
</tr>
<tr>
<td>Fungal infection</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sudden death</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
blasts obtained on day 3 from two (P1 and P19) of four AML patients tested who achieved CR (P1, P19, P20, and P33) showed simultaneous down-regulation of multiple flavopiridol target proteins (Bcl-2, phospho-RNA polymerase II, and Mcl-1) relative to blasts obtained before flavopiridol day 0. Cyclin D1, measurable only in a few of the patients, was decreased in P1. In contrast, day 3 marrow blasts from four nonresponding patients (P2, AML; P9, ALL; P27, AML; and P31, AML) exhibited increases in at least one cycle regulatory protein.

Vascular endothelial growth factor and endothelial cell proliferation. To establish that flavopiridol could inhibit the expression and secretion of VEGF from leukemic cells, we quantified changes in VEGF levels in medium conditioned by VEGF-expressing U937 cells following 24-hour exposure to escalating doses of flavopiridol (Fig. 4). As depicted in Fig. 4A, flavopiridol inhibited VEGF secretion by U937 cells in a dose-dependent fashion, with an IC₅₀ of 3 nmol/L. Flavopiridol also inhibited the proliferation of BAEC cells cultured in the presence of escalating doses of drug (Fig. 4B), with an IC₅₀ of 30 nmol/L. Maximal inhibition of BAEC proliferation was achieved at 125 nmol/L.

VEGF levels were determined in marrow cell lysates and supernatants derived from pretreatment marrow aspirates obtained from six individual patients. Cell lysate VEGF levels ranged from 60 to 750 pg/mL and marrow supernatant VEGF levels ranged from 60 to 1,200 pg/mL, without a clear relationship between cell lysate and supernatant levels. To determine the effects of in vivo levels of flavopiridol on BAEC proliferation, sera were obtained longitudinally from nine patients treated at dose levels 1 and 2 on day 0 before flavopiridol, day 3 at the end of flavopiridol, and day 6 before ara-C and assayed for their ability to inhibit BAEC proliferation.

---

### Table 3. Clinical outcome of TST with flavopiridol, ara-C, and mitoxantrone according to flavopiridol dose level

<table>
<thead>
<tr>
<th>Flavopiridol dose level</th>
<th>Diagnosis (no. patients)</th>
<th>CR (%)</th>
<th>Priority report (%)</th>
<th>NR (%)</th>
<th>NE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (40 mg/m²)</td>
<td>AML (4)</td>
<td>1 (25)</td>
<td>—</td>
<td>3 (75)</td>
<td>—</td>
</tr>
<tr>
<td>II (50 mg/m²)</td>
<td>AML (19)</td>
<td>5 (26)</td>
<td>2 (11)</td>
<td>12 (63)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ALL (6)</td>
<td>1 (17)</td>
<td>—</td>
<td>3 (50)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Total (25)</td>
<td></td>
<td>6 (24)</td>
<td>2 (8)</td>
<td>15 (60)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>III (60 mg/m²)</td>
<td>AML (3)</td>
<td>—</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ALL (2) *</td>
<td>—</td>
<td>0 (0)</td>
<td>4 (80)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Total (5)</td>
<td></td>
<td>6 (23)</td>
<td>2 (8)</td>
<td>17 (65)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Total AML (26)</td>
<td></td>
<td>1 (12.5)</td>
<td>0 (0)</td>
<td>5 (62.5)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Total ALL (8)</td>
<td></td>
<td>7 (21)</td>
<td>2 (6)</td>
<td>22 (65)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Overall response (34)</td>
<td></td>
<td>1 (12)</td>
<td>0 (0)</td>
<td>5 (62.5)</td>
<td>2 (25)</td>
</tr>
</tbody>
</table>

*Includes one patient with CML-LBC.

---

**Fig. 1.** Predicted versus observed concentrations of flavopiridol. Population-predicted (A) and individual-predicted (B) plasma concentrations of flavopiridol plotted as a function of the observed concentrations. •, individual data; line, unity line.
in vitro (Fig. 5). Sera obtained on day 3 from five patients who eventually achieved CRs inhibited endothelial cell proliferation relative to day 0 plasma, with a mean inhibition of 32% (range, 10-80%; Fig. 5A), whereas sera obtained on day 6 before ara-C administration showed a return toward baseline activity. In contrast, sera obtained from four patients with clinically resistant (progressive) leukemia revealed no inhibitory/recovery pattern (Fig. 5B). We then sought to determine the pharmacokinetic relationship of flavopiridol levels and BAEC growth looking at three different variables of flavopiridol concentration in vivo (Cp at 2 hours, Cmax, and AUC). BAEC proliferation was inversely proportional to the level of flavopiridol in the sera with R² values of 0.7809 (Cp), 0.7981 (Cmax), and 0.7216 (AUC). This decrease in proliferation was modest, ~30% at the maximum achieved flavopiridol exposure, but statistically significant (P < 0.05), with the change in flavopiridol concentration accounting for 70% of the variability in response.

Discussion

The design of our phase I trial of flavopiridol followed by ara-C and mitoxantrone for adults with relapsed and refractory acute leukemias was based on findings from our preceding in vitro studies (27, 42); that is, flavopiridol can exert cytotoxic effects on cell lines and cultured human leukemic marrow blasts followed by recruitment and priming of remaining cells for enhanced ara-C sensitivity. Further, the use of flavopiridol as the initial drug in sequence afforded us the opportunity to examine whether flavopiridol could exert direct antileukemic effects in vivo, independent of known antileukemic cytotoxic agents. The clinical results of the trial support the contention that flavopiridol is directly cytotoxic to leukemic cells and, when followed by ara-C and mitoxantrone, exerts measurable biological and clinical effects in patients with relapsed and refractory acute leukemias. This direct cytotoxicity was manifested by a significant drop in peripheral blood blast count in almost half of the patients, with evidence of a preceding flavopiridol-induced tumor lysis syndrome in 26% of those treated. The ability of flavopiridol to induce tumor lysis has been shown in chronic lymphocytic leukemia by Byrd et al. (34) and Donehower et al. (43), raising the possibility that flavopiridol causes cell necrosis as well as apoptosis in selected malignant cell populations.

The DLT for flavopiridol in this regimen occurred at 60 mg/m²/day given as a 1-hour infusion daily for 3 days, with marrow aplasia of >40 days occurring in two patients treated at that dose level. This finding is similar to that of Tan et al. (33), who observed that dose-limiting marrow aplasia occurred at flavopiridol doses ≥52.5 mg/m²/day in solid tumor patients. The depth of myelosuppression in both of the leukemia patients treated at 60 mg/m²/day extended beyond that commonly seen in other TST regimens (30–32, 36) and may relate to the refractory nature of the leukemias or to stem cell toxicity from previous cytotoxic therapies (e.g., treatment-related AML and heavily pretreated ALL). Alternatively, the use of flavopiridol to recruit leukemic blasts into cell cycle may have resulted in recruitment of normal stem cells at the same time, with a resultant prolongation of myelosuppression in patients with preexisting marrow damage.

Pharmacokinetic analyses show that the plasma levels of flavopiridol achieved in our patients are similar to those obtained in earlier studies (2, 33, 34, 38). We were able to describe flavopiridol pharmacokinetics with a two-compartment model using Nonlinear Mixed Effects Modeling Program. As depicted in Table 4, the high intersubject variability in clearance (64%) is a reflection of the high variability in the plasma concentrations. Although no relationship was found between clinical and demographic covariates and clearance (or volume of distribution) to explain the high variability in clearance, the role of the breast cancer resistance protein cannot be

![Fig. 2. Representative plasma concentration versus time plot.](image1)

**Fig. 2.** Representative plasma concentration versus time plot. Plasma flavopiridol concentration versus time plot for a representative patient receiving 60 mg/m² flavopiridol as a 1-hour infusion once daily for 3 days. Solid line, model-predicted concentration; ●, measured concentrations.

![Fig. 3. Effects of flavopiridol therapy on leukemia cell polypeptides isolated from 11 patients.](image2)

**Fig. 3.** Effects of flavopiridol therapy on leukemia cell polypeptides isolated from 11 patients. Relative amounts of cellular polypeptides were assessed by immunoblotting, with total cellular proteins from 2 x 10⁶ patient mononuclear cells loaded in each lane. Analogous results obtained from immunoblotting of HL60 total cellular proteins are shown for comparison purposes. D0, pretherapy; D3, following flavopiridol therapy.
ruled out. In this regard, Nakanishi et al. (42) detected resistance to flavopiridol-induced cell death in primary human leukemic marrow blast populations with relatively high (>10,000 copies/pg β-actin) breast cancer resistance protein mRNA expression as measured by real-time reverse transcription-PCR. Interestingly, no such relationship could be detected between MDR1 mRNA expression and resistance to flavopiridol-induced apoptosis. It is likely that mechanisms in addition to high breast cancer resistance protein expression (either pretreatment or induced by flavopiridol exposure) are involved in flavopiridol resistance, because marked variation in cell apoptosis and survival in the presence of flavopiridol was detected in blast cell populations with relatively low breast cancer resistance protein expression.

The cytotoxicity of flavopiridol relates to its ability to modulate the activity of diverse proteins that regulate cycle progression and cell survival. We were able to show that the in vivo administration of flavopiridol was associated with changes in one or more cell cycle or survival proteins in leukemic marrow blasts from some of the patients in whom we were able to measure such protein levels. Unfortunately, the data are difficult to analyze quantitatively for several reasons, including disparities in cell numbers and survival among specimens. Quantitation and correlation with clinical outcome would be important to pursue in future correlative clinical laboratory trials involving flavopiridol. In addition, our in vitro studies support the notion that flavopiridol inhibits expression and secretion of VEGF in the VEGF-expressing monoblastic leukemia cell line U937, with inhibition detected at very low drug concentrations. The link between flavopiridol and VEGF suppression is further substantiated by our demonstration that flavopiridol inhibits endothelial cell proliferation at clinically achievable concentrations in vitro. Interestingly, patient sera obtained at the end of the day 3 flavopiridol infusion and containing peak clinically achievable concentrations of flavopiridol had inhibitory effects on endothelial cell proliferation in vitro. Further, pharmacokinetic analyses showed significant dose response with regard to inhibitory effects of day 3 flavopiridol-containing sera on BAEC growth, with the endothelial cell proliferation being inversely proportional to the serum level of flavopiridol. This inhibitory activity could reflect any number of flavopiridol-induced changes in the activities of cyclin-dependent kinases, cyclin D1, or target proteins involved in regulating apoptosis. Nonetheless, it is interesting to speculate that at least part of the propapoptotic effects of flavopiridol might stem from the ability of the drug to decrease VEGF production, which in turn may prevent the observed up-regulation of Mcl-1 expression that has been documented in multiple myeloma cells and has been shown to protect myeloma cells from apoptosis in response to cellular stress (5, 41, 44).

Overall, this clinical trial shows a moderate efficacy of this timed sequential regimen for adults with relapsed and refractory leukemias, particularly AML. We did not examine alternate drug schedules in either our in vitro TST model or our in vivo phase I clinical trials. As such, our data do not preclude the possibility that another sequence of flavopiridol and ara-C might be
equally effective in terms of net antileukemic cytotoxicity. In this regard, recent studies in lung cancer cell lines show that flavopiridol causes mitochondrial damage and apoptosis in S phase (45, 46). This induction of apoptosis in S phase is dependent on the ability of flavopiridol to block phosphorylation of E2F1 (45), which in turn stabilizes E2F1/DP-1 hybrid dimer binding to DNA and thereby represses Mcl-1 transcription (45, 46). This induction of apoptosis in S phase is equally effective in terms of net antileukemic cytotoxicity. In this regard, recent studies in lung cancer cell lines show that flavopiridol causes mitochondrial damage and apoptosis in S phase (45, 46). This induction of apoptosis in S phase is dependent on the ability of flavopiridol to block phosphorylation of E2F1 (45), which in turn stabilizes E2F1/DP-1 hybrid dimer binding to DNA and thereby represses Mcl-1 transcription (45, 46). Combinations with other cytotoxics may require different schedules depending on the maximal cycle-dependent effects of the specific cytotoxic agent and the growth kinetics of the target cell population (3, 26, 47). Additionally, flavopiridol exhibits synergistic antitumor activities when combined with mechanistically diverse agents, such as the topoiserase inhibitor imatinib (48), the histone deacetylase inhibitor suberoylanilide hydroxamic acid (49), proapoptotic agent, such as tumor necrosis factor–related apoptosis-inducing ligand/Apo2L (50), and phosphatidylinositol 3-kinase inhibitor, such as wortmannin or LY294002 (6). Such synergy may be to some extent independent of cell cycle status (3) and more linked to non-cross-resistant mechanisms of mitochondrial damage (6, 10, 41, 48–50). Finally, alternative schedules of flavopiridol administration are important to consider. Byrd et al. (34) have shed important light on the schedule dependency of flavopiridol in patients with chronic lymphocytic leukemia related at least in part to the extensive binding of flavopiridol to human plasma proteins. Their data suggest that a combination of a 30-minute i.v. bolus followed by a more sustained (4-hour) infusion might enhance overall flavopiridol cytotoxicity and therefore clinical responses (34, 43). The mechanistic versatility of flavopiridol and its ability to induce leukemic cell death directly support its continuing development in combination with cytotoxic and biological agents for acute leukemias.

Acknowledgments

We thank Yean Kit Lee, Crescent R. Isbarn, Ngo Dai, and David Loegering for assistance with the immunoblotting.

References


Table 4. Mean pharmacokinetic variables and estimates of intersubject variability

<table>
<thead>
<tr>
<th>Variables (units)</th>
<th>Mean value (95% confidence interval)</th>
<th>Intersubject variability (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/h)</td>
<td>27.7 (18.5-36.9)</td>
<td>64 (33.9-84)</td>
</tr>
<tr>
<td>V1 (L)</td>
<td>5.74 (43.6-71.2)</td>
<td>44.3 (10.5-56.3)</td>
</tr>
<tr>
<td>Q (L/h)</td>
<td>25.3 (15.6-34)</td>
<td>33.8 (19-62.7)</td>
</tr>
<tr>
<td>V2 (L)</td>
<td>392 (245-538.9)</td>
<td>NE</td>
</tr>
<tr>
<td>Proportional error (%)</td>
<td></td>
<td>Residual variability (%)</td>
</tr>
</tbody>
</table>

33 (28.2-45.6)


Phase I and Pharmacokinetic Study of Flavopiridol followed by \(1-\beta-d\)-Arabinofuranosylcytosine and Mitoxantrone in Relapsed and Refractory Adult Acute Leukemias


Updated version Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/11/23/8403

Cited articles This article cites 50 articles, 36 of which you can access for free at: http://clincancerres.aacrjournals.org/content/11/23/8403.full.html#ref-list-1

Citing articles This article has been cited by 9 HighWire-hosted articles. Access the articles at: /content/11/23/8403.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.