The Lethal Effects of Pharmacological Cyclin-dependent Kinase Inhibitors in Human Leukemia Cells Proceed through a Phosphatidylinositol 3-Kinase/Akt-dependent Process^1

Chunrong Yu, Mohamed Rahmani, Yun Dai, Daniel Conrad, Geoffrey Krystal, Paul Dent, and Steven Grant^2

Departments of Medicine [C. Y., M. R., Y. D., G. K., S. G.], Biochemistry [S. G.], Pharmacology [S. G.], Microbiology [S. G., D. C.], and Radiation Oncology [P. D.], Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

ABSTRACT

The impact of disruption of the PI3K (phosphatidylinositol 3-kinase) pathway on the response of human leukemia cells to pharmacological cyclin-dependent kinase (CDK) inhibitors has been examined. Exposure of U937 monocyte leukemia cells to minimally toxic concentrations of flavopiridol (FP), roscovitine, or CGP74514A for 3 h in conjunction with the PI3K inhibitor LY294002 (abbreviated LY in the article) resulted in a marked decrease in Akt phosphorylation. Coexposure of cells to LY and CDK inhibitors also resulted in an early (i.e., within 3 h) and striking increase in mitochondrial damage [e.g., cytochrome c, second mitochondria-derived activator of caspases/direct inhibitor of apoptosis (IAP)-binding protein with low isoelectric point (Smac/DIABLO), and apoptosis-initiating factor (AIF) release], caspase activation, and apoptosis. Similar interactions were observed in a variety of other leukemia cell types (e.g., HL-60, Jurkat, Raji, and NB4). Apoptosis, induced by FP/LY, was substantially blocked by ectopic expression of Bel-2, but to a considerably lesser extent by dominant-negative caspase-8. FP-induced apoptosis was not enhanced by agents that inhibited protein kinase (PK) A (H89), PKC (GFX), mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase (MEK1/2; U0126), p38 MAP kinase (MAPK; SB202190), m-target of rapamycin (TOR; rapamycin), or ataxia-telangiectasia mutation (ATM; caffeine), whereas the PI3K inhibitor wortmannin exerted effects similar to those of LY. The dramatic potentiation of CDK inhibitor-induced apoptosis by LY was accompanied by diminished Bad phosphorylation, induction of Bel-2 cleavage, and down-regulation of X-linked IAP (XIAP) and Mcl-1. Cells exposed to CDK inhibitors + LY also exhibited reduced phosphorylation of glycogen synthase kinase (GSK)-3, forkhead transcription factor (FKHR), p70^S6K^, and ERK, but also exhibited reduced phosphorylation of glycogen synthase kinase (GSK), levels of which fluctuate throughout the cell cycle, as well as the CDKs, e.g., CDK1 1, 2, 5, 4/6, 7, and 9 (2). CDKs 2 and 4/6, in association with cyclins D, E, and A, are primarily involved through sequential phosphorylation of the retinoblastoma protein, in governing the G1-S phase transition as well as progression through S phase (3). In contrast, CDK1, in association with cyclins B and A, regulates progression of cells through G2-M (4). The central importance of cell cycle dysregulation in the malignant phenotype has prompted intensive efforts to develop small-molecule inhibitors of various cell cycle regulatory components. The first of these to enter clinical trials has been the semisynthetic flavonoid FP (NSC 649890), which binds to the CDK ATP binding site, resulting in the inhibition of catalytic activity (5). FP is a broadly acting CDK antagonist and inhibits, to various degrees, the activities of CDKs 1, 2, 4/6, and 7 (6). More recently, FP has been shown to interfere with the CDK9/T1 complex and, thereby, to function as a transcriptional repressor (7). Other pharmacological CDK inhibitors in preclinical development include olomoucine, roscovitine, butyrolactone, the paullonines, and CGP74514A, among others (8, 9).

In addition to their capacity to induce cell cycle arrest, CDK inhibitors are also lethal to tumor cells, a phenomenon that may reflect the close relationship that exists between the apoptotic and cell cycle machinery (10). For example, FP triggers apoptosis in lung cancer cells (11), and is a particularly potent inducer of apoptosis in malignant cells of hematopoietic origin (12). The induction of apoptosis by other CDK inhibitors has also been widely reported (13, 14). In the case of FP, the induction of cell death has been linked to perturbations in cell cycle proteins (i.e., down-regulation of cyclin D1; Ref. 15), diminished expression of certain antiapoptotic proteins (i.e., Mcl-1 and XIAP; Ref. 16), or induction of mitochondrial injury (17). However, the mechanism by which such agents activate the apoptotic cascade remains, for the most part, unclear.

In addition to a continuously expanding family of pro- and antiapoptotic proteins (18), apoptosis is also regulated by multiple signal transduction cascades, of which the MAPK pathways have received considerable attention. The MAPK pathways represent three parallel serine-threonine kinase modules that are triggered by various mitogen- and stress-related stimuli (19). The MAPK pathways consist of

INTRODUCTION

Disregulation of the cell cycle pathway represents one of the hallmarks of malignant transformation (1). Cell cycle progression is regulated by a highly complex network of proteins that include the cyclins, levels of which fluctuate throughout the cell cycle, as well as the CDKs, e.g., CDKs 1, 2, 5, 4/6, 7, and 9 (2). CDKs 2 and 4/6, in association with cyclins D, E, and A, are primarily involved through sequential phosphorylation of the retinoblastoma protein, in governing the G1-S phase transition as well as progression through S phase (3). In contrast, CDK1, in association with cyclins B and A, regulates progression of cells through G2-M (4). The central importance of cell cycle dysregulation in the malignant phenotype has prompted intensive efforts to develop small-molecule inhibitors of various cell cycle regulatory components. The first of these to enter clinical trials has been the semisynthetic flavonoid FP (NSC 649890), which binds to the CDK ATP binding site, resulting in the inhibition of catalytic activity (5). FP is a broadly acting CDK antagonist and inhibits, to various degrees, the activities of CDKs 1, 2, 4/6, and 7 (6). More recently, FP has been shown to interfere with the CDK9/T1 complex and, thereby, to function as a transcriptional repressor (7). Other pharmacological CDK inhibitors in preclinical development include olomoucine, roscovitine, butyrolactone, the paullonines, and CGP74514A, among others (8, 9).

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In addition to a continuously expanding family of pro- and antiapoptotic proteins (18), apoptosis is also regulated by multiple signal transduction cascades, of which the MAPK pathways have received considerable attention. The MAPK pathways represent three parallel serine-threonine kinase modules that are triggered by various mitogen- and stress-related stimuli (19). The MAPK pathways consist of
the ERK, JNK, and p38 MAPK kinase modules (20). Although exceptions exist, ERK activation is generally associated with cell survival, whereas JNK and p38 MAPK have been linked to proapoptotic events (21). Recently, however, the Ras/PI3K/Akt signal transduction pathway has become the focus of intense interest as a critical regulator of cell survival/death decisions (22). Activation of PI3K results in the generation of the lipid second messenger PIP3 from its precursor, phosphatidylinositol-4,5-bisphosphate (PIP2; Ref. 23). PIP3 recruits to the plasma membrane through interactions with a pleckstrin homology domain, the serine/threonine kinase Akt, which is then phosphorylated by phosphoinositide-dependent kinase 1 (24). Phosphorylation of Akt leads to the modulation of the expression/function of multiple downstream targets involved in apoptosis regulation, which include, but are not restricted to, Bad, procaspase-9, FKHRs, m-TOR, p70S6K, nuclear factor κB, CREB, and p21(CIP1), among others (25–28). Attempts to elucidate the role of the PI3K pathway in apoptosis regulation have been facilitated by the availability of agents such as wortmannin, a PI3K antagonist that also inhibits DNA PK (29), as well as LY (30).

Currently, little information exists concerning the signaling events responsible for apoptosis induction by FP or other pharmacological CDK inhibitors. Moreover, essentially nothing is known of the role of the PI3K pathway in regulating apoptosis in cells subjected to CDK inhibition. To address these issues, the effects of established PI3K inhibitors have been examined in relation to the response of human leukemia cells to FP and other pharmacological CDK inhibitors. Here we report that exposure of such cells to CDK inhibitors, in conjunction with PI3K antagonists such as LY, diminishes Akt phosphorylation and triggers a very early (e.g., within 2 h) and striking increase in mitochondrial damage, caspase activation, and apoptosis. In contrast, blockade of the PKC, ERK, p38 MAPK, PKA, or m-TOR cascades exerts minimal effects on apoptosis induction by CDK inhibitors. Moreover, inducible expression of a constitutively active, myristylated Akt mutant significantly, albeit partially, attenuates the lethal effects of FP as well as the FP/LY regimen. Taken together, these findings indicate that PI3K inhibitors dramatically increase the lethality of pharmacological CDK inhibitors in human leukemia cells and suggest that this phenomenon involves, at least in part, interference with Akt-related cytoprotective functions.

**MATERIALS AND METHODS**

**Cells.** U937, Jurkat, CCRF, HL60, and NB4 human leukemia cells were purchased from American Type Culture Collection, Manassas, VA. All of the cells were cultured in RPMI 1640 supplemented with sodium pyruvate, MEM essential vitamins, t-glutamate, penicillin, streptomycin, and 10% heat-inactivated FCS (Hyclone, Logan, UT). They were maintained in a 37°C, 5% CO2, fully humidified incubator, passed twice weekly, and prepared for experimental procedures when in log-phase growth (cell density 105 cells/ml).

U937 cells, stably transfected with constructs expressing Bcl-2, a phosphorelay loop-deleted Bcl-2 mutant, or dominant-negative caspase-8, were generated and maintained as described previously in detail (17, 31).

**Reagents.** FP (L86 8275; NCS 69890) was kindly provided by Dr. Edward Sauvageau (Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, MD), formulated in DMSO as a 10−2 M stock solution, and was stored at −20°C. LY, CGP74514A, rosuvastatin, DiOC4, and wortmannin were purchased from Sigma (St. Louis, MO), dissolved in DMSO, and were stored at −20°C. Rapamycin was purchased from Cell Signaling Technology (Beverly, MA); SB20190, H-89, SP600125, U0126, and SP600125 were purchased from BioMol Research Laboratories (Plymouth, MA); The PKC inhibitor bisindolylmaleimide I (GF109203X; Calbiochem, San Diego, CA) was formulated in DMSO and was added 0.5 h before the addition of FP. The pan-caspase inhibitor BOC-D-fmk was purchased from Enzyme System Products (Livermore, CA) and dissolved in DMSO.

**Experimental Format.** Logarithmically growing cells were transferred to sterile plastic T-flasks (Corning, Corning, NY) to which were added the designated drugs, after which the flasks were replaced in the incubator for various intervals. At the end of the incubation period, cells were transferred to sterile centrifuge tubes, pelleted by centrifugation at 400 × g for 10 min at room temperature, and prepared for analysis as described below.

**Assessment of Apoptosis.** After drug treatment, cytotoxicity preparations were stained with Wright-Giemsa and viewed by light microscopy to evaluate the extent of apoptosis (i.e., cell shrinkage, nuclear condensation, formation of apoptotic bodies) as described previously (32). For these studies, the percentage of apoptotic cells was determined by evaluating ≥500 cells/condition in triplicate. To confirm the results of morphological analysis, Annexin V/PI staining was used. Annexin V/PI (BD Pharmingen, San Diego, CA) analysis of cell death was carried out as per the manufacturer's instructions. For these experiments, 1–2 × 106 cells per condition were harvested. Analysis was carried out using a Becton-Dickinson FACScan flow cytometer (Burlington, MA).

**Determination of Loss of MMP (ΔΨm).** Loss of MMP (∆Ψm) was monitored using the fluorochrome DiOC6 as previously described (33). For each condition, 4 × 105 cells were incubated for 15 min at 37°C in 1 ml of 40 mM DiOC6 (Calbiochem) and subsequently analyzed using a Becton Dickinson FACScan flow cytometer with excitation and emission settings of 488 and 525 nm, respectively. Control experiments documenting the loss of ∆Ψm were performed by exposing cells to 5 μM of carbamyl cyanide m-chlorophenylhydrazone (Sigma Chemical Co.; 15 min, 37°C), an uncoupling agent that abolishes the MMP.

**Preparation of S-100 Fractions for Assessment of Cytochrome c, Smac/DIABLO, and AIF Release.** U937 cells were harvested after drug treatment as described previously (33) by centrifugation at 600 × g for 10 min at 4°C and washed in PBS. Cells (4 × 107) were lysed by incubating them for 3 min in 100 μl of lysis buffer containing 75 mM NaCl, 8 mM Na2HPO4, 1 mM NaH2PO4, 1 mM EDTA, and 350 μg/ml digitonin. The lysates were centrifuged at 12,000 × g for 5 min, and the supernatant was collected and added to an equal volume of 2× Laemmli buffer. The protein samples were quantified and separated by 15% SDS-PAGE. Expression of the mitochondrial proteins cytochrome c, Smac/DIABLO, and AIF was assessed by Western blot analysis as described below.

**Immunoblot Analysis.** Immunoblotting was performed as described previously (33). In brief, after drug treatment, cells were pelleted by centrifugation and were lysed immediately in Laemmli buffer [1× = 30 mM Tris-base (pH 6.8), 2% SDS, 2.88 mM β-mercaptoethanol, and 10% glycerol], and were briefly sonicated. Homogenates were quantified using Coomassie protein assay reagent (Pierce, Rockford, IL). Equal amounts of protein (20 μg) were boiled for 10 min, separated by SDS-PAGE (5% stacker and 10% resolud), and electroblotted to nitrocellulose membrane. After blocking in TBS-T (0.05%) and 5% milk for 1 h at 22°C, the blots were incubated in fresh blocking solution with an appropriate dilution of primary antibody for 4 h at 22°C. Sources of primary antibodies were as follows: Bcl-2 and Bid, mouse monoclonal (Dako, Carpenteria, CA); Bcl-xL, rabbit polyclonal (Santa Cruz Biotechnology); XIAP, rabbit polyclonal (R&D Systems, Minneapolis, MN); Mcl-1: mouse monoclonal (PharMingen, San Diego, CA); cyclins A, E, D1, CDK2 phosphospecific site, rabbit polyclonal (Biosource); caspase 3, mouse monoclonal (Transduction Laboratories, Lexington, KY); PARP (C-2-10), mouse monoclonal (Santa Cruz Biotechnology); phospho-p38 MAPK, rabbit polyclonal (Cell Signaling Technology); phospho-cdk2 and phospho-p70S6K (Th439), rabbit polyclonal (Cell Signaling Technology); phospho-Akt (Ser473), rabbit polyclonal (Cell Signaling Technology); phospho-GSK (Cell Signaling Technology); phospho-FKHR, rabbit polyclonal (Cell Signaling Technology); phospho-p70S6K, rabbit polyclonal (Cell Signaling Technology); phospho-CREB, rabbit polyclonal (Upstate Biotechnology, Lake Placid, NY); phospho-Bad and total Bad, rabbit polyclonal (Cell Signaling Technology); pRB, mouse monoclonal (PharMingen); under-phosphorylated-pRb, mouse monoclonal (PharMingen); phospho-p72TSP1, mouse monoclonal (Transduction Laboratories, Lexington, KY); PARP (C-2-10), mouse monoclonal (BioMol Research Laboratories, Plymouth, MA); cytochrome c, mouse monoclonal; caspase 8, rabbit polyclonal (PharMingen); and α-tubulin

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(Calbiochem). Blots were washed three times for 15 min each time in TBS-T and then were incubated with a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody (Bio-Rad Laboratories, Hercules, CA) for 1 h at 22°C. Blots were again washed three times for 15 min in TBS-T and then were developed by enhanced chemiluminescence (Pierce, Rockford, IL).

**Tet-On Inducible Jurkat Cell Lines.** A stable Jurkat lymphoblastic leukemia cell line inductively expressing a myristoylated Akt (myr-Akt), which is constitutively active, was generated as follows. Myc-tagged myr-AKT (Upstate Biotechnology, Lake Placid, NY) was subcloned into the pTRE2-hygro expression vector (Clontech) according to standard techniques. Jurkat “Tet-On” cells that stably express an rtTA (reverse tet transactivator) regulator protein (Clontech) were transfected with Myc-tagged myr-AKT-pTRE2-hygro by electroporation (600 V, 60 ms) using 0.4 μg μl cuvettes. Stable clones derived from single cells were selected by limiting dilution in RPMI 1640 supplemented with 10% of Tet-System-approved FBS (Clontech) in the presence of 400 μg/ml hygromycin. To test for induced expression of the myc-AKT, stable clones were left untreated or treated for 24 h with 2 μg/ml doxycycline, after which they were harvested and analyzed for myc-AKT and phosphorylated (activated) Akt expression by Western blot as described above.

**AML Patient-derived Cells and Normal Peripheral Blood Mononuclear Cells.** Leukemic cells were obtained with informed consent and as described previously (34) from the peripheral blood of a patient with AML (subtype M2) who relapsed after induction therapy. These studies have been sanctioned by the Investigational Review Board of Virginia Commonwealth University/Medical College of Virginia. For these studies, the percentage of blasts in the peripheral blood was >90%. Blood was collected into sterile tubes containing preservative-free heparin, layered over Ficoll-Hypaque (Sigma; specific gravity, 1.077–1.081) in a sterile 50-cc plastic centrifuge tube. The diluted cells were layered over a cushion of Ficoll-Hypaque (Sigma), when LY was administered in combination with the CDK inhibitors U937 cells were exposed to a sublethal concentration of FP (i.e., 75 μM) for 6 h in the presence of various concentrations of LY, after which apoptosis was assessed. As shown in Fig. 1 A, coadministration of LY at concentrations ≥10 μM, which were minimally toxic alone, resulted in apoptosis in the majority of FP-treated cells. A FP dose-response curve revealed that when cells were exposed to a fixed concentration of LY (15 μM) in conjunction with FP concentrations ≥50 nM, a marked increase in apoptosis was observed (Fig. 1B). Similar patterns were noted when LY was administered in combination with the CDK inhibitors CGP74514A and roscovitine (Fig. 1, C and D). Thus, interruption of the PI3K pathway resulted in an early and striking increase in apoptosis in U937 cells exposed to several CDK inhibitors.

These results were confirmed using annexin/PI analysis, which distinguishes between early apoptosis (in which cells stain positively for annexin) and late apoptosis (in which cells stain positively for both annexin and PI; Fig. 2A). It is noteworthy that even at an early exposure interval (e.g., 6 h), a modest increase in late apoptosis was observed in LY/CDK inhibitor-treated cells. In accord with these results, coadministration of each of the CDK inhibitors with FP for 6 h

![Fig. 1](image-url)
resulted in a marked loss of MMP ΔΨm; Fig. 2B), a hallmark of mitochondrial injury. A time course study of U937 cells exposed to 75 nM FP and 15 μM LY revealed a modest increase in apoptosis in FP/LY-treated cells by 3 h, and extensive cell death by 6–9 h (Fig. 2C). After 12 h of combined drug exposure, virtually all of the cells were apoptotic. Very similar results were obtained when LY was combined with roscovitine (10 μM) or with CGP (1.5 μM; data not shown). Finally, Median Dose Effect analysis of apoptosis induction in U937 cells exposed to a range of LY and FP concentrations for 6 h yielded C.I. values considerably less than 1.0, corresponding to a highly synergistic interaction (Fig. 2D).

As shown in Fig. 3A, treatment of U937 cells with LY (15 μM) in

Fig. 2. A, U937 cells were exposed to 15 μM LY ± 75 nM FP, 1.5 μM CGP74514A (CGP), or 10 μM roscovitine (Ros) for 6 h, after which the extent of apoptosis was assessed by Annexin V/PI staining as described in “Materials and Methods.” B, early apoptotic cells (Annexin V⁺ only), late apoptotic cells (Annexin V/API⁺). C, cells were treated with LY ± FP, CGP74514A (CGP), or roscovitine (Ros) as above for 6 h, after which the percentage of cells exhibiting loss of ΔΨm, reflected by “low” uptake of the fluorochrome DiOC₆, was determined by flow cytometry as described in “Materials and Methods.” C, cells were exposed to the designated concentrations of FP ± LY for the indicated intervals, after which the extent of apoptosis was determined as above. For A–C, values represent the means ± SD for three separate experiments. D, cells were exposed for 24 h to various concentrations of LY (10–25 μM) ± FP (50–125 nM) at a fixed ratio (200:1), after which the percentage of apoptotic cells was determined as above. Median Dose Effect analysis was used to determine the C.I. for each F.A. C.I. values <1.0 correspond to synergistic interactions. Values for a representative experiment are shown; two others yielded equivalent results. con, control.
experiments from 12 to 24 h, after which the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained cytospin preparations under light microscopy as described in “Materials and Methods.” Values represent the means for three separate experiments ± SD.

Table 1 Induction of apoptosis by FP and LY in human leukemia cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control</th>
<th>LY</th>
<th>FP</th>
<th>FP + LY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat</td>
<td>0.6 ± 0.3</td>
<td>1.2 ± 0.8</td>
<td>2.6 ± 1.2</td>
<td>74.3 ± 6.3</td>
</tr>
<tr>
<td>CCFR</td>
<td>0.8 ± 0.4</td>
<td>2.1 ± 0.7</td>
<td>4.5 ± 1.4</td>
<td>78.8 ± 4.7</td>
</tr>
<tr>
<td>NB4</td>
<td>1.2 ± 0.5</td>
<td>2.2 ± 1.2</td>
<td>4.7 ± 1.7</td>
<td>52.1 ± 2.9</td>
</tr>
<tr>
<td>HL-60</td>
<td>2.1 ± 0.6</td>
<td>3.5 ± 1.4</td>
<td>3.8 ± 1.3</td>
<td>57.5 ± 3.5</td>
</tr>
</tbody>
</table>

Various human leukemia cell lines were exposed to LY ± FP for intervals ranging from 12 to 24 h, after which the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained cytospin preparations under light microscopy as described in “Materials and Methods.” Values represent the means for three separate experiments ± SD.

Combination with each of the CDK inhibitors for 6 h resulted in a marked increase in PARP degradation and cleavage of caspases-9, -3, and -8, as well as of Bid. The caspase-dependent nature of these events was documented by the observation that coadministration of the broad caspase inhibitor BOC-fmk, as well as the caspase-3 inhibitor DEVD-fmk, blocked FP/LY-mediated apoptosis, reflected by a marked decrease in Annexin/PI positivity (Fig. 3B) and loss of ΔΨm (Fig. 3C). Such findings also indicate that the loss of MMP in FP/LY-treated cells represents a secondary, caspase-dependent phenomenon. In addition, ectopic expression of a dominant-negative caspase-8 construct resulted in a modest, although statistically significant, reduction in FP/LY-induced apoptosis (Fig. 3D), suggesting that activation of the extrinsic pathway plays a secondary role in the lethality of this regimen.

To determine whether this interaction was restricted to U937 cells, parallel studies were conducted using several other human leukemia cell lines (Table 1). Although the drug doses and exposure intervals associated with maximal induction of cell death differed somewhat from those used in U937 cells, a marked increase in apoptosis was observed in LY/FP-treated HL-60 and NB4 promyelocytic leukemia cells, as well as Jurkat and CCRF lymphoblastic leukemia cells. Thus, combined CDK and PI3K inhibition represented a potent apoptotic stimulus in multiple leukemia cell types.

To gain insight into early mitochondrial event-associated interactions, cytochrome c release into the S-100 cytosolic fraction was monitored at early intervals in U937 cells treated with LY in combination with each of the CDK inhibitors (Fig. 4). As shown in Fig. 4A, the combined exposure of cells to 75 nM FP and 15 μM LY resulted in a clear increase in cytosolic cytochrome c release within 2 h of drug treatment. In contrast, treatment of cells with each drug alone induced minimal mitochondrial injury. Parallel results were obtained when cells were exposed to 2–6 h to roscovitine or CGP74514A in combination with LY (Fig. 4, B and C). Thus, combined exposure of human leukemia cells to pharmacological CDK inhibitors and the PI3K inhibitor LY resulted in a very early and dramatic increase in mitochondrial injury.

Consistent with evidence that antiapoptotic proteins act by attenuating mitochondrial injury (36), U937 cells ectopically expressing full-length Bcl-2 protein were completely resistant to FP/LY-mediated apoptosis at 6 h, and were very largely (although not completely) resistant at 24 h (Table 2). Moreover, cells ectopically expressing an NH2-terminal loop deleted Bcl-2 protein, which confers a high degree of resistance to various agents, including FP (37), were completely resistant to this drug combination at both early and late intervals.

Effects of coexposure (3 h) of U937 cells to CDK inhibitors and LY were then examined in relation to expression of a variety of proteins implicated in mitochondrial injury and apoptosis regulation (Fig. 5). As noted previously, coexpression of cells to each of the CDK inhibitors in combination with LY resulted in a marked increase in cytosolic release of cytochrome c, as well as the proapoptotic mitochondrial proteins Smac/DIABLO and AIF. In addition, LY/CDK inhibitor-cotreated cells displayed Bcl-2 cleavage, little change in the expression of Bcl-xL, and substantial down-regulation of the antiapoptotic proteins Mcl-1 and XIAP. Lastly, coexpression of cells to LY and each of the CDK inhibitors for 3 h resulted in reductions in levels of phospho-Bad, whereas total Bad expression remained unperturbed.

Table 2: Induction of apoptosis by FP and LY in leukemia cells ectopically expressing full-length and loop-deleted Bcl-2

<table>
<thead>
<tr>
<th>Time</th>
<th>pCEP4</th>
<th>pCEP4 ΔBcl-2</th>
<th>D9(Bcl-2)</th>
<th>D9(Bcl-2) ΔBcl-2</th>
<th>pSFFV</th>
<th>pSFFV ΔBcl-2</th>
<th>% apoptotic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>2.3 ± 0.8</td>
<td>0.8 ± 0.6</td>
<td>1.7 ± 0.9</td>
<td>0.9 ± 0.3</td>
<td>74 ± 5.4</td>
<td>1.8 ± 0.7</td>
<td>69 ± 4.4</td>
</tr>
<tr>
<td>24 h</td>
<td>2.6 ± 0.6</td>
<td>0.9 ± 0.7</td>
<td>1.8 ± 0.7</td>
<td>0.8 ± 0.6</td>
<td>87 ± 4.2</td>
<td>1.4 ± 0.3</td>
<td>84 ± 3.1</td>
</tr>
</tbody>
</table>

U937 cells ectopically expressing full-length Bcl-2 (D9(Bcl-2)), a NH2-terminal phosphorylation loop deleted mutant (ΔBcl-2), and their respective empty-vector controls (pCEP4; pSFFV) were exposed to 75 nM FP ± 15 μM LY for 6 and 24 h, after which the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained cytospin preparations under light microscopy as described in “Materials and Methods.” Values represent the means for three separate experiments ± SD.

Fig. 4. A, U937 cells were exposed to 75 nM FP ± 15 μM LY for the indicated interval, after which S-100 cytosolic fractions were isolated and cytochrome c (cyto c) expression was monitored by Western analysis as described in “Materials and Methods.” B, cells were exposed to 15 μM LY ± 75 nM FP, 15 μM CGP75417A (CGP), or 10 μM roscovitine (Ros) for 1–3 h (B) or 6 h (C), after which cytochrome c release into the S-100 cytosolic fraction was determined as described above. For A–C, each lane contained 25 μg protein; blots were subsequently stripped and reprobed with antibodies to tubulin to ensure equivalent loading and transfer of protein. In each case, the results of a representative study are shown; an additional experiment yielded equivalent results.

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Interactions between CDK inhibitors and LY were then examined in relation to effects on several cell cycle-related proteins, with an emphasis on pRb, the phosphorylation status of which is known to be regulated by cyclin D in association with various CDKs. As shown in Fig. 6, coexposure of cells to each of the CDK inhibitors in conjunction with LY for 3 h resulted in a marked diminution in pRb phosphorylation on CDK2 and CDK4 phosphorylation sites. Western analysis of total pRb protein revealed an increase in the abundance of the slowly migrating hypophosphorylated form in LY/CDK inhibitor-treated cells, as well as evidence of pRb cleavage. These findings were confirmed in studies using an antibody specific for the under-phosphorylated form of pRb. Combined treatment with LY and CDK inhibitors resulted in a marked diminution in expression of cyclin D1, but no changes in expression of cyclins A or E. In separate studies, no differences could be detected between levels of activity of CDK2/cyclin E versus CDK2/cyclin A, or in the amount of cyclin A versus cyclin E coimmunoprecipitating with CDK2 (data not shown). Finally, coexposure of cells to LY and CDK inhibitors was associated with a modest diminution in p34\textsuperscript{cdk2} phosphorylation. Thus, the exposure of cells to CDK inhibitors in combination with a PI3K inhibitor resulted in multiple perturbations in cell cycle-related proteins, including pRb dephosphorylation and cleavage, p34\textsuperscript{cdk2} activation, and cyclin D1 down-regulation.

An attempt was then made to characterize the effects of LY and CDK inhibitors, alone and in combination, on the status of various signaling pathways in U937 cells. Treatment with LY alone (3 h) modestly reduced phosphorylation of Akt on the Ser473 site (Fig. 7A), whereas the CDK inhibitors produced little effect. However, the coadministration of LY with each of the CDK inhibitors essentially abrogated expression of the phosphorylated Akt species. Total Akt expression was unchanged by any of these treatments. Combined treatment with LY and CDK inhibitors also resulted in a marked reduction in phosphorylation of GSK and FKHR, both well-established downstream targets of Akt (25). Coadministration of LY and CDK inhibitors also induced cleavage and diminished expression of phosphorylated p70\textsuperscript{S6k}, another Akt downstream target (26).

As shown in Fig. 7B, the cotreatment of cells with LY ± each of the CDK inhibitors (3 h) induced a slight reduction in levels of phosphorylated (activated) MAPK and minor effects on JNK phosphorylation. In addition, exposure of cells to LY alone resulted in a very marked increase in p38 MAPK phosphorylation/activation, a response that was not modified by coadministration of CDK inhibitors. Combined treatment of cells with LY/CDK inhibitors also did not alter the expression of other potential PI3K downstream targets, including IkB-α, IkB-β, p21\textsuperscript{Cip1}, or p27\textsuperscript{Kip1}.

To evaluate the functional significance of some of these events, and to determine whether the potentiation of FP-mediated lethality was restricted to interruption of the PI3K pathway, interactions between FP and a variety of other pharmacological inhibitors were examined in U937 cells (Table 3). Cotreatment of cells for 6 h with FP (75 nM) in combination with the PI3K inhibitor wortmannin (300 nM) also resulted in a marked increase in apoptosis. Similar results were observed in Jurkat cells. For example, in this line, exposure to 300 nM wortmannin or 100 nM FP for 12 h induced apoptosis in 3.2 ± 1.2 and 2.8 ± 1.6% of cells, respectively, whereas combined exposure resulted in apoptosis in 52.3 ± 4.7% of cells (data not shown). In contrast, potentiation of apoptosis was minimal or absent in cells exposed to the combination of FP and the MEK1/2 inhibitor U0126.
studies were performed in cells exposed to the broad caspase inhibitor 
represent secondary events stemming from caspase activation, parallel not play a critical role in enhanced lethality. MAPK, p38 MAPK, and m-TOR pathways in FP/LY-treated cells do 
cascade, and also suggest that the observed perturbations in the 
FP lethality is specifically linked to the disruption of the PI3K 
Together, these findings support the notion that early potentiation of 
but it was significantly less than that observed in the case of LY. 
to tubulin to ensure equivalent loading and transfer of protein. The results of a representative study are shown; two additional experiments yielded equivalent results. 

Fig. 7. A, U937 cells were exposed to 15 μM LY ± 75 nM FP, 1.5 μM CGP74514A (CGP), or 10 μM roscovitine (Ros) for 3 h, after which they were lysed and the proteins separated by SDS-PAGE. The blots were then probed with antibodies directed against phospho-Akt (p-Akt; Ser473), phospho-GSK 3 (p-GSK 3), phospho-FKHR (p-FKHR), or phospho p70S6K [residue threonine-389; p-p70S6K] (389)). B, cells were cultured as above, and Western analysis was used to monitor expression of phospho-ERK1/2 (p-ERK1/2), phospho-INK (p-JNK), phospho-p38 MAPK (p-p38), IκB-α, IκB-β, p27KIP1, and p21CIP1. For both A and B, lanes contained 25 μg protein; blots were subsequently stripped and reprobed with antibodies to tubulin to ensure equivalent loading and transfer of protein. The results of a representative study are shown; two additional experiments yielded equivalent results.

Table 3  Effect of inhibitors of various signal transduction pathways on FP lethality in leukemia cells

<table>
<thead>
<tr>
<th></th>
<th>% apoptotic cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>FP (75 nM)</td>
</tr>
<tr>
<td>Control</td>
<td>0.6 ± 0.3</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>Wortmannin (300 nM)</td>
<td>2.3 ± 1.1</td>
<td>57.4 ± 3.8</td>
</tr>
<tr>
<td>U0126 (25 μM)</td>
<td>0.8 ± 0.4</td>
<td>4.3 ± 1.1</td>
</tr>
<tr>
<td>SB202190 (10 μM)</td>
<td>0.7 ± 0.5</td>
<td>5.4 ± 1.3</td>
</tr>
<tr>
<td>SP600125 (25 μM)</td>
<td>1.2 ± 0.7</td>
<td>12.4 ± 2.6</td>
</tr>
<tr>
<td>Rapamycin (10 nM)</td>
<td>1.1 ± 1.6</td>
<td>6.2 ± 2.3</td>
</tr>
<tr>
<td>H-89 (5 μM)</td>
<td>1.2 ± 0.9</td>
<td>7.2 ± 2.6</td>
</tr>
<tr>
<td>Caffeine (5 mM)</td>
<td>3.3 ± 1.9</td>
<td>6.4 ± 3.0</td>
</tr>
<tr>
<td>GFX (1 μM)</td>
<td>0.6 ± 0.4</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>LY294002 (15 μM)</td>
<td>1.1 ± 0.9</td>
<td>68.4 ± 4.7</td>
</tr>
</tbody>
</table>

U937 cells were exposed for 6 h to the indicated concentration of various inhibitors (e.g., U0126-MEK1/2; wortmannin-P13K; SB202190-p38 MAPK; SP600125-JNK; rapamycin-mTOR; H-89-PI3K; caffeine-ATM; LY294002-P13K) in the absence or presence of 75 nM FP. At the end of the incubation period, the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained cytoxin preparations under light microscopy as described in “Materials and Methods.” Values represent the means for three separate experiments ± SD.

(38), the p38 MAPK inhibitor SB202190 (39), the m-TOR inhibitor rapamycin (40), the PKA inhibitor H-89 (41), or the PKC inhibitor GFX (42). In addition, the lethal effects of FP were not enhanced by coadministration of caffeine, arguing against the possibility that the effects of LY or wortmannin involve the ATM/Chk1 axis. Importantly, coadministration of FP with the JNK inhibitor SP600125 (25 μM; Ref. 43) did lead to a slight increase in apoptosis (e.g., ~12%), but it was significantly less than that observed in the case of LY. Together, these findings support the notion that early potentiation of FP lethality is specifically linked to the disruption of the PI3K pathway, and that the observed perturbations in the MAPK, p38 MAPK, and m-TOR pathways in FP/LY-treated cells do not play a critical role in enhanced lethality.

Because perturbations in LY/CDK inhibitor-treated cells could represent secondary events stemming from caspase activation, parallel studies were performed in cells exposed to the broad caspase inhibitor BOC-fmk (Fig. 8). As anticipated, the addition of BOC-fmk blocked procaspase-3 cleavage. It did not, however, prevent the release of cytochrome c or AIF, indicating that these represent primary, caspase-independent events (Fig. 8A). In contrast, Sma/MADIBLO release in FP/LY-treated cells was blocked by BOC-fmk, demonstrating the caspase-dependence of this phenomenon. Also, as anticipated, Bcl-2 cleavage was abrogated by BOC-fmk, although down-regulation of Mcl-1 and XIAP were unaltered. Down-regulation of cyclin D1 and cleavage of phospho-p70S6K were also blocked by BOC-fmk. Finally, cleavage of total and underphosphorylated pRb was prevented by caspase inhibition, as expected, whereas the attenuation of pRb phosphorylation on CDK2- and CDK4-specific sites was only partially reversed by the administration of the caspase inhibitor (Fig. 8B). These findings indicate that FP/LY-mediated perturbations in the cell cycle and apoptotic regulatory events proceed through both caspase-dependent and -independent pathways.

To characterize the functional role of perturbations in Akt in the apoptotic response of leukemia cell to FP, a Jurkat cell line that inducibly expresses a constitutively active myristolated Akt mutant in the presence of doxycycline was used. As shown in Fig. 9A, addition of doxycycline to the medium resulted in expression of the Myc tag and induction of activated (phosphorylated) Akt on the Ser473 site. Moreover, in the presence of doxycycline, marked Akt phosphorylation was noted in cells exposed to LY ± FP (12 h), in effect circumventing the blockade of Akt activation by LY (Fig. 9B). After exposure to 250 nM FP alone for 12 h, apoptosis was partially but significantly attenuated when cells were cultured in the presence of doxycycline, which resulted in a clear increase in Akt phosphorylation (P < 0.02; Fig. 9C). Moreover, an even greater protective effect was observed when cells were exposed to the combination of LY + FP (P < 0.002 versus cells exposed to drugs in the absence of doxycycline). Together, these findings suggest that FP-mediated lethality is regulated, at least in part, by Akt, and that interruption of the Akt pathway contributes to the potentiation of FP-induced apoptosis by LY.
Finally, to determine whether such events were restricted to continuously cultured cell lines, parallel studies were performed in primary leukemic blasts obtained from the peripheral blood of a patient with AML (M2 subtype). As shown by the histograms in Fig. 10A, 24-h exposure to 15 μM LY or 100 nM FP alone resulted in a modest degree of apoptosis, reflected by annexin V and annexin V/PI positivity (i.e., 3 and 6%, respectively). However, cotreatment of blasts with FP and LY induced apoptosis in the large majority of leukemic blasts (i.e., 80%; Fig. 10B). These findings indicate that the dramatic potentiation of cell death in leukemic cells that are simultaneously exposed to a CDK inhibitor in conjunction with a PI3K inhibitor occurs in at least some primary AML specimens. On the other hand, treatment of normal peripheral blood mononuclear cells with identical concentrations of FP and LY, either alone or in combination, induced little apoptosis at either the 6- or 24-h interval (Fig. 10C), raising the possibility that this strategy may offer the potential for therapeutic selectivity.

**DISCUSSION**

In view of evidence linking dysregulation of cell cycle progression to apoptosis (10), it is tempting to invoke this mechanism to explain the ability of FP and other pharmacological CDK inhibitors to induce cell death in leukemia and other neoplastic cell types. However, identification of the specific cell cycle events responsible for CDK inhibitor-mediated lethality remains an elusive goal. An alternative possibility is that FP and similar agents (e.g., UCN-01) act by modulating expression of certain antiapoptotic proteins (e.g., Mcl-1 and XIAP; Ref. 16). However, it is unlikely that such a mechanism could entirely explain the early induction of mitochondrial injury and apoptosis by FP and other CDK inhibitors (e.g., CGP74514A) that has been reported (9). A third possibility is that the induction of cell death by CDK inhibitors like FP is regulated by the activity of one or more signal transduction pathways, which are known to play critical roles in cell survival decisions (21). Of the various possibilities, activation of the MEK1/2/MAPK and the PI3K signaling pathways, both of which are intimately involved in promoting cell survival (21, 22), represent two plausible candidates. In this context, exposure of human leukemia cells to UCN-01, which inhibits CDKs in addition to PKC and Chk1 (44), has recently been shown to trigger an early and dramatic increase in MAPK activity (45). Moreover, interference with the latter process (e.g., by MEK1/2 inhibitors) results in a striking potentiation of UCN-01-mediated mitochondrial injury and apoptosis (45). However, in marked contrast to these findings, the CDK inhibitors FP, CGP74514A, and roscovitine failed to elicit a major MAPK response, nor did coadministration of MEK1/2 inhibitors enhance CDK-mediated lethality. Together, these findings suggest that the PI3K cytoprotective signaling pathway, rather than the MEK1/2/MAPK pathway, plays a potentially specific role in attenuating mitochondrial damage and apoptosis in leukemic cells subjected to dysregulated CDK function.

In contrast to the failure of the MEK1/2 inhibitor U0126 to promote FP-mediated lethality, LY and wortmannin, both of which inhibit
PI3K (29, 30), strikingly enhanced the lethal effects of each of the CDK inhibitors. These interactions were noteworthy for (a) the extent of synergism; (b) the very early induction of mitochondrial damage (i.e., within 2–3 h); and (c) the ability to extend this phenomenon to include multiple leukemic cell types. Whereas FP acts broadly to inhibit CDKs 1, 2, 4/6, 7, and 9 (5–7), roscovitine and CGP are somewhat more specific for CDK1 (46, 47). Whether inhibition of this CDK is required or sufficient for synergistic interactions with PI3K inhibitors remains to be determined. Although LY and wortmannin are viewed as PI3K inhibitors, they also inhibit several other enzymes involved in cell cycle regulation and DNA repair, including ATM/ATM and Rad3-related (48) and DNA-PK (49). However, the failure of the ATM inhibitor caffeine to potentiate FP-induced lethality argues against a role for dysregulation of ATM in the CDK/PI3K inhibitor interaction. Nevertheless, the possibility that the other actions of LY or wortmannin contribute to the potentiation of CDK inhibitor lethality cannot be ruled out. Lastly, the striking increase in p38 MAPK activation in LY-treated cells (Fig. 7) is consistent with previous reports in the literature (50). However, the inability of the p38 MAPK inhibitor SB202190 (39) to promote FP toxicity suggests...
The finding that FP/LY-induced apoptosis was substantially blocked by ectopic expression of Bcl-2 or by an NH2-terminal phosphoerbory loop-deleted protein is consistent with a mitochondrial basis for the lethality of this drug combination. Although the precise mechanism by which Bcl-2 and related proteins block activation of the apoptotic cascade remains the subject of some controversy, there is strong evidence that Bcl-2 and Bcl-xL interfere with interactions between BAX-only members of the Bcl-2 family and proapoptotic multidomain family members such as Bak and Bak, thereby preventing the release of proapoptotic mitochondrial proteins (51). The roles that such mitochondrial proteins play in triggering apoptosis may vary between cell types, and even within the same cell depending on the stimulus. For example, in multiple myeloma cells, dexamethasone and ionizing radiation exerted divergent effects on the release of cytochrome c and Smac/DIABLO (52), a pro-apoptotic protein that acts by antagonizing the actions of members of the XIAP family of caspase inhibitors (53). In contrast, Smac-DIABLO release has recently been shown to play a critical role in facilitating cytochrome c release in prostate carcinoma cells exposed to LY (54). In the present study, coadministration of FP with LY in U937 cells induced the early release of each of the major proapoptotic mitochondrial proteins, i.e., cytochrome c, Smac/DIABLO, and AIF. However, the observation that release of Smac/DIABLO, in contrast to the other mitochondrial proteins, was inhabitable by caspase inhibitors suggests that, in these cells, Smac/DIABLO redistribution plays a secondary role in the initiation of the apoptotic cascade. Lastly, the observation that ectopic expression of dominant-negative caspase-8 exerted only modest effects on LY/FP-mediated apoptosis, at least compared with that of Bcl-2, suggests that engagement of the intrinsic, mitochondrial cell death pathway is primarily involved in the lethality of this drug combination. Nevertheless, the possibility that activation of the extrinsic pathway plays a role in amplification of the apoptotic process, i.e., through activation of BID, as occurs in the case of certain cytotoxic drugs (55), cannot be excluded.

Because Akt represents a major downstream target of PI3K (21), and has been linked, through both indirect and direct mechanisms, to a wide variety of antia apoptotic functions (25, 26, 56), it is tempting to speculate that LY promotes the lethal effects of CDK inhibitors by blocking the activation of Akt and one or more of its downstream targets, thereby lowering the threshold for mitochondrial damage and apoptosis. For example, Akt has been implicated in posttranslational modification of Bad (57), regulation of the expression of antia apoptotic proteins including Bcl-2 and XIAPs (58), and modulation of diverse pathways governing cell survival decisions, including those associated with GSK (59), m-TOR/p70S6K (60), and nuclear factor-κB (61), among numerous others (23–25). In this regard, the finding that rapamycin, an inhibitor of m-TOR, and by extension, p70S6K, failed to enhance FP lethality argues against a critical role for this pathway in the regulation of apoptosis. FP/LY did not substantially modify expression of the antia apoptotic proteins Bcl-2 or Bcl-xL, although cells exposed to both agents did exhibit marked down-regulation of XIAP and Mcl-1. Such actions are similar to those described in chronic lymphocytic leukemia and multiple myeloma cells exposed to FP alone (16, 62) and raise the possibility that the interruption of the PI3K pathway may increase the ability of FP to down-regulate expression of certain antia apoptotic proteins. Co-administration of CDK inhibitors and LY also led to diminished Bad phosphorylation, an event that spares Bad from proteasomal degradation and permits it to associate with and antagonize the ability of antia apoptotic Bcl-2 family members such as Bcl-xL to protect cells from mitochondrial injury (63). It is, therefore, conceivable that reduced phosphorylation of Bad contributed to the potentiation of CDK inhibitor-mediated apoptosis by LY.

The observation that enforced expression of Akt attenuated, at least in part, LY/FP-mediated lethality is consistent with reports that the interruption of the PI3K pathway can potentiate the response of tumor cells to various conventional cytotoxic agents. For example, LY has been shown to enhance the activity of multiple conventional cytotoxic agents (e.g., paclitaxel, gemcitabine, VP-16, doxorubicin) in diverse neoplastic cell types (e.g., ovarian, pancreatic, leukemia, non-small cell lung cancer; Refs. 64–67) However, to the best of our knowledge, enforced activation of Akt has not previously been shown to protect cells from the lethal actions of agents that specifically target the cell cycle, i.e., pharmacological CDK inhibitors. In addition, the striking ability of minimally toxic concentrations of LY to induce mitochondrial injury and apoptosis in cells exposed to subtoxic or nontoxic concentrations of FP may have particular significance. It is noteworthy that, whereas the induction of Akt exerted a very significant protective effect against LY/FP-related lethality, protection was not complete, raising the possibility that factors other than, or in addition to, Akt inhibition may be involved in this phenomenon. In this context, other examples of PI3K-dependent survival functions that operate independently of Akt have been described. For example, the ability of glucagon-like peptide 2 to circumvent LY-mediated lethality in baby hamster kidney cells transfected with the glucagon-like peptide 2 receptor was associated with Bad phosphorylation but occurred independently of Akt activation (68). Interestingly, in this system, survival events were blocked by the PKA inhibitor H89, whereas in the present studies, H89 failed to mimic the potentiation of CDK inhibitor lethality by LY. Together, these findings suggest that signaling cascades that regulate the lethal response to PI3K interruption vary with cell type and/or the inciting stimulus. Other recently described PI3K-dependent, Akt-independent downstream targets include the small GTP-binding proteins CDC42 and RAC1 as well as the serum and glucocorticoid-inducible kinases (SGK), including the cytokine-independent survival kinase (CISK; Refs. 69–71). Whether any of these plays a role in the potentiation of CDK inhibitor-mediated lethality by PI3K interruption will require further investigation.

In summary, the present findings indicate that the pharmacological interruption of the PI3K pathway dramatically lowers the threshold for the early initiation of mitochondrial injury, caspase activation, and apoptosis in multiple leukemia cell types in response to a variety of pharmacological CDK inhibitors. Such findings suggest that PI3K, and one or more of its downstream targets, play a particularly important role in preventing apoptosis in leukemic cells experiencing CDK dysregulation. In addition, these and earlier studies focusing on interactions between the Chk1 inhibitor UCN-01 and MEK1/2 inhibitors (45) provide further evidence that leukemic cells are particularly vulnerable to a strategy in which cell cycle and survival signaling events are simultaneously disrupted. In this regard, it may not be coincidental that both the UCN-01/MEK inhibitor and FP/LY regimens induced dephosphorylation of p34cdc2, unscheduled activation of which has been associated with enhanced lethality (72). The frequency with which dysregulation of the PI3K pathway is associated with malignant transformation (22, 25, 26), as well as evidence that PI3K inhibitors can enhance the lethality of conventional chemotherapeutic drugs (64–67) have prompted the search for clinically relevant agents that interrupt the PI3K/Akt pathway (73). In this context, the dramatic potentiation of CDK inhibitor-mediated cytotoxicity by pharmacological PI3K antagonists, and the striking synergism of this regimen against multiple leukemia cell types, suggest that efforts to interrupt PI3K/Akt signaling cascades may be particularly effective when combined with novel, clinically relevant inhibitors of cell cycle.
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progression. Given these considerations, attempts to identify the specific PI3K targets responsible for such actions are clearly warranted. Accordingly, such studies are currently underway.

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The Lethal Effects of Pharmacological Cyclin-dependent Kinase Inhibitors in Human Leukemia Cells Proceed through a Phosphatidylinositol 3-Kinase/Akt-dependent Process

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