DOT1L Inhibitor EPZ-5676 Displays Synergistic Antiproliferative Activity in Combination with Standard of Care Drugs and Hypomethylating Agents in MLL-Rearranged Leukemia Cells

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List of nonstandard abbreviations:

ALL      Acute Lymphoblastic Leukemia
AML      Acute Myeloid Leukemia
BSA      Bovine Serum Albumin
CI       Combination index
DMSO     Dimethyl sulfoxide
DNMT     DNA methyltransferase
Fa       Fractional effect
<table>
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<th>Acronym</th>
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<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
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<tr>
<td>H3K79</td>
<td>Histone H3 at Lysine 79</td>
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<tr>
<td>HDAC</td>
<td>Histone deacetylase</td>
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<td>HDM</td>
<td>Histone demethylase</td>
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<td>HMT</td>
<td>Histone methyltransferase</td>
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<tr>
<td>IMDM</td>
<td>Iscove’s Modified Dulbecco’s Medium</td>
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<tr>
<td>MLL-r</td>
<td>MLL-rearranged</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<tr>
<td>PMT</td>
<td>Protein methyltransferase</td>
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<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute Medium</td>
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<td>SOC</td>
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**Recommended section assignment:** Drug Discovery and Translational Medicine
Abstract

EPZ-5676, a small molecule inhibitor of the protein methyltransferase DOT1L, is currently under clinical investigation for acute leukemias bearing MLL-rearrangements (MLL-r). In this study, we evaluated EPZ-5676 in combination with standard of care (SOC) agents for acute leukemias as well as other chromatin modifying drugs in cellular assays with three human acute leukemia cell lines MOLM-13 (MLL-AF9), MV4-11 (MLL-AF4) and SKM-1 (non-MLL-r). Studies were performed to evaluate the anti-proliferative effects of EPZ-5676 combinations in a co-treatment model where the second agent was added simultaneously with EPZ-5676 at the beginning of the assay or, in a pre-treatment model where cells were incubated for several days in the presence of EPZ-5676 prior to the addition of the second agent. EPZ-5676 was found to act synergistically with the acute myeloid leukemia (AML) SOC agents, cytarabine or daunorubicin in MOLM-13 and MV4-11 MLL-r cell lines. EPZ-5676 is selective for MLL-r cell lines as demonstrated by its lack of effect either alone or in combination in the non-rearranged SKM-1 cell line. In MLL-r cells, the combination benefit was observed even when EPZ-5676 was washed out prior to the addition of the chemotherapeutic agents, suggesting that EPZ-5676 sets up a durable, altered chromatin state that enhances the chemotherapeutic effects. Our evaluation of EPZ-5676 in conjunction with other chromatin modifying drugs also revealed a consistent combination benefit including synergy with DNA hypomethylating agents. These results indicate that EPZ-5676 is highly efficacious as a single agent and synergistically acts with other chemotherapeutics including AML SOC drugs and DNA hypomethylating agents in MLL-r cells.
Introduction

Leukemias bearing recurrent translocations at the 11q23 locus are collectively referred to as MLL-r leukemia and the occurrence of this genetic lesion is associated with a poor prognosis. Overall survival at 5 years for adult AML patients harboring balanced 11q23 translocations is between 10 and 20% (Byrd et al., 2002). Patients with this form of acute leukemia are currently treated by chemotherapy, most commonly using cytarabine (Ara-C) and daunorubicin, or by hematopoietic stem cell transplantation (Burnett et al., 2011; Mrozek et al., 1997; Tamai and Inokuchi, 2010). Either treatment modality is associated with a relatively poor response rate of approximately 45% among MLL-r patients (Byrd et al., 2002; Balgobind et al., 2009; Mrozek et al., 1997). Therefore, new treatment modalities for MLL-r have been of great interest to the clinical community.

Translocations at the 11q23 locus target the MLL gene and result in the expression of an oncogenic fusion protein comprising the amino-terminus of MLL fused in frame to one of over 70 potential fusion partners. The vast majority of such fusion partners are derived from the AF or ENL families of proteins (Hess, 2004; Kristov and Armstrong, 2007; Neff and Armstrong, 2013; Slany, 2009). The MLL protein is a transcriptional regulator with histone methyltransferase activity specific for histone H3 at lysine 4 (H3K4). In the context of the MLL-r associated fusion proteins, MLL loses its catalytic domain. However, a unifying feature of many of the common MLL fusion partner proteins is the ability to bind to another histone methyltransferase (HMT) known as DOT1L (Bitoun et al., 2007; Biswas et al., 2011; Mohan et al., 2010; Mueller et al., 2007; Okada et al., 2005; Park et al., 2010; Yokoyama et al., 2010). In this manner, DOT1L is recruited to gene locations normally under the control of MLL (Monroe et al., 2011, Mueller et
al., 2007; Okada et al., 2005). DOT1L catalyzes the specific methylation of histone H3 at lysine 79 (H3K79), and this site-specific marking of histone H3 leads to transcriptional activation (Guenther et al., 2008; Kristov et al., 2008; Milne et al., 2005, Monroe et al., 2011, Mueller et al., 2009; Nguyen et al., 2011; Okada et al., 2005; Thiel et al., 2010). It has therefore been speculated that DOT1L enzymatic activity represents an oncogenic driver of MLL-r leukemia and that inhibition of the DOT1L enzyme would represent a cogent approach to therapeutic intervention for MLL-r patients. This hypothesis has gained significant support recently through the demonstration that genetic knock down of DOT1L message or small molecule inhibition of DOT1L catalysis lead to robust anti-proliferative effects in MLL-r leukemia cells, with minimal effect on non-MLL-r cells, both in vitro and in vivo (Basavapathruni et al., 2012; Bernt et al., 2011; Chen et al., 2013; Daigle et al., 2011, 2013; Desphande et al., 2013; Nguyen et al., 2011; Yu et al., 2012). Daigle et al. (2013) reported the discovery of an extremely potent and selective DOT1L inhibitor, EPZ-5676. EPZ-5676 represents the first PMT inhibitor to be tested in human clinical trials. In 2012, phase 1 clinical testing of EPZ-5676 was initiated.

EPZ-5676 has demonstrated robust, single-agent activity as an anti-proliferative drug in preclinical in vitro and in vivo models of MLL-r leukemias (Daigle et al., 2013). Nevertheless, there is a growing trend in clinical practice of combining anti-cancer agents to affect maximal benefit for cancer patients. With this in mind, we wished to understand how EPZ-5676 might behave in the context of combination with current SOC treatments for AML, as well as in combination with another class of epigenetic-targeted drugs, such as the currently approved DNA methyltransferase (DNMT) inhibitors. Here we report the results of these studies. We find that EPZ-5676 acts synergistically with the AML SOC chemotherapeutic agents Ara-C and
daunorubicin as well as with the DNMT inhibitors azacitidine and decitabine. Remarkably, this synergy is observed not only when the EPZ-5676 and the second drug are co-administered to cells, but also when cells are pretreated with EPZ-5676, this compound is washed out, and then the second drug is applied to the cells. Consistent with the hypothesized mechanism of action of EPZ-5676, we did not observe single-agent activity for this compound, nor did we observe any synergy with other agents in leukemia cells that do not bear an 11q23 chromosomal rearrangement.
Methods

Cell Lines

The biphenotypic leukemia cell line MV4-11 (MLL-AF4) (CRL-9591) was obtained from American Type Culture Collection (Manassas, VA). AML cell lines MOLM-13 (MLL-AF9) (ACC 554) and SKM-1 (ACC 547) were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). MV4-11 cells were maintained in Iscove’s Modified Dulbecco’s Medium (IMDM) supplemented with 10% fetal bovine serum (FBS). MOLM-13 and SKM-1 cells were maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% FBS. They were cultured in flasks or plates in a humidified 37 °C, 5% CO2 atmosphere.

Compounds

EPZ-5676 was synthesized by Epizyme. Ara-C, vincristine, and prednisolone were purchased from SelleckChem (Houston, TX). Mitoxantrone, daunorubicin and azacitidine were purchased from Sigma (St. Louis, MO). LSD1-inhibitor II and tranylcypromine were purchased from EMD-Millipore (Billerica, MA). Mafosfamide was purchased from Santa Cruz Biotechnology (Dallas, TX). JQ-1 was purchased from Cayman Chemical (Ann Arbor, MI).

Proliferation Assays

Proliferation studies were performed using MOLM-13, MV4-11 and SKM-1 cell lines in vitro to evaluate both the combinatorial effect of compounds on cell killing and the durability of the effect by washing out one of the agents. For all assays described below, the cell counts were
measured by ATP quantitation using the CellTiter-Glo® reagent from Promega (Madison, WI) and luminescence values corresponded to the amount of ATP in a given well.

SOC drugs were tested in combination with EPZ-5676 to study their effect on cell proliferation in a pre-treatment model, with or without washout, or in a co-treatment model. The co-treatment model was used to further investigate the combinatorial effect of EPZ-5676 with other agents of interest.

Prior to performing the combination experiments, the IC₅₀ value of each compound was determined in each cell line which served to appropriately bracket the concentration ranges around this half-maximal inhibition value in the combination matrix.

In the pre-treatment model exponentially growing cells were seeded in flasks (5 x 10⁴ cells per ml for MV4-11 and 3 x 10³ cells per ml for MOLM-13) and pre-treated with seven concentrations of EPZ-5676 or DMSO for several days followed by co-treatment with EPZ-5676 and SOC. MV4-11 assays were a 4+3 model and MOLM-13 assays were a 7+3 model. Pre-treatment concentrations of EPZ-5676 were tested by performing a series of two-fold dilutions of EPZ-5676 with a top concentration of 500 nM and 650 nM for MV4-11 and MOLM-13 cells, respectively. On day four (MV4-11) or seven (MOLM-13), cell densities were normalized to 5 x 10⁴ cells per ml and cells were then plated to a 96 well plate for the three day co-treatment phase, containing EPZ-5676 with the second agent in a matrix format. Assay ready plates were prepared with the HP-D300 digital compound dispenser (Tecan, Männedorf, Switzerland). The combinatorial matrix allowed for several constant ratios to be interrogated simultaneously. The constant ratio was calculated by normalizing the concentration of the second test compound to EPZ-5676, e.g. EPZ-5676 at 50 nM to the second combination agent at
100 nM, was calculated as a 1:2 constant ratio. The concentration matrix was designed to evaluate the single agent activity by treating cells with one agent paired with DMSO, which was normalized to 0.1% v/v across the plate. When washout experiments were performed, EPZ-5676 was excluded in the 3-day incubation after pre-treatment.

The co-treatment model was established to understand the effect of simultaneous dosing of both agents beginning at time zero and ending on day seven. In the co-treatment model, exponentially growing MV4-11, MOLM-13 or SKM-1 cells were seeded in 96-well plates at 1.25 x 10⁴, 3 x 10³, and 6 x 10³ cells per ml, respectively, and treated for seven days with the combination of EPZ-5676 and a compound of interest in a matrix format as previously described. The top concentration of EPZ-5676 tested was 50 nM, 250 nM and 4000 nM for MV4-11, MOLM-13 and SKM-1, respectively, and compounds were pre-diluted in DMSO in a two-fold dilution series for a total of eight concentrations tested.

A third model was established to study the effect of addition of Ara-C by measuring the ten day proliferation of cells pretreated with Ara-C in a 3 + 7 model. This experiment was performed by first pretreating MOLM-13 cells with increasing concentrations of Ara-C for three days (concentration ranges: 1.9-250 nM). Ara-C was then washed out, the cell numbers were normalized and either treated with EPZ-5676 alone or co-treated with EPZ-5676 and Ara-C in a matrix format for additional seven days.

For all experimental models, maximum and minimum inhibition (dimethyl-sulfoxide (DMSO) alone) controls were used in each plate to calculate fraction affected (Fa) of a test well. DMSO concentration was kept at 0.1% v/v. The drug combination analysis was performed as described in the Data Analysis section.
Cell Treatment for Analysis of Mechanism of Cell Death Studies

To understand the mechanism of cell death, MOLM-13 cells were seeded in flasks at $3 \times 10^3$ cells per mL and pre-treated with EPZ-5676 (625 nM to 10 nM, two-fold serial dilutions) for seven days. On day seven, cells were split back and normalized to a seeding density of $5 \times 10^4$ cells per mL. Cells were then treated with EPZ-5676 (78 and 156 nM) alone, in combination with Ara-C (31 and 63 nM) or in combination with daunorubicin (7.5 and 15 nM) to induce a synergistic anti-proliferative response. Again, on day ten, cells were normalized and redosed with individual compound or a combination of EPZ-5676 and SOC. Samples of cells were taken at day 7, 10 and 14.

Flow Cytometric Analysis of Cell Cycle and Annexin V

Flow cytometric analysis was performed to evaluate the fraction of cells in each phase of the cell cycle and to determine cell death by apoptosis. Cells were treated (as described in the Cell Treatment for Analysis of Mechanism of Cell Death section) alone or in combination with EPZ-5676 and SOC and harvested on days 7, 10 and 14 and samples were divided to allow simultaneous analysis of cell cycle and Annexin V staining. Apoptosis was determined using the Guava Nexin Assay (Millipore, Billerica, MA) and cells were prepared according to the manufacturer’s recommendations. Samples were analyzed using the Guava EasyCyte Plus System (Millipore, Billerica, MA). For cell cycle analysis, cells were pelleted by centrifugation at 200 x g for 5 minutes at 4 °C, washed twice with ice cold phosphate buffered saline (PBS) then fixed with 70% ice cold ethanol. Following fixation, cells were washed with PBS and stained with the Guava cell cycle reagent (Millipore) for 30 minutes. Samples were analyzed using the Guava EasyCyte Plus System.
**Analysis of CD11b and CD14 Expression by Flow Cytometry**

MOLM-13 cells were treated in the presence of EPZ-5676, Ara-C and daunorubicin as single agents or in combination of EPZ-5676 and Ara-C or daunorubicin (as described in the *Cell Treatment for Analysis of Mechanism of Cell Death* section). On day 7, 10, and 14, cells were collected for analysis. Samples were washed twice with PBS followed by fixation in 4% formaldehyde for ten minutes at 37°C. After fixation, cells were washed and blocked with blocking buffer (0.5% bovine serum albumin in PBS) for 10 minutes at room temperature. Cells (5 x 10^5) were then incubated in the presence of mouse anti-CD14 antibody, mouse anti-CD11b antibody or mouse anti-IgG isotype control, all FITC labeled (purchased from Millipore, Billerica, MA) for 1 hour at room temperature while rotating. Cells were washed, resuspended in PBS and 5,000 events were analyzed using ExpressPro software on the Guava EasyCyte Plus System.

**Quantitative PCR**

MOLM-13 cells were treated with DMSO, 78 nM or 40 nM of EPZ-5676 for 7 days. Cells were split to 50,000 cells/mL and retreated with DMSO, 78 nM or 40 nM of EPZ-6438 alone or in combination with 78 nM EPZ-5676 + 31 nM Ara-C or 40 nM EPZ-6438 + 3.8 nM Daunorubicin for 3 additional days. Cells were harvested and total mRNA was extracted from cell pellets using the RNeasy Plus Mini Kit (Qiagen; 74134). cDNA was made using the High Capacity cDNA Reverse Transcriptase Kit (Invitrogen; 4368813). TaqMan probe based qPCR was carried out with the ViiA 7 Real-Time PCR Systems (Applied Biosystems [AB]) using TaqMan Fast Advanced Master Mix (AB; 4444964) and TaqMan primer/probe sets for β-actin, CD11b, and CD14 (Invitrogen; 4333762F, Hs00355885_m1, Hs02621496_s1). Gene expression
was normalized to housekeeping gene RPLP0 (AB; 4333761F) and fold change compared to DMSO was calculated using the ΔΔCt method.

Data Analysis

The drug combination analysis was performed using the Chou-Talalay method (Chou TC, 2006). Synergy quantification is performed using the Calcusyn for Windows version 2.1 software (Biosoft, Cambridge, UK). The Combination Index equation offers a quantitative definition for additivity (CI=1), synergism (CI < 1), and antagonism (CI > 1). This equation uses fractional effect (Fa) values from a constant ratio of drug combination to determine CI values. The resulting plot (Fa-CI) plot shows the resultant CI values bracketed by 95% confidence intervals. CI values < 1 with confidence interval lines also below 1 indicate statistically significant synergism. For combination studies in the MOLM-13 and MV4-11 cell lines the 50% inhibitory concentrations of the single agents were referred to as Dm (rather than IC50). Additionally, synergism and antagonism were described based on the CI values according to the Chou and Talalay guidelines (CI values 0-0.1 Very strong synergism, 0.1–0.3 Strong synergism, 0.3–0.7 Synergism, 0.7–0.85 Moderate synergism, 0.85–0.90 Slight synergism, 0.90–1.10 Nearly additive, 1.10–1.20 Slight antagonism, 1.20–1.45 Moderate antagonism, 1.45–3.3 Antagonism, 3.3–10 Strong antagonism, 10 Very strong antagonism).

For combinations of EPZ-5676 and a test compound in the SKM-1 cell line, where only the latter compound showed more than 50% inhibition as single agent, dose responses were plotted using Graphpad Prism and 50% inhibitory concentrations were interpolated from the 4-parameter logistic model. The quantitation of the anti-proliferative effect between the two compounds was calculated as fold potency shift of the test compound in the presence of several concentrations of EPZ-5676. Combination benefit or enhancement of the anti-proliferative activity was
demonstrated when the presence of EPZ-5676 produced a leftward shift of the IC₅₀. In contrast, antagonism was demonstrated by a rightward shift of the IC₅₀. This shift was considered significant when confidence intervals for the calculated IC₅₀ values in the presence of EPZ-5676 did not overlap with those of the IC₅₀ value of the test compound alone. For experiments in the SKM-1 cell line with combinations of EPZ-5676 with Ara-C, daunorubicin or azacitidine, the 1/α constant was calculated to quantitate further the combinatorial effect (methods for calculation of α and its reciprocal value are described in the supplemental text).
Results

EPZ-5676 Induces a Synergistic and Durable Anti-Proliferative Effect in Combination with AML Standard of Care Drugs

We evaluated the effect of EPZ-5676 on the proliferation of the MLL-r leukemia cell lines MOLM-13 (AML) and MV4-11 (biphenotypic leukemia) when used in combination with the SOC drugs for AML, Ara-C and daunorubicin. We have previously shown that the anti-proliferative effects of EPZ-5676 on cultured cells require several days to manifest (Daigle et al., 2013). Proliferation assays were, therefore, performed over a period of 7 – 10 days. We have previously demonstrated that EPZ-5676 treatment of MV4-11 cells decreased histone H3 lysine 79 methylation and expression of key MLL fusion target genes such as HOXA9 (Daigle et al Blood 2013). We confirmed that this was also the case in MOLM-13 cells (Supplemental Figure 4). To evaluate combination effects cells were treated according to the pre-treatment model described in Materials and Methods (i.e. treatment with EPZ-5676 alone for 4 – 7 days followed by co-treatment with EPZ-5676 plus SOC agent for 3 days). Synergistic anti-proliferative activity was observed with both Ara-C and daunorubicin under this regimen (Figure 1). The synergistic anti-proliferative activity of EPZ-5676 in combination with AML SOC agents was also observed when cells were treated according to the co-treatment model described in Materials and Methods (i.e., treatment with EPZ-5676 plus SOC agent for 7 days; see Table 1). Intriguingly, synergistic anti-proliferative activity, albeit somewhat reduced when compared with co-treatment, was observed in MOLM-13 and MV4-11 MLL-r cells even when EPZ-5676 was removed (i.e., washed out) prior to the addition of the SOC agent (Figure 2). These data are remarkable in that they imply a durable reprogramming of the epigenetic status of
these cells by EPZ-5676 that renders them more acutely sensitive to chemotherapeutic agents, even when the DOT1L inhibitor has been removed from the cellular environment. This result is consistent with the kinetics of EPZ-5676 effect on histone methylation at the DOT1L substrate site, H3K79. In previous studies, we have shown that 4 days of treatment with EPZ-5676 is sufficient to deplete cellular levels of H3K79me2 by ≥ 80% (Daigle et al., 2013). H3K79 methylation remained depleted for 3 days following subsequent removal of EPZ-5676 by wash out. After this 3-day latency period, the level of H3K79me2 slowly returned to pre-treatment levels over the course of an additional 4 days. Hence, treatment of MLL-r cells with EPZ-5676 results in durable inhibition of H3K79 methylation, which in turn results in sensitization of these cells to chemotherapy-induced cell killing.

To further test the flexibility of dosing schedules that might afford synergistic cell killing, we pre-treated MOLM-13 cells with the chemotherapeutic agent Ara-C for 3 days, washed this drug out and then treated the cells with EPZ-5676 for an additional 7 days. As illustrated in Figure 3, this sequential treatment schedule resulted in essentially the same level of synergistic cell killing as seen when both drugs were co-administered to cells simultaneously. These results offer the possibility of a highly flexible dosing schedule for combinations of EPZ-5676 and chemotherapies. While both single agent activity and strong synergy with Ara-C and daunorubicin were seen for EPZ-5676 in the MLL-r cell lines MV4-11 and MOLM-13, no effect of EPZ-5676 was observed in the non-MLL-r AML cell line SKM-1. EPZ-5676 showed no single agent activity in SKM-1 cells, nor did it affect the anti-proliferative activity of either chemotherapeutic agent in this cell line (Supplemental Figure 1). The lack of activity of EPZ-5676 in SKM-1 cells is completely consistent with the proposed mechanism of action of this drug. In previous studies we have demonstrated that while EPZ-5676 and related
compounds also inhibit intracellular DOT1L activity – as evidenced by concentration-dependent inhibition of H3K79 methylation – in non-MLL-r leukemia cell lines, this enzyme inhibition only translates into an anti-proliferative effect for those leukemia cells bearing an 11q23 chromosomal translocation (Daigle et al., 2011, 2013).

*EPZ-5676 Increases Expression of Differentiation Markers and Apoptosis as Single Agent and in Combination with AML Standard of Care Drugs*

We wished to describe the phenotypic effects underlying synergistic anti-proliferative activity observed with EPZ-5676 and AML SOC agent combinations in more detail. EPZ-5676 and related compounds have previously been reported to promote apoptosis and differentiation in MLL-r cells. Consistent with this, EPZ-5676 alone induces a concentration-dependent increase in apoptotic cells (as measured by Annexin-V staining) after 7 days of treatment of MOLM-13 cells (Figure 4A). The total content of viable cells decreases with EPZ-5676 concentration according to a classic Langmuir isotherm, with a midpoint value (EC50) of 364 ± 18 nM and this trend is exactly mirrored by the increasing content of apoptotic cells (sum of early and late stage apoptosis). We next evaluated the kinetics for induction of apoptosis at fixed time points over a 14 day course of treatment for MOLM-13 cells treated with DMSO (as a control), 156 nM EPZ-5676, 63 nM Ara-C or a combination of EPZ-5676 and Ara-C (at the same concentrations as for the single agent treatments). Ara-C by itself induced a modest increase in apoptotic cell population over the co-treatment period, while EPZ-5676 treatment led to much more robust induction of apoptosis over the same time course. The combination of the two drugs led to enhanced apoptosis in MOLM-13 cells (Figure 4B). Apoptotic cell content was also assessed by measuring the percent of cells in the sub-G1 phase of the cell cycle. The data for the sub-G1 cell
population is graphed as a kinetic plot in Figure 4C. This plot makes clear that Ara-C treatment alone has minimal effect of the sub-G1 population of MOLM-13 cells over the 7 day treatment course, while treatment with EPZ-5676 leads to a moderate, time-dependent increase in sub-G1 population. When EPZ-5676 and Ara-C are combined, a significant increase in the population of sub-G1 cells at 10 and 14 days is realized with a concomitant increase in the rate of sub-G1 population growth as well. Figure 4D, E and F illustrate the distribution of the G1, S and G2/M cell cycle stages respectively at various time points for MOLM-13 cells treated with DMSO (control), 156 nM EPZ-5676, 63 nM Ara-C or a combination of EPZ-5676 and Ara-C. Similar results were observed when EPZ-5676 was combined with daunorubicin (data not shown).

In addition to driving apoptotic cell death, EPZ-5676 and Ara-C either as single agents or in combination, promote time and concentration dependent up-regulation of the differentiation markers CD11b and CD14 (Figure 5) in MLL-r MOLM-13 cells. The same effect was observed by day 10 with daunorubicin as a single agent and in combination with EPZ-5676 (Supplemental Figure 2). In all cases the degree of differentiation marker up-regulation was greater with the combination of agents than with either agent alone. This significant up-regulation was also shown by gene expression analysis of differentiation markers in MOLM-13 cells treated alone or in combination with EPZ-5676 with Ara-C or daunorubicin (Supplemental Figure 5). Taken together, these results demonstrate that the synergistic anti-proliferative activity observed by combining EPZ-5676 with AML SOC agents is due to an enhanced ability of drug combinations over single agents to induce apoptosis and differentiation in MLL-r cells.

**EPZ-5676 Demonstrates Combination Benefit with ALL Standard of Care Drugs**
MLL-r is also found in acute lymphoblastic leukemia (ALL) and is primarily associated with infants (children younger than 12 months). This subset of ALL has a poor prognosis when compared to the ALL patients without the 11q23 translocation. Long term event free survival in infants harboring MLL-r has been reported to be between 28 and 45%. These rates are much lower than non-MLL-r patients who have survival rates approaching 90% (Bhojwani et al., 2009; Pieters et al., 2007; Inaba et al., 2013). Similar to the AML SOC we wanted to evaluate combination of EPZ-5676 with current ALL therapies that include mitoxantrone, methotrexate, mafosfamide, prednisolone and vincristine (Pieters et al., 2007; Inaba et al., 2013). The results of these combinations are summarized in Table 1 (see also Supplemental Table 1). We observed synergism or additive effects with all the ALL SOC agents in combination with EPZ-5676 with the exception of prednisolone, where antagonism was observed in MLL-r cell lines. No enhancement of the anti-proliferative single agent activity of ALL SOC drugs was seen when combined with EPZ-5676 in the non-MLL-r cell line SKM-1 with the exception of prednisolone, where enhanced anti-proliferative activity was observed in the presence of EPZ-5676 concentrations of greater than 1000 nM. The basis for this enhancement in prednisolone activity is unknown, however we note that these EPZ-5676 concentrations are much higher than those required for maximal efficacy in preclinical MLL-r models.

EPZ-5676 Demonstrates Strong Synergy with DNMT Inhibitors and Chromatin Modifying Agents in MLL-Rearranged Cell Lines

There is considerable evidence that epigenetic regulation of gene transcription results from the combinatorial effects of distinct covalent modifications of chromatin components, including histone methylation, histone acetylation, other covalent histone modifications and direct
methylation of chromosomal DNA at CpG islands by the DNA methyltransferases (DNMTs) (Arrowsmith et al., 2012; Kouzarides, 2007). With this in mind, we tested the impact of combining the HMT inhibitor EPZ-5676 in combination with compounds that affect their pharmacology by inhibition of other chromatin regulators, such as histone deacetylases (HDAC), histone demethylases (HDMs), acetyl-lysine reader domains (bromodomains) and DNA methyltransferases (DNMTs). The results of these combinations are summarized in Table 1 (see also Supplementary Table 1). Most of the conditions tested demonstrated synergy or additivity between EPZ-5676 and these compounds, but there were also examples of antagonism, including HDAC inhibitors in the context of MOLM-13 cells. The DNMT inhibitors decitabine and azacitidine demonstrated synergistic anti-proliferative activity in MLL-r cells when combined with EPZ-5676. In contrast, and again consistent with the mechanism of action of EPZ-5676, this compound had no impact on the anti-proliferative activity of either DNMT inhibitor when tested in the non-MLL-r leukemia cell line SKM-1 (Table 1). Figure 6 illustrates representative data for the strong synergistic effects of combining azacitidine and EPZ-5676 in MV4-11 and MOLM-13 cell lines. No potency enhancement was seen when SKM-1 cells were treated with a combination of EPZ-5676 and azacitidine (Supplemental Figure 3).
Discussion

Advances in genomic understandings of human cancers have provided a rational basis for the identification of oncogenic, driver alterations in specific cancer types. Surveys of these oncogenic lesions have led to a broad understanding of the molecular underpinnings of cancer, both in terms of metabolic and signaling pathways that are commonly coopted in disease (Hanahan and Weinberg, 2011), as well as specific, molecular targets that are genetically altered in a manner that causes or facilitates the hyperproliferative phenotype of cancer. Thus, today a significant portion of basic cancer research and applied drug discovery research is focused on the identification and validation of specific molecular targets for therapeutic intervention in cancer (Jabbour et al., 2013; Konopleva et al., 2014; Patel et al., 2012). Indeed, recent cancer clinical trials have been largely dominated by targeted therapeutic approaches.

Despite this paradigm shift in cancer treatment, clinical oncology today continues to rely heavily on more traditional chemotherapeutic approaches. While these traditional chemotherapies are less targeted, and therefore prone to greater safety concerns, they have nevertheless demonstrated broad activity in the treatment of cancer patients. Most commonly, cancer therapies of varying modality are combined in clinical use to offer the best compromise between potent, anti-cancer efficacy and patient safety and comfort. Hence, it is imperative that as new, targeted therapies enter the clinic, the research community understands how these new therapeutic modalities may combine with existing drugs that are in current clinical use for a specific cancer indication. The goals of this type of research are two-fold. First, it is critical that one understands any antagonistic relationship between a new therapeutic agent and current SOC therapies that might contraindicate the co-administration of the two. Second, preclinical and clinical studies of drug
combinations may reveal a greater-than-additive effect of the combined drugs that would provide significant benefit to patients; drugs that when combined produce greater efficacy than would be expected from summing of their individual activities are said to behave *synergistically*.

EPZ-5676 is a highly selective and potent inhibitor of the PMT enzyme DOT1L and is being pursued as a targeted cancer therapeutic for patients with 11q23 chromosomal translocations as the driver alteration in *MLL*-r leukemia. This compound has demonstrated potent, single-agent activity as a selective cytotoxic for *MLL*-r leukemia cells with minimal impact on non-*MLL*-r cells in preclinical models and has recently begun Phase 1 clinical testing (Daigle et al., 2013). AML patients, including those bearing *MLL*-rearrangements, are most commonly treated today with the chemotherapies Ara-C and daunorubicin, or by hematopoietic stem cell transplantation (Burnett et al., 2011; Mroz et al., 1997; Tamai and Inokuchi, 2010). Our goal in this current study was, therefore, to determine the impact of combining EPZ-5676 with these SOC chemotherapeutics on the proliferation of *MLL*-r leukemia cells in culture. As illustrated in Figure 1 and Table 1, the combination of EPZ-5676 with either Ara-C or daunorubicin resulted in strongly synergistic anti-proliferative activity when tested against the *MLL*-r leukemia cell lines MV4-11 and MOLM-13. These cell lines are derived from AML and acute biphenotypic leukemia, respectively, and express the two most commonly found MLL fusion proteins. MV4-11 cells bear a (t(4;11)(q21;q23)) translocation in which the MLL fusion partner protein is AF4, whereas MOLM-13 cells bear a (t(9;11)(q23;p22p23)) translocation in which the MLL fusion partner protein is AF9. In stark contrast to the strong synergy seen between EPZ-5676 and these chemotherapies in cells harboring 11q23 chromosomal translocations, no effect of EPZ-5676 was observed in the non-*MLL*-r leukemia cell line SKM-1, either as a single agent or in combination. This lack of activity in non-*MLL*-r leukemia is
completely consistent with the proposed mechanism of action of EPZ-5676 and with previous studies of the drug as a single-agent in a broad spectrum of leukemia cell lines (Daigle et al., 2013).

A universal characteristic of acute leukemias, such as MLL-r, is the accumulation of highly proliferative myeloblasts or lymphoblasts that have lost their ability to differentiate fully into mature leukocytes. In previous studies, we have demonstrated that EPZ-5676 and related compounds effect a reprogramming of chromatin modification and downstream transcriptomic activities that result in MLL-r leukemia cells starting down a path of differentiation/maturation and apoptotic cell death (Daigle et al., 2011, 2013). Differentiation/maturation can be characterized and quantified by cytometric assessment of cell surface markers of cell state, such as CD11b and CD14, two markers of leukocyte differentiation. In combination with Ara-C, both apoptotic (Figure 4) and differentiation/maturation responses to EPZ-5676 treatment were augmented on day 10 with the greatest expression measured on Day 14 (Figure 5).

Differentiation/maturation responses measured on Day 10 show an increase in the expression of CD11b and CD14 when cells were cotreated with EPZ-5676 and daunorubicin, compared with daunorubicin alone. In combination with Ara-C or daunorubicin, both the apoptotic and differentiation/maturation responses to EPZ-5676 treatment were augmented (Figure 5 and Supplemental Figure 2). Induction of blast differentiation and/or apoptosis are established approached to resolution of human leukemias (Bruserud et al., 2000; Nowak et al., 2009). The ability of EPZ-5676 to cause blast differentiation and apoptosis by itself, and the enhancement of these effects by Ara-C and daunorubicin, bode well for the effective use of this compound alone, or with chemotherapy as a clinical treatment for MLL-r patients. It is noteworthy that the
antileukemic effect of EPZ-5676 in combination with chemotherapy was tolerant of variation in
dose schedule. In particular, the ability to pretreat cells with EPZ-5676, remove this compound
by washout, and then dose the chemotherapeutic with retention of synergistic effect offers
considerable flexibility in the clinical utilization of such combinations. This potential dosing
schedule flexibility may prove quite useful not only in optimizing the efficacy of the
combination therapy but also in mitigation of any chemotherapy-associated safety issues.

MLL-rearrangements are also found in a subset of ALL patients. We therefore tested the effect
of combining EPZ-5676 with current ALL therapies that include mitoxantrone, methotrexate,
mafosfamide, prednisolone and vincristine (Pieters et al., 2007; Inaba et al., 2013). With the
exception of prednisolone, all of these SOC agents demonstrated synergy or additivity with
EPZ-5676, thereby providing a rationale for combining EPZ-5676 with current ALL SOC drugs
for the treatment of MLL-r patients suffering from ALL.

Beyond current acute leukemia SOC chemotherapies, we also wished to explore the possibility
of synergy between EPZ-5676, a HMT inhibitor, and other epigenetic-targeted compounds for
treatment of MLL-r leukemias. Preclinical small molecule inhibitor and genetic knockdown
studies have provided some evidence for a role for other chromatin regulators such as HDACs,
DNMTs, bromodomains and LSD1 in the pathogenesis of MLL-r leukemia (Neff and
Armstrong, 2013; Deshpande et al., 2012). We therefore studied the impact on MLL-r leukemia
cell proliferation of combinations of EPZ-5676 with DNMT inhibitors, HDAC inhibitors, HDM
inhibitors and bromodomain inhibitors. As summarized in Table 1, we observed a range of
combination effects among these various inhibitors. With bromodomain and LSD1 inhibitors we
saw synergy or additivity depending on the specific inhibitor used. In the case of HDAC
inhibitors, we saw antagonism with EPZ-5676 in some cases and mere additivity in other cases (Table 1). These data suggest that combinations of EPZ-5676 and HDAC inhibitors are unlikely to provide greater benefit to MLL-r patients in a clinical setting. On the other hand, we saw consistent synergy between EPZ-5676 and DNMT inhibitors for selective inhibition of MLL-r leukemia cell proliferation. Further studies will be required to elucidate the detailed molecular mechanisms underlying these observations. In summary, the data presented here provides a rational foundation for combining EPZ-5676 with acute leukemia SOC chemotherapeutics for the treatment of MLL-r leukemia. The data further suggest that combinations of EPZ-5676 and DNMT inhibitors may also provide additional benefit to patients with MLL-r leukemia. These preclinical data provide a basis for rational hypothesis generation that must ultimately be tested in human clinical trials.
Acknowledgments

We wish to thank the compound management group at Epizyme, Christina Majer, Andrew Santospago and Elizabeth Admirand for their tireless support in providing compounds for experiments.
Authorship Contributions

*Participated in research design:* Klaus, Iwanowicz, Johnston, Campbell, Smith, Moyer, Copeland, Olhava, Porter Scott, Pollock, Daigle, and Raimondi

*Conducted experiments:* Klaus, Iwanowicz, Johnston, Campbell, and Raimondi

*Contributed new reagents or analytic tools:* Klaus, and Raimondi

*Performed data analysis:* Klaus, Iwanowicz, Campbell, Copeland and Raimondi

*Wrote or contributed to the writing of the manuscript:* Klaus, Iwanowicz, Copeland, Porter Scott, Pollock, Daigle, and Raimondi
References


Footnotes:

C. R. Klaus and D. Iwanowicz contributed equally to this work.
Legends for Figures

Figure 1. Synergistic anti-proliferative activity of EPZ-5676 in combination with SOC drugs for AML in *MLL*-r leukemia cell lines

MOLM-13 and MV4-11 cells were treated according to the pre-treatment model described under *Materials and Methods* with EPZ-5676 followed by co-treatment with a combination of EPZ-5676 and each SOC drug. EPZ-5676 concentrations spanned a range known to be efficacious in preclinical models. Data is representative of two biological experiments. The gray oval on the Fa-CI plots indicates a region of synergy. A) Fa-CI plot shows synergistic combination of EPZ-5676 and Ara-C at a 1.25:1 constant ratio in MOLM-13 cells (data plotted represents a concentration range of 19.5-313 nM for EPZ-5676 and 15.6-250 nM for Ara-C).

B) Fa-CI plot shows synergistic combination of EPZ-5676 and daunorubicin at a 20:1 constant ratio in MOLM13 cells (data plotted represents a concentration range of 39.1-156 nM for EPZ-5676 and 1.95-7.8 nM for daunorubicin).

C) Fa-CI plot shows synergistic combination of EPZ-5676 and Ara-C at a 1:8 constant ratio in MV4-11 cells (data plotted represents a concentration range of 7.8-500 nM for EPZ-5676 and 62.5-4000 nM for Ara-C).

D) Fa-CI plot shows synergistic combination of EPZ-5676 and daunorubicin at a 40:1 constant ratio in MV4-11 cells (data plotted represents a concentration range of 31.25-250 nM for EPZ-5676 was and 0.78-6.3 nM for daunorubicin).

For reference, in MOLM-13 cells, the Dₘ for EPZ-5676 is 0.15 µM, the IC₅₀ for Ara-C is 0.053 µM, and the Dₘ for daunorubicin was 0.0038 µM. In MV4-11 cells, the Dₘ for EPZ-5676 is 0.029 µM, the Dₘ for Ara-C was 0.66 µM, and the Dₘ for daunorubicin was 0.0018 µM.
Figure 2. Synergistic anti-proliferative activity between EPZ-5676 and AML SOC agents is maintained following EPZ-5676 washout prior to the addition of the SOC agents in MLL-r leukemia cell lines

MOLM-13 and MV4-11 MLL-r cells were treated according to the pre-treatment model described in the Material and Methods (with EZP-5676 washout). EPZ-5676 concentrations spanned a range known to be efficacious in preclinical models. Data is representative of two biological experiments. The gray oval on the Fa-CI plots indicates a region of synergy. A) Fa-CI plot shows synergistic combination of EPZ-5676 and Ara-C at a 1.25:1 constant ratio in MOLM-13 cells (data plotted represents a concentration range of 19.5-625 nM for EPZ-5676 and 15.6-500 nM for Ara-C). B) Fa-CI plot shows synergistic combination of EPZ-5676 and daunorubicin at a 20:1 constant ratio in MOLM13 cells (data plotted represents a concentration range of 39.5-312.5 nM for EPZ-5676 and 1.95 -15.6 nM for daunorubicin). C) Fa-CI plot shows synergistic combination of EPZ-5676 and Ara-C at a 1:8 constant ratio in MV4-11 cells (data plotted represents a concentration range of 15.6-500 nM for EPZ-5676 and for Ara-C was 125-4000 nM). D) Fa-CI plot shows synergistic combination of EPZ-5676 and daunorubicin at a 40:1 constant ratio in MV4-11 cells (data plotted represents a concentration range of 31.25-500 nM for EPZ-5676 and 0.78 -12.5 nM for daunorubicin). For reference, in MOLM-13 cells, the $D_m$ for EPZ-5676 is 0.21 µM, the $D_m$ for Ara-C is 0.053 µM, and the $D_m$ for daunorubicin was 0.0040 µM. In MV4-11 cells, the $D_m$ for EPZ-5676 is 0.16 µM, the $D_m$ for Ara-C was 0.33 µM, and the $D_m$ for daunorubicin was 0.0022 µM.

Figure 3. Synergistic anti-proliferative activity between EPZ-5676 and AML SOC agents in MOLM-13 MLL-r cells
are pretreated with Ara-C prior to co-treatment with EPZ-5676 and the effect is durable upon washout of Ara-C prior to the addition of the EPZ-5676

MOLM-13 cells were pretreated with several doses of Ara-C (2-fold serial dilutions, 1.9-250 nM) as described under Materials and Methods followed by co-treatment with EPZ-5676 and Ara-C or treatment only with EPZ-5676 (Ara-C washout). EPZ-5676 was tested in 2-fold dilutions, ranging from 9.8 to 1250 nM. Gray oval on the Fa-CI plots indicates a region of synergy. A) Fa-CI plot shows combination benefit when cells are pretreated with Ara-C followed by co-treatment with EPZ-5676 and Ara-C at a 10:1 constant ratio. Concentrations of Ara-C in excess of 31.25 nM produced cell death and therefore could not be included in the calculation of combination index. B) Fa-CI plot shows that combination benefit is durable when Ara-C is removed from the cultures prior to treatment with EPZ-5676. Concentrations of Ara-C in excess of 31.25 nM produced cell death and therefore could not be included in the calculation of combination index.

(For reference, the Dₘ for EPZ-5676 for the 7 day co-treatment phase as single agent was 0.052 µM, the Dₘ for Ara-C in the 10 day assay was 0.017 µM (no Ara-C washout) and 0.036 µM (Ara-C washout).

Figure 4. EPZ-5676 and Ara-C act synergistically to enhance apoptosis in MLL-r

MOLM-13 cells.

EPZ-5676 and Ara-C act synergistically to enhance apoptosis in MLL-rearranged MOLM-13 cells. Compound treatments for mechanism of cell death studies were performed as described under Materials and Methods. EPZ-5676 was tested at a concentration of 156 nM and Ara-C was tested at a concentration of 62 nM and in combination at the same concentrations as with the
single agents. In panels B) through F) triangles represent treatment with the combination of EPZ-5676 and Ara-C, circles represent treatment with EPZ-5676 as single agent and squares represent treatment with Ara-C as single agent.

A) Dose dependent increase in the percent of the population of cells in early and late apoptosis (Filled circles, Annexin V positive) and concomitant decrease in cell viability expressed as % of total cells (Empty circles, Annexin V negative/PI negative) after seven day treatment with EPZ-5676 as single agent. B) Synergistic time-dependent increase in apoptosis is seen when cells are treated with a combination of EPZ-5676 and Ara-C over the course of seven days following EPZ-5676 pre-treatment. Points on the progress curve represent the mean of percentage of gated cells in early and late apoptosis (Annexin-V positive, mean +/- S.D., n=3). C) Synergistic time-dependent increase of cells in sub-G1 phase of the cell cycle is seen when cells are treated with a combination of EPZ-5676 and Ara-C over the course of seven days after pre-treatment with EPZ-5676 for seven days. D) Synergistic time-dependent decrease in the population of cells in G1 treated with a combination of EPZ-5676 and Ara-C. E) Cell population in S phase is reduced by treatment with the combination of EPZ-5676 and Ara-C. F) Cell population in G2/M phase decreases over the seven day co-treatment period with the combination of EPZ-5676 and Ara-C. (For reference, concentrations of EPZ-5676 and Ara-C tested were shown to induce synergy with combination index of 0.48 and Fa 0.95.)

**Figure 5.** EPZ-5676 and Ara-C as single agents and in combination promote time and concentration dependent up-regulation of the differentiation markers CD11b and CD14 in *MLL*-rearranged leukemia cells
MOLM-13 cells were treated as described under *Materials and Methods* for mechanism of cell death studies. EPZ-5676 and Ara-C as single agents and in combination promote time and concentration dependent up-regulation of the differentiation markers CD11b and CD14. Histograms in black represent data from naïve cells. Red histograms represent data from DMSO treated cells. Histograms in magenta and green represent the indicated concentrations of single agent or combination of the two agents. Results are representative of two biological experiments.

A) Flow cytometry analysis for cell surface expression of CD11b shows time and dose dependent up-regulation of the marker in cells treated with EPZ-5676 and Ara-C as single agents and in combination. B) Flow cytometry analysis for cell surface expression of CD14 shows time and dose dependent upregulation of the marker in cells treated with EPZ-5676 and Ara-C as single agents and in combination. C) Flow cytometry analysis for cell surface expression IgG isotype control. Similar results were obtained in two independent experiments.

**Figure 6. EPZ-5676 and azacitidine synergistically induce an anti-proliferative effect in MLL-rearranged leukemia cells**

MOLM-13 and MV4-11 cells were treated with EPZ-5676 and azacitidine as single agents and in combination according to the co-treatment model described under *Materials and Methods*. The gray oval on the Fa-CI plots indicates a region of synergy. A) Fa-CI plot shows synergistic anti-proliferative activity when MOLM-13 cells were co-treated with EPZ-5676 and azacitidine (data plotted represents a concentration range of 39.5-625 nM for EPZ-5676 and 156 -2500 nM for azacitidine). B) Fa-CI plot shows synergistic anti-proliferative activity when MV4-11 cells were co-treated with EPZ-5676 and azacitidine (data plotted represents a concentration range of 1.9-125 nM for EPZ-5676 and 19.5 -1250 nM for azacitidine). For reference, the D$_m$ for
EPZ-5676 was 0.20 µM and 0.0097 µM in the MOLM-13 and MV4-11 cell lines, respectively. The $D_m$ for azacitidine was 0.58 µM and 1.50 µM in the MOLM-13 and MV4-11 cell lines, respectively.
Tables

Table 1. Summary of combination studies of EPZ-5676 with AML and ALL standard of care drugs and chromatin modifying agents

Compounds were tested in combination with EPZ-5676 according to the co-treatment model described under Materials and Methods. The anti-proliferative effect of the combinations in the MOLM-13 and MV4-11 cell lines was evaluated using the Calcusyn software. In these studies the lowest CI value obtained corresponding to a Fα value greater than 0.5 was used to assess the degree of synergism or antagonism as described in the Data Analysis section. For the SKM-1 cell line the potency shift of the compound tested in the presence of EPZ-5676 was calculated from the dose response curves as described in the Data Analysis section and used to define the combination effect of the two drugs.
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\(^a\) No enhancement was observed based on analysis of the IC\(_{50}\) shift of the test compound in the presence of EPZ-5676. Analysis of IC\(_{50}\) shifts is described under Materials and Methods.

\(^b\) IC\(_{50}\) of test compound not achieved.

\(^c\) Methotrexate showed antagonistic effect in combination with EPZ-5676 at some constant ratios.

\(^d\) Enhancement or shift in IC\(_{50}\) was observed at concentrations of EPZ-5676 of 2000 nM and above.

N/D: not determined.
Figure 1

A.

EPZ-5676 + Ara-C

B.

EPZ-5676 + Daunorubicin

C.

EPZ-5676 + Ara-C

D.

EPZ-5676 + Daunorubicin
Figure 2

A

EPZ-5676 + Ara-C

B

EPZ-5676 + Daunorubicin

C

EPZ-5676 + Ara-C

D

EPZ-5676 + Daunorubicin
Figure 3

Ara-C + EPZ-5676

Fractional Effect

CI +/- 1.96 s.d.

0 0.2 0.4 0.6 0.8 1.0

0 1.0 2.0 3.0 4.0

Fractional Effect

A

B

Ara-C + EPZ-5676

CI +/- 1.96 s.d.
Figure 4

**A**

![Graph A](image1)

- Percent of Total Cells
- log [EPZ-5676], nM
- 100%, 80%, 60%, 40%, 20%, 0%
- EPZ-5676 Pretreatment

**B**

![Graph B](image2)

- Annexin Positive (% of gated)
- Time (Days)
- 80%, 60%, 40%, 20%
- EPZ-5676 Pretreatment

**C**

![Graph C](image3)

- Sub-G1 population (% of gated)
- Time (Days)
- 80%, 60%, 40%, 20%
- EPZ-5676 Pretreatment

**D**

![Graph D](image4)

- GI population (% of gated)
- Time (Days)
- 80%, 60%, 40%, 20%
- EPZ-5676 Pretreatment

**E**

![Graph E](image5)

- S population (% of gated)
- Time (Days)
- 40%, 30%, 20%
- EPZ-5676 Pretreatment

**F**

![Graph F](image6)

- G2/M population (% of gated)
- Time (Days)
- 40%, 30%, 20%
- EPZ-5676 Pretreatment
Figure 5

A  CD11b

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B  CD14

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C  IgG

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</tr>
<tr>
<td>EPZ/5676 156 nM</td>
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</tr>
</tbody>
</table>
Figure 6

A

EPZ-5676 + Azacitidine

Fractional Effect

CI +/- 1.96 s.d.

B

EPZ-5676 + Azacitidine

Fractional Effect

CI +/- 1.96 s.d.