Binding of Released Bim to Mcl-1 is a Mechanism of Intrinsic Resistance to ABT-199 which can be Overcome by Combination with Daunorubicin or Cytarabine in AML Cells

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Abstract

Purpose: To investigate the molecular mechanism underlying intrinsic resistance to ABT-199.

Experimental Design: Western blots and real-time RT-PCR were used to determine levels of Mcl-1 after ABT-199 treatment alone or in combination with cytarabine or daunorubicin. Immunoprecipitation of Bim and Mcl-1 were used to determine the effect of ABT-199 treatment on their interactions with Bcl-2 family members. Lentiviral short hairpin RNA knockdown of Bim and CRISPR knockdown of Mcl-1 were used to confirm their role in resistance to ABT-199. JC-1 assays and flow cytometry were used to determine drug-induced apoptosis.

Results: Immunoprecipitation of Bim from ABT-199–treated cell lines and a primary patient sample demonstrated decreased association with Bcl-2, but increased association with Mcl-1 without corresponding change in mitochondrial outer membrane potential. ABT-199 treatment resulted in increased levels of Mcl-1 protein, unchanged or decreased Mcl-1 transcript levels, and increased Mcl-1 protein half-life, suggesting that the association with Bim plays a role in stabilizing Mcl-1 protein. Combining conventional chemotherapeutic agent cytarabine or daunorubicin with ABT-199 resulted in increased DNA damage along with decreased Mcl-1 protein levels, compared with ABT-199 alone, and synergistic induction of cell death in both AML cell lines and primary patient samples obtained from AML patients at diagnosis.

Conclusions: Our results demonstrate that sequestration of Bim by Mcl-1 is a mechanism of intrinsic ABT-199 resistance and supports the clinical development of ABT-199 in combination with cytarabine or daunorubicin for the treatment of AML.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by rapid clonal growth of myeloid lineage blood cells. This year, there will be an estimated 20,830 new AML cases and an estimated 10,400 deaths from this deadly disease in the United States (1). Overall survival rates remain low despite advances in treatment with overall survival rates of 25% for adults (1) and 65% for children (2). Resistance to front-line chemotherapy remains a major cause of treatment failure (3), highlighting the need for new therapies.

Overexpression of the antiapoptotic Bcl-2 family members is associated with chemoresistance in leukemic cell line models and with poor clinical outcome (4–7). Antiapoptotic Bcl-2 family members, such as Bcl-2, Bcl-xl, and Mcl-1 sequester proapoptotic BH3-only proteins, such as Bim, preventing activation of the proapoptotic proteins Bax and Bak, ultimately preventing mitochondrial outer membrane permeabilization, cytochrome c release, and apoptosis (8). Thus, inhibition of antiapoptotic Bcl-2 family members represents a promising approach for the treatment of AML. ABT-199, a Bcl-2–selective inhibitor, has demonstrated encouraging results in AML, acute lymphoblastic leukemia, chronic lymphocytic leukemia, mantle cell lymphoma, multiple myeloma, and breast cancer (9–15). However, it has limited efficacy in Bcl-xl– and Mcl-1–dependent malignancies (9). Thus, intrinsic drug resistance remains a concern. Understanding the molecular mechanisms of resistance to ABT-199 will allow for rationally designed combination regimens to increase its antileukemic efficacy.

In this study, we identified Bim sequestration by Mcl-1 as a mechanism of resistance to ABT-199 in AML cells. Binding to...
Translational Relevance

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy, and its overall survival rate remains low. Therefore, new therapies are urgently needed. ABT-199 is a Bcl-2–selective inhibitor that has shown promising preclinical activities against some subtypes of AML cells. However, intrinsic resistance has limited its efficacy. In this study, we demonstrate that sequestration of Bim by Mcl-1 contributes to ABT-199 resistance. We also demonstrate that this mechanism of resistance can be overcome by combining ABT-199 with DNA damaging agents daunorubicin or cytarbine, which reduce Mcl-1 levels and enhance ABT-199 activity in AML cell lines. In addition, we demonstrate that these combinations are synergistic in cell lines and primary patient samples independent of their sensitivities to ABT-199, thus providing evidence that screening for ABT-199 resistance is not necessary, therefore supporting clinical testing regardless of ABT-199 sensitivity.

Bim likely contributed to increased Mcl-1 protein stability and presumably decreased levels of free Bim, preventing its induction of apoptosis. To overcome this resistance, AML cells were treated with ABT-199 in combination with daunorubicin or cytarbine, which increases DNA damage and decreases Mcl-1 protein levels, resulting in synergistic induction of cell death in both ABT-199–resistant and ABT-199–sensitive cell lines. In addition, our studies using primary patient samples further support the clinical development of combined ABT-199 and daunorubicin or cytarbine treatment as well as provide guidance for designing other ABT-199 combination therapies for the treatment of AML.

Materials and Methods

Drugs

ABT-199 and MG-132 were purchased from Selleck Chemicals. Daunorubicin, cytarbine, and cycloheximide were purchased from Sigma-Aldrich.

Cell culture

MV4-11, THP-1, and U937 cell lines were purchased from the ATCC (2006, 2014, and 2002, respectively). OCI-AML3 cell line was purchased from the German Collection of Microorganisms and Cell Cultures (2011; DSMZ). The CMS cell line was a gift from Dr. A Fuse from the National Institute of Infectious Diseases (2004; Tokyo, Japan). The cell lines have not been authenticated since they were received in our laboratory. The cell lines were cultured in RPMI1640 (or αMEM for OCI-AML3 cells) media with 10% to 20% FBS (Life Technol-
Quantification of gene expression by real-time RT-PCR

Total RNA was extracted using TRIzol (Life Technologies) and cDNAs were prepared from 2 μg total RNA using random hexamer primers and a RT-PCR Kit (Life Technologies), and then purified using the QiAquick PCR Purification Kit (Qiagen) as described previously (12, 20, 23). McI-1 (Hs01050896_m1) transcripts were quantitated using TaqMan probes (Life Technologies) and a LightCycler 480 real-time PCR machine (Roche Diagnostics), based on the manufacturer's instructions. Real-time PCR results were expressed as means from three independent experiments and were normalized to GAPDH transcripts. Fold changes were calculated using the comparative C_ method (25).

Assessment of mitochondrial membrane potential

Determination of mitochondrial membrane potential (MMP) following the indicated drug treatments was performed as described previously (26). Briefly, 5 × 10^5 cells were resuspended in fresh growth media containing 1 μM/L JC-1 (Sigma-Aldrich) and incubated for 15 minutes at 37°C. The samples were washed and plated in a black-walled 96-well plate. JC-1 monomer fluorescence was measured on a microplate reader with excitation 485 nm and emission 535 nm.

Immunoprecipitation

AML cells were treated for 24 hours and then the cells were lysed using 1% CHAPS, 5 mM/L MgCl2, 150 mM/L NaCl, 1 mM/L EDTA, 1 mM/L EGTA, 20 mM/L Tris, and 0.5% Tween-20 in the presence of protease inhibitors. Immunoprecipitation (IP) of Bim and McI-1 was performed as described previously (27) using 2 μg of anti-Bim (2819, Cell Signaling Technology) or anti-McI-1 (SC-819, Santa Cruz Biotechnology) antibody, 1 mg protein lystate, and Protein A agarose beads (Roche Diagnostics). Proteins were eluted using 50 mM/L glycine, pH 2.0, and then analyzed by Western blotting.

shRNA knockdown

The pMD-VSV-G and delta 8.2 plasmids were gifts from Dr. Dong at Tulane University. Bin and nontarget control (NTC) short hairpin (shRNA) lentiviral vectors were purchased from Sigma-Aldrich. Lentivirus production and transduction were carried out as described previously (28). Briefly, TLA-HEK293T cells were transduced with pMD-VSV-G, delta 8.2, and lentiviral shRNA constructs using Lipofectamine and Plus reagents (Life Technologies) according to the manufacturer's instructions. Virus-containing culture medium was harvested 48 hours posttransfection. Cells were transduced overnight using 1 ml of virus supernatant and 4 μg of polybrene and then cultured for an additional 48 hours prior to selection with puromycin.

CRISPR knockdown

The lentCRISPRv2 plasmid was a gift from Feng Zhang at the Broad Institute of MIT and Harvard [Addgene plasmid #52961 (29)]. Guide RNAs were designed using the CRISPR design tool (http://crispr.mit.edu). The NTC and McI-1 vectors were generated using Feng Zhang’s protocol, which is available on Addgene's website (www.addgene.org). Lentivirus production and transduction were carried out as described above in “shRNA Knockdown,” except that psPAX2 (gift from Didier Trono at the Swiss Institute of Technology, Addgene plasmid #12260) was used instead of delta 8.2.

Alkaline comet assay

U937 cells were treated for 8 hours with ABT-199 and/or daunorubicin and subjected to alkaline comet assay as described previously (28). Slides were stained with SYBR Gold (Life Technologies), and then imaged on an Olympus BX-40 microscope equipped with a DP72 microscope camera and Olympus cellsDension Software (Olympus America Inc.). Approximately 50 comets per gel were scored using CometScore (TriTek Corp).

Statistical analysis

Differences were compared using the two-sample t test. Statistical analyses were performed with GraphPad Prism 5.0. Error bars represent ± SEM. The level of significance was set at P < 0.05.

Results

ABT-199 induces apoptosis in ABT-199-sensitive AML cells

First, we treated MV4-11 (a relatively sensitive cell line, with an ABT-199 IC_{50} of 137.5 nmol/L, as determined previously by MTT
ABT-199 treatment increases Mcl-1 protein levels in ABT-199–resistant AML cells

To begin to determine the mechanism of resistance to ABT-199, we treated OCI-AML3 cells (a relatively resistant AML cell line, with an ABT-199 IC50 of ~15 μmol/L, as determined previously; ref. 12) with clinically achievable concentrations of ABT-199 (10) for 24 hours and measured MMP. ABT-199 treatment did not result in loss of MMP in OCI-AML3 cells (Fig. 1A), indicating that ABT-199 did not induce apoptosis. In contrast to the sensitive cells, there was an overall increase of Mcl-1 in OCI-AML3 cells treated with ABT-199 (Fig. 1B). IP of Bim revealed decreased association of Bcl-2 with Bim (Fig. 1C). Interestingly, ABT-199 treatment in OCI-AML3 cells resulted in increased Mcl-1 that coimmunoprecipitated with Bim, which was confirmed by reciprocal immunoprecipitation of Mcl-1 (Fig. 1C and D). These results were further confirmed in an ABT-199–resistant primary AML patient sample (ABT-199 IC50 = 10.4 μmol/L, as determined by MTT assay; Supplementary Table S1; Fig. 1E–G). In addition, increased Mcl-1 levels were detected in three additional ABT-199–resistant cell lines (ABT-199 IC50 >2.4 μmol/L, as determined previously; ref. 12) and one primary patient sample (ABT-199 IC50 = 3.6 μmol/L, as determined by MTT assay; Supplementary Table S1; Fig. 1H). Densitometry measurements revealed that the increase in Mcl-1 protein levels were significant (Fig. 1I). Consistent with the parental cells, expression of NTC shRNA in THP-1 and U937 cells showed similar increase in Mcl-1 after ABT-199 treatment (Fig. 1I). In contrast, expression of the Bim shRNA, which decreased Bim expression by 50%, partially abolished ABT-199 treatment–induced increase of Mcl-1 protein levels, suggesting that Bim contributes to increased Mcl-1 protein levels after ABT-199 treatment. Furthermore, knockdown of Mcl-1 blocked the increase of Mcl-1 levels following ABT-199 treatment and resulted in increased cell death [Fig. 1K and L, repeated attempts using shRNA did not yield significant knockdown of Mcl-1 (data not shown), thus CRISPR was used instead]. These results demonstrate that Mcl-1 plays an important role in ABT-199 resistance.

ABT-199 treatment results in increased Mcl-1 protein stability in ABT-199–resistant AML cells

To elucidate the molecular mechanism by which ABT-199 increases Mcl-1 protein levels in the resistant cell lines, we first determined Mcl-1 transcript levels after ABT-199 treatment. Following ABT-199 treatment, Mcl-1 transcript levels remained the same or decreased (Fig. 2A), suggesting that transcriptional regulation of Mcl-1 did not play a prominent role. Then we treated U937 cells with the protein translation inhibitor cycloheximide for up to 90 minutes in the absence or presence of ABT-199. Mcl-1 levels decreased slower in the presence of ABT-199 (Fig. 2B), resulting in a significantly longer half-life (127 minutes vs. 83 minutes, P = 0.0369), demonstrating that ABT-199 likely affected Mcl-1 protein stability. To determine whether the ubiquitin–proteasome pathway played a role, U937 cells were treated with the proteasome inhibitor MG-132 for 24 hours. There was a concentration-dependent increase in Mcl-1 protein levels and little to no decrease of viable cells, as assayed by Trypan blue staining (Supplementary Figs. S2 and Fig. 2C). One-hour treatment with 1 μmol/L MG-132 resulted in increased Mcl-1 protein level and no further enhancement was detected when treated with combined MG-132 and ABT-199 (Fig. 2D). Similar results were obtained when the cells were treated with a lower concentration of MG-132 for 24 hours (Fig. 2E). These results demonstrate that ABT-199 likely affects Mcl-1 protein stability through the ubiquitin–proteasome pathway.

Daunorubicin enhances ABT-199–induced cell death in AML cells

It has been demonstrated that DNA damage can cause down-regulation of Mcl-1, which plays an important role in DNA damage-induced apoptosis (30). Thus, combination with a DNA damaging agent may overcome the stabilization of Mcl-1 by ABT-199 and enhance sensitivity. To test this possibility, we treated U937 cells with daunorubicin and ABT-199, alone or in combination, for 8 and 24 hours. We detected increased γH2AX in the combined drug treatment compared with single drug treatment (γH2AX is an established biomarker for DNA double-strand breaks; ref. 31; Fig. 3A). Daunorubicin treatment inhibited the increase of Mcl-1 protein levels as seen with ABT-199 treatment alone. Furthermore, the combined drug treatment resulted in synergistic induction of cell death in U937 cells after 24 hours (Fig. 3B), which was accompanied by cleavage of PARP and caspase-3 (Fig. 3A). It is important to note that cleavage of PARP and caspase-3 were not detected in the 8-hour treatment, yet increased γH2AX levels were detected in the combined drug treatment compared with single drug treatments, suggesting that γH2AX levels were indicative of DNA damage and were not likely the result of apoptosis. To further confirm that the combined drug treatment did indeed result in increased DNA damage, U937 cells were treated for 8 hours and subjected to the alkaline comet assay. As shown in Fig. 3C and D, combined drug treatment resulted in significantly more DNA damage, as measured by percent DNA in the tail, than daunorubicin alone.

To elucidate the mechanism of antileukemic interaction, we treated U937 cells for 24 hours with ABT-199 alone or in combination with daunorubicin. ABT-199 treatment resulted in increased Bim that coimmunoprecipitated with Mcl-1, which was mitigated by the addition of daunorubicin and was confirmed by reciprocal IP using a Bim antibody (Fig. 3E, left). In addition, ABT-199 treatment alone or in combination with daunorubicin resulted in decreased Bcl-2 that coimmunoprecipitated with Bim (Fig. 3E, right). Overall total protein levels of Bim remained unchanged regardless of drug treatment (Fig. 3F). These results provide evidence to suggest that with the combined drug treatment there was a decrease of Bim...
sequestered by both Mcl-1 and Bcl-2, presumably leading to increased levels of free Bim. Knockdown of Bim in U937 cells resulted in a small, yet significant decrease of cell death induced by the combined drug treatment, but not by daunorubicin alone (Fig. 3G). Consistent with the parental cells, U937/NTC cells had increased levels of Mcl-1 following ABT-199 treatment which was reduced in the combined drug treatment (Fig. 3H). In contrast, the increase of Mcl-1 levels after ABT-199 treatment was blunted in the Bim knockdown cells, although the levels were decreased after daunorubicin treatment, either alone or in combination with ABT-199.

Cytarabine synergizes with ABT-199 to induce AML cell death

Next, we tested cytarabine in combination with ABT-199. Cytarabine synergized with ABT-199 in U937 cells to induce cell death, which was accompanied by cleavage of PARP and caspase-3, indicative of apoptosis (Fig. 4A and B). Similar to daunorubicin, addition of cytaraabine abolished the increase of Mcl-1 as early as 8 hours postdrug treatment and resulted in increased γH2AX compared with single drug treatments. Cleavage of caspase-3 and PARP were not detected at 8 hours postdrug treatment. Bim knockdown significantly reduced induction of cell death by both cytarabine alone as well as in combination with ABT-199 (Fig. 4C and D). Then, we tested ABT-199 in combination with cytaraabine or daunorubicin in an ABT-199–sensitive cell line, MV4-11, and found synergistic induction of cell death accompanied by cleavage of PARP and caspase-3 (Fig. 4E and F).

Next, we sought to determine how cytarabine and daunorubicin abrogate induction of Mcl-1 by ABT-199 treatment. We treated U937 cells for 8 hours, which was prior to cleavage of caspase-3 and PARP, and determined that transcript levels for Mcl-1 increased or remained unchanged for individual and combined drug treatments (Fig. 4G), demonstrating that downregulation of Mcl-1 was likely not due to transcriptional regulation. While ABT-199 in combination with cytarabine or daunorubicin resulted in downregulation of Mcl-1, addition of MG-132 resulted in partial rescue of Mcl-1 (Fig. 4H), suggesting that the proteasome pathway plays a role in the combined drug treatment.

ABT-199 synergizes with daunorubicin or cytarabine in primary AML samples

Next, we tested the antileukemic effects of ABT-199 in combination with daunorubicin or cytarabine in primary patient samples. ABT-199 combined with daunorubicin or cytarabine resulted in synergistic induction of cell death in AML1009 and...
Figure 3.
Daunorubicin (DNR) synergizes with ABT-199 in U937 AML cell line. A, U937 cells were treated with ABT-199 and daunorubicin, alone or in combination, for 8 and 24 hours. Whole cell lysates were subjected to Western blotting and probed with the indicated antibody. The fold changes for γH2AX and Mcl-1 densitometry measurements, normalized to β-actin and then compared with no drug treatment control, are indicated. Whole cell lysate from the 24-hour ABT + daunorubicin treatment was used as the positive control for the 8-hour treatment. B, U937 cells were treated with ABT-199 and daunorubicin, alone or in combination, for 24 hours and then subjected to Annexin V/PI staining and flow cytometry analyses. ***, P < 0.0005. CI values were calculated using CompuSyn software. C, U937 cells were treated with ABT-199 and daunorubicin, alone or in combination, for 8 hours and then subjected to alkaline comet assay analyses. Representative images are shown. D, comet assay results are graphed as median percent DNA in the tail from four replicate gels ± SEM. *, P < 0.05. E, U937 cells were treated with ABT-199 and daunorubicin, alone or in combination, for 24 hours. Mcl-1 (left) or Bim (right) was immunoprecipitated from whole cell lysates and then subjected to Western blotting. Western blots were probed with anti-Mcl-1, anti-Bim, or anti-β-actin antibody. The fold changes for Mcl-1 and Bim densitometry measurements, normalized to β-actin and then compared with no drug treatment control, are indicated. F, whole cell lysates from E were subjected to Western blotting and probed with anti-Bim or anti-β-actin antibody. G, whole cell lysates were subjected to Western blotting and probed with anti-Bim or anti-β-actin antibody. The fold changes for Bim and Mcl-1 densitometry measurements, normalized to β-actin and then compared with no drug treatment control, are indicated. Niu et al. Clin Cancer Res; 22(17) September 1, 2016
AML1010 or AML1003 and AML1008, respectively (Fig. 5A–D). AML1008 was sensitive to ABT-199 with an MTT IC₅₀ of 369 nmol/L, whereas AML1003, AML1009, and AML1010 had ABT-199 IC₅₀s of >1 µmol/L (Supplementary Table S1). Finally, we tested the combined drug treatments in primary patient samples by MTT assays. The combined drug treatments resulted in...
synergistic antileukemic activity in AML (Fig. 5). Although cytarabine and daunorubicin combined with ABT-199 resulted in additive to slightly synergistic interaction in two nonmalignant bone marrow samples, the impact of ABT-199 on daunorubicin or cytobrine IC50 was minor when compared with malignant samples.
Discussion

The Bcl-2–selective inhibitor ABT-199 has demonstrated promising preclinical results (10, 11, 26, 32), although it has limited efficacy in Bcl-xl– and Mcl-1–dependent malignancies (9, 33). Thus, it is important to understand the mechanisms of resistance and develop therapies to overcome resistance. In this study, we demonstrated that ABT-199 treatment resulted in increased Mcl-1 protein levels in intrinsically resistant AML cell lines, likely due to increased protein stability. Recently, Choudhary and colleagues demonstrated that cells with acquired resistance to ABT-199 exhibited increased Mcl-1 and Bcl-xl levels, leading to increased sequestration of Bim in lymphoma cell lines (33). In addition, Alford and colleagues found that high levels of Mcl-1 can maintain survival of ABT-199–treated ALL cells in part through sequestering Bim (34). Bogenberger and colleagues demonstrated that siRNA knockdown of Mcl-1 in THP1 and OCI-AML3 cells (ABT-199–resistant cell lines) resulted in increased ABT-199 sensitivity (35) and we demonstrated previously that ectopic overexpression of Mcl-1 attenuated ABT-199–induced apoptosis (12). In agreement with all of these studies, our data demonstrated that intrinsically resistant AML cells have increased levels of Mcl-1 following ABT-199 treatment, resulting in increased sequestration of Bim by Mcl-1 (Figs. 1 and 3). Our Bim knockdown data demonstrated that Bim played a role in the increased Mcl-1 levels after ABT-199 treatment in resistant cells (Fig. 1I). Choudhary and colleagues determined that increased mRNA levels and increased Mcl-1 protein stability in their acquired resistance cell line models was responsible for the increased protein levels (33). Similarly, we found that in ABT-199–resistant AML cell lines, ABT-199 treatment resulted in increased Mcl-1 protein stability, however, we did not observe an increase of mRNA, possibly due to the differences between acquired resistance and intrinsic resistance and/or differences between leukemia and lymphoma cell lines. Our Mcl-1 knockdown data further confirmed that Mcl-1 played an important role in ABT-199–induced cell death (Fig. 1K and L).

Although the exact mechanism by which Bim protects Mcl-1 is still unclear, we speculate that ABT-199 treatment releases Bim from Bcl-2, allowing for sequestration of Bim by Mcl-1, stabilizing Mcl-1, and ultimately resulting in survival in the ABT-199–resistant cells (Fig. 6). The redistribution of Bim to Mcl-1 may displace ubiquitin ligases, such as MULE, β-TRCP, or FBW7 (36, 37), resulting in increased Mcl-1 protein stability. Interestingly, our data demonstrated a time-dependent increase of Mcl-1 after ABT-199 treatment, potentially due to the continued production of Mcl-1 protein in addition to the increased stability via interaction with Bim. Alternatively, deubiquitinases, such as USP5X (38), may also play a role in the increased Mcl-1 protein stability. Studies to determine the exact mechanism by which ABT-199 treatment results in increased Mcl-1 protein stability in ABT-199–resistant cells are currently underway.

To overcome resistance to ABT-199, various chemotherapeutic drugs have been used in combination with ABT-199 and have demonstrated synergistic anticancer activity. For example, ABT-199 was demonstrated to synergize with cytarabine in acute lymphoblastic leukemia (39) and lymphoma cell lines, though the mechanism was not investigated (40). It has also been demonstrated to synergize with 5-azacytidine in ex vivo AML and myelodysplastic syndrome/chronic myelomonocytic leukemia samples (32). In addition, synergistic interactions have been demonstrated for ABT-199 in combination with doxorubicin, bortezomib, and YM155 in lymphoma cell lines (40), although the mechanism of action for the combined treatments was not investigated in these studies. Our studies not only demonstrated that daunorubicin and cytarabine can synergize with ABT-199 in both ABT-199–sensitive and ABT-199–resistant cells, but also revealed that these DNA damaging agents cooperate with ABT-199 in inducing DNA damage, leading to abrogation of Mcl-1 stabilization induced by ABT-199 treatment.

We previously reported an inverse correlation between the ratio of Bcl-2/Mcl-1 transcript levels and ABT-199 IC50 (12). In a larger cohort of patient samples, we further confirmed these results (Supplementary Fig. S3). Thus, it is plausible that in the ABT-199–sensitive cells more Bim is released from Bcl-2 than can be sequestered by Mcl-1 (high Bcl-2/Mcl-1 transcript ratios), resulting in free Bim which would then activate the canonical apoptosis pathway. While in ABT-199–resistant cells (those with low Bcl-2/Mcl-1 transcript ratios) it is conceivable that there is enough Mcl-1 to sequester Bim and inhibit apoptosis. For the combined drug treatments in resistant cells, Mcl-1 downregulation likely results in free Bim, allowing for activation of Bak/Bax and apoptosis (Fig. 6). Interestingly, both combinations were also synergistic in inducing cell death in both ABT-199–sensitive and ABT-199–resistant AML cell lines and primary patient samples (Fig. 5D). In addition, the
mechanism of action. We previously demonstrated that ectopic expression of Mcl-1 attenuated ABT-199–induced apoptosis (12), suggesting that other Bcl-2 family members may also contribute to intrinsic ABT-199 resistance. Bim knockdown had a small yet significant impact on combined daunorubicin and ABT-199–induced cell death (Fig. 3G), suggesting that although Bim played a role, other factors likely contributed to the synergistic interaction. It is also important to note that Bim knockdown had no effect on daunorubicin-induced cell death. In contrast, Bim knockdown had a more significant impact on cytarabine as well as combined cytarabine and ABT-199–induced cell death (Fig. 4C). Additional studies are underway to determine the mechanisms underlying cell death induced by these drug combinations in AML cells.

In summary, binding of released Bim by Mcl-1 following ABT-199 treatment in AML cells is a mechanism of intrinsic resistance. Our study demonstrates that this mechanism of resistance can be overcome by combining ABT-199 with daunorubicin or cytarabine in AML cells. In addition, we demonstrate that these combinations are synergistic in cell lines and primary patient samples independent of their sensitivities to ABT-199, thus providing evidence that screening for ABT-199 resistance is not necessary, therefore supporting clinical testing regardless of ABT-199 sensitivity. Our findings, though in a limited number of primary patient samples, provide new insights into the mechanism of ABT-199 resistance in AML cells and support the clinical development of the combination of daunorubicin or cytarabine and ABT-199 in the treatment of AML.

References


Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The funders had no role in study design, data collection, analysis and interpretation of data, decision to publish, or preparation of the manuscript.

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