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# Bortezomib and sphingosine kinase inhibitor interact synergistically to induces apoptosis in BCR/ABl$^+$ cells sensitive and resistant to STI571 through down-regulation Mcl-1

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ABSTRACT

Interactions between the proteasome inhibitor, bortezomib, and the sphingosine kinase (SPK1) inhibitor, SKI, were examined in BCR/ABL human leukemia cells. Coexposure of K562 or chronic myeloid leukemia (CML) cells from patients to subtoxic concentrations of SKI (10 μM) and bortezomib (100 nM) resulted in a synergistic increase in caspase-3 cleavage and apoptosis. These events were associated with the down-regulation of BCR–ABL and Mcl-1, and a marked reduction in SPK1 expression. In imatinib mesylate-resistant K562 cells that displayed decreased BCR–ABL expression, bortezomib/SKI treatment markedly increased apoptosis and inhibited colony-formation in association with the downregulation of Mcl-1. Finally, the bortezomib/SKI regimen also potently induced the downregulation of BCR/ABL and Mcl-1 in human leukemia cells. Collectively, these findings suggest that combining SKI and bortezomib may represent a novel strategy in leukemia, including apoptosis-resistant BCR–ABL$^+$ hematologic malignancies.


## 1. Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by the Chr. 9;22 translocation, which results in the expression of a fusion oncoprotein, BCR/ABL. The BCR/ABL kinase activates a variety of downstream survival pathways, including the mitogen activated protein kinase (MEK)/extracellular signal regulating kinase (ERK) cascade, Akt, signal transducers and activators of transcription (STATs), and sphingosine kinase 1, among others [1–3]. Activation of these pathways in BCR/ABL$^+$ cells results in increased expression of several anti-apoptotic proteins, such as Mcl-1 [4]. Collectively, these events provide BCR/ABL$^+$ cells with a survival advantage over their normal counterparts, thereby contributing to the leukemic phenotype. Moreover, BCR/ABL$^+$ cells display varying degrees of resistance against conventional cytotoxic drugs [5].

The discovery that the BCR/ABL kinase not only promotes the proliferation of leukemic cells but is also necessary for their survival prompted the search for specific inhibitors of this kinase. Such efforts culminated in the development of the BCR/ABL kinase inhibitor, imatinib mesylate (IM; Gleevec, STI571), which has revolutionized the treatment of CML. IM has proved highly active in patients with chronic-phase CML and, to a lesser extent, in patents with accelerated and blastic-phase disease. Unfortunately, the preexistence or development of IM resistance, generally through BCR/ABL amplification or mutation, ultimately leads to disease progression. More recently, IM resistance has been associated with diminished BCR/ABL expression and activation of other kinases [6]. In view of the continuing problem of IM resistance, new approaches to the treatment of BCR/ABL$^+$ leukemia remain a high priority.

Sphingosine 1-phosphate (S1P) is a bioactive lipid that has an important role in regulating the growth, survival, and migration of mammalian cells. Sphingosine kinase (SPK1) is an oncogenic sphingolipid-metabolizing enzyme that catalyzes the formation of S1P at the expense of pro-apoptotic ceramide. Thus, SPK1 is an attractive target for cancer therapy because blockage of S1P formation leads to inhibition of proliferation, as well as the induction of apoptosis in cancer cells [7]. Compelling evidence indicates the role of SPK1 deregulation in the processes of carcinogenesis and the acquisition of drug resistance, which provides a rationale for an effective anti-cancer therapy. SPK1 inhibitors, including DMS

*Abbreviations:* SPK1, sphingosine kinase 1; S1P, sphingosine 1-phosphate; SKI, sphingosine kinase inhibitor; DMS, *N*,*N*-dimethylsphingosine; CML, chronic myelogenous leukemia; MEK, mitogenactivated protein kinase; ERK, extracellular signal regulating kinase; Mcl-1, myeloid-cell-leukemia.

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(*N*,*N*-dimethylsphingosine) and SKI (2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl) thiazole) [8], have been tested in cancer cell lines. In the current study, we used SKI and DMS to interrogate the role of SPK1 in human CML BCR–ABL$^+$ cells. These findings suggest that SKI and DMS may be more clinically efficacious than was predicted.

Bortezomib (Velcade; previously known as PS-341) is an inhibitor of the 20S proteasome, which is responsible for the degradation of diverse intracellular proteins. The ubiquitin/proteasome system plays a critical role in cellular homeostasis and contributes to the control of multiple proteins, including those implicated in the regulation of cell proliferation, survival, and differentiation [9,10]. Proteasome inhibitors trigger apoptosis in malignant cells through a mechanism that has not been fully elucidated [11]. Bortezomib, a boronic acid anhydride proteasome inhibitor, has recently shown remarkable activity in patients with multiple myeloma, including those with refractory disease [12–14].

Bortezomib has been reported to downregulate and inhibit BCR/ABL$^+$ and induce cell death in CML cells [11]. Moreover, combined exposure to bortezomib and sphingosine kinase inhibitors potently induces apoptosis in BCR/ABL$^+$ human leukemia cells. As BCR/ABL$^+$ cells are relatively resistant to apoptosis induced by conventional agents and depend upon unique BCR/ABL-dependent signaling pathways for their survival, the question arises as to whether such cells might also be susceptible to such a strategy. To address this issue, we examined interactions between SKI and bortezomib in BCR/ABL$^+$ cells, including those resistant to IM. Our results indicate that combined administration of SKI and bortezomib effectively triggers apoptosis in BCR/ABL$^+$ cells that are both sensitive and resistant to IM through a mechanism that involves the downregulation of Mcl-1 expression.

## 2. Materials and methods

### 2.1. Cells

K562 cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in culture in RPMI 1640 medium that contained 10% fetal bovine serum. The cells were passaged once a week. To establish imatinib-resistant sublines, logarithmically growing cells were exposed to increasing concentrations of imatinib, starting with a concentration of 0.05 μM and increasing gradually by increments of 0.1 μM. After the cells acquired the ability to grow in the presence of a specific concentration of IM, the level of resistance was determined. A proportion of cells were then frozen, and the remaining cells were grown at the next highest drug level. All experiments were performed using logarithmically growing cells (4–6 $\times$ $10^5$ cells/ml).

### 2.2. Reagents and antibodies

The proteasome inhibitor, bortezomib, was provided by Millennium Pharmaceuticals (Cambridge, MA). The SPK inhibitor, *N*,*N*-dimethylsphingosine (DMS) was purchased from CalBiochem (San Diego, CA, USA), and SKI (2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl) thiazole) was purchased from Echelon (Echelon Bioscience, Salt Lake City, UT). These agents were dissolved in dimethyl sulfoxide (DMSO) as a stock solution, and stored at −80 °C. In all experiments, the final concentration of DMSO did not exceed 0.1%. S1P was purchased from CalBiochem. PE-conjugated active caspase-3 antibodies were purchased from BD Pharmingen. MethCult® (4445, StemCell Technologies, Vancouver, Canada). Anti-Bcl-2, anti-polyadenosine diphosphate ribose polymerase (anti-PARP, which recognized the full-length product and the N-terminal cleavage product), anti-BCR–ABL and anti-actin were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit anti-human Mcl-1 antibody and rabbit polyclonal antibody to SPK1 was from Abcam (Cambridge, UK). EasySep® Human CD34 Positive Selection Kit was from StemCell Technologies (Vancouver, BC, Canada). The protein assay reagent was from Bio-Rad Laboratories (Hercules, CA). FITC-labeled annexin V staining kit for apoptosis was from BD Biosciences (San Jose, CA). HRP-conjugated goat anti-rabbit antibody was from Jackson ImmunoResearch (West Grove, PA, USA).

### 2.3. Patient samples

Patient samples were leukapheresis products taken at the time of diagnosis with CP CML, with informed consent from each patient and approval of the Local Research Ethics Committee. Blood was collected in heparinized syringes, diluted to a ration of 1:3 with RMPI 1640 medium, and transferred as an overlayer to centrifuge tubes containing 10 ml Ficoll-Hypaque (specific gravity, 1.077–1.081; Sigma, St. Louis, MO). After centrifugation at room temperature for 30 min, the interface layer that contained the mononuclear cells, was extracted with a sterile Pasteur pipette, suspended in RPMI medium, and washed three times. The CD34$^+$ population was enriched using EasySep® Human CD34 Positive Selection Kit (Stemcell, Vancouver, Canada) according to the standard protocols, before storage as aliquots at −150 °C. For particular experiments, CML CD34$^+$ samples were stained with CD34-PE (BD Biosciences) and tested using a FACS (BD Biosciences) to obtain the CD34 positive ratio.

### 2.4. Total RNA extraction and reverse transcriptase real-time polymerase chain reaction

Total RNA was extracted with TRIzol reagent (Gibco, Invitrogen) according to the manufacturers instruction real-time PCR was carried out in iCycler IQ detection system (Bio-Rad, Herlev, Denmark) by using SYBR® Green I as a double-strand DNA-specific binding dye. PCRs were performed in triplicate for each. The mRNAs of target genes and the house-keeping gene β-2-microglobulin (B2M) mRNA were quantified in separate tubes. The value of $100 \times 2^{-\Delta C_t}$ represents the relative level of target gene expression.

### 2.5. Colony-forming assay

K562R cells were added to MethCult® or MethCult® with 100 nM bortezomib ± 10 μM SPK inhibitor (SKI) and vortexed. Then cells were dispensed into pre-tested Petri dishes using syringe and blunt-end needle. The cells were incubated for 14 days in a humidified incubator at 37 °C and 5% $CO_2$. Colonies were stained with MTT and counted using an inverted microscope and scoring dishes with grids. Colonies with at least 50 cells were scored.

### 2.6. Western blot analysis

Cells were washed twice with 1× PBS and suspended in lysis buffer (20 mM Tris, 150 mM NaCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride, 40 mM glycerophosphate, 125 μM $Na_3VO_4$, 50 mM NaF, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml pepstatin, and 1 mM dithiothreitol. After 40 min on ice, lysates were cleared by centrifugation at 13,000*g*/min for 30 min at 4 °C. Then, 40 μg of the sample was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose. After blocking with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST), the nitrocellulose membrane was incubated with primary antibody in 5% bovine serum albumin in TBST overnight at

4 °C. The membrane was then washed three times with TBST and incubated with the secondary antibody in 5% milk in TBST. After five washes with TBST, the membrane was developed with enhanced chemiluminescence (ECL, Amersham Pharmacia).

### 2.7. Apoptosis assay

Apoptotic cells were detected using FITC-conjugated annexin V (annexin V-FITC; Caltag laboratories, Burlingame, CA) and propidium iodide (PI). Cells were washed twice with cold PBS and resuspended in annexin V binding buffer (10 mM Hepes, 140 mM NaCl, 5 mM $CaCl_2$) at a concentration of $1 \times 10^7$ cells/ml. One hundred microliters ($1 \times 10^6$ cells) were added to a 5 ml culture tube, and 5 μl of annexin V-FITC and 10 μl of PI were added; the tube was gently vortexed and incubated for 15 min at room temperature in the dark. Binding buffer (400 μl) was then added to each tube and the cells were analyzed with a flow cytometer (Becton–Dickinson, Mountain View, CA, USA).

### 2.8. Statistical analysis

The data were reported as mean ± SE (standard error of the mean) obtained from at least three separate experiments in which each time point analysis was performed in triplicate. The means were compared by an analysis of variance and paired *t*-test. A *P*-value <0.05 was used to define statistical significance.

## 3. Results and discussion

While the introduction of the BCR/ABL kinase inhibitor, STI571, into the clinical armoury represents a major advance in the treatment of CML, the development of drug resistance constitutes a major barrier to the cure of this disease. Thus far, the major mechanisms of clinical resistance appear to involve either increased expression of the BCR/ABL protein through gene amplification [15] or the development of mutations in the BCR/ABL catalytic domain, which interfere with IM binding to BCR/ABL [16]. Compelling evidence indicates that there is a role for SPK1 deregulation in carcinogenesis and the acquisition of drug resistance, which provides the rationale for an effective anti-cancer therapy.

In this report, human CML $CD34^+$ cells and $BCR/ABL^+$ cells that were sensitive and resistant to STI571 were examined using real-time PCR analysis and Western blotting (Fig. 1A and B). Overexpression of SPK1 was observed in human CML $CD34^+$ cells and $BCR/ABL^+$ cells that were sensitive and resistant to STI571. The SPK1 inhibitor affected the clonogenic potential and viability of primary cells from CML patients, including one that harbored the IM-insensitive *Abl* kinase domain mutation, T315I [7].

SPK1 is a downstream effector of the BCR–ABL/Ras/ERK pathway inhibited by IM [2]. SPK1 is a critical regulator of the sphingolipid balance and is involved in the susceptibility of either sensitive or multidrug-resistant acute myeloid leukemia cells to antineoplastic agents [17]. We used the SPK1 inhibitor, *N*,*N*-dimethylsphingosine (DMS), to treat K562 cells, and showed that DMS inhibited the expression of SPK1 (Fig. 1C) and induced the apoptosis of K562 cells (Fig. 1D).

Due to the fact that the phenomenon of resistance to IM remains a major issue in the treatment of patients with CML, the identification of alternative targets and new drugs may be of clinical relevance. However, the effect of bortezomib/SKI on apoptosis of human $BCR/ABL^+$ cells has not been reported to date. To assess bortezomib/SKI interactions in $BCR/ABL^+$ cells, K562 cells were

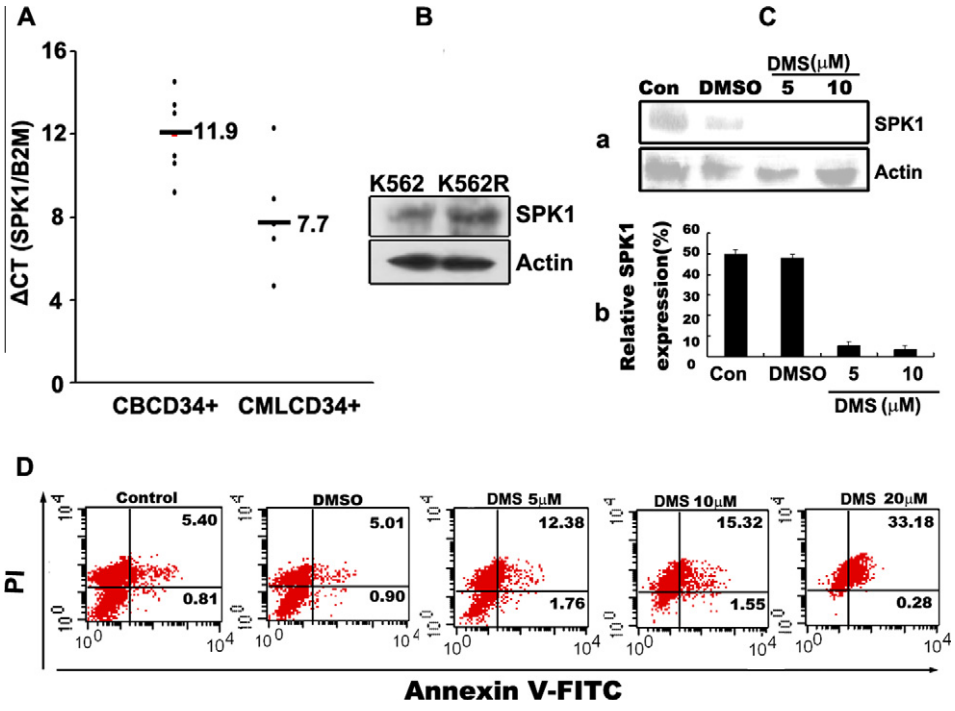


**Fig. 1.** Overexpression of SPK1 by human CML $CD34^+$ cells and $BCR/ABL^+$ cells that were sensitive and resistant to imatinib; inhibition of SPK1 induces apoptosis in $BCR/ABL^+$ cells. (A) Expression of SPK1 in human CML $CD34^+$ cells was detected by real-time PCR. B2M was used as the internal reference. (B) The expression of SPK1 in $BCR/ABL^+$ cells sensitive and resistant to imatinib was detected by Western blotting. Total cell lysates from K562 and K562R cells were separated by 12% SDS–PAGE and analyzed by immunoblotting. (C) DMS inhibited SPK1 expression; (a) K562 cells were cultured overnight in RPMI 1640 supplemented with 5 or 10 μM DMS, and DMSO served as the control. Cells were lysed and SPK1 expression was determined by Western blotting. Actin served as the loading control; (b) densities of signals were determined by densitometry and were shown to be relative to the control that was arbitrarily normalized to '1'. Results are representative of triplicate experiments. (D) DMS induces apoptosis in $BCR/ABL^+$ cells. K562 cells were cultured overnight in RPMI 1640 supplemented with 5, 10, or 20 μM DMS, and DMSO served as the control. Cells were collected and washed with cold PBS, then dual stained with annexin V-FITC and PI. The percentages of apoptotic cells were determined by flow cytometry analysis. The data is a representative of three experiments.

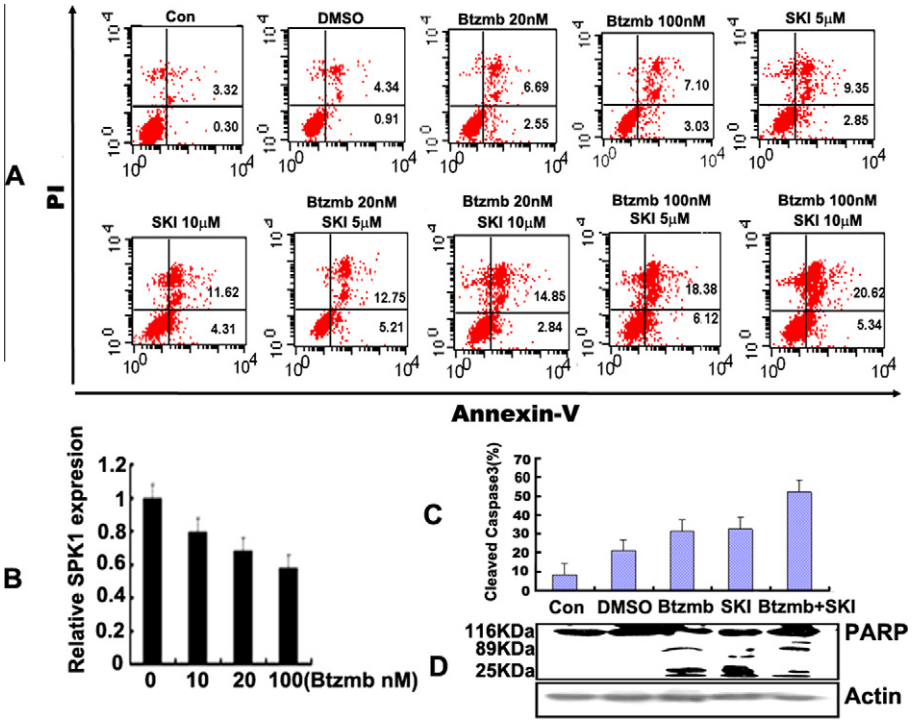


**Fig. 2.** Cotreatment with bortezomib/SPKI synergistically induces apoptosis in BCR/ABL$^+$ cells, accompanied by caspase-3 activation and PARP cleavage. (A) Bortezomib/SPKI synergistically induces apoptosis in BCR/ABL$^+$ cells. K562 cells were exposed to 20 nM (or 100 nM) bortezomib (Btzmb) ± 5 μM (or 10 μM) SPK inhibitor (SKI) for 24 h. Cells were collected and washed with cold PBS, then dual stained with annexin V-FITC and PI. The percentages of apoptotic cells were determined by flow cytometry analysis. The data is a representative of triplicate experiments. (B) Bortezomib inhibits the expression of SPK1. K562 cells were exposed to different concentrations of bortezomib (0, 10, 20, 100 nM) for 24 h. Results represent the means ± SDs for three separate experiments performed in triplicate. (C) Bortezomib/SKI synergistically prompts caspase-3 activation. K562 cells were exposed to 100 nM bortezomib (Btzmb) ± 10 μM SPK inhibitor (SKI) for 24 h. Cells were collected and washed with cold PBS, then stained with PE-conjugated active caspase-3 antibodies. The percentages of apoptotic cells were determined by flow cytometry analysis. Results represent the means ± SDs for three separate experiments performed in triplicate. (D) Bortezomib/SKI synergistically prompts PARP cleavage. K562 cells were exposed to 100 nM bortezomib (Btzmb) ± 10 μM SPK inhibitor (SKI) for 24 h. Cell lysates were prepared and subjected to Western blot analysis. Results are representative of three separate experiments.

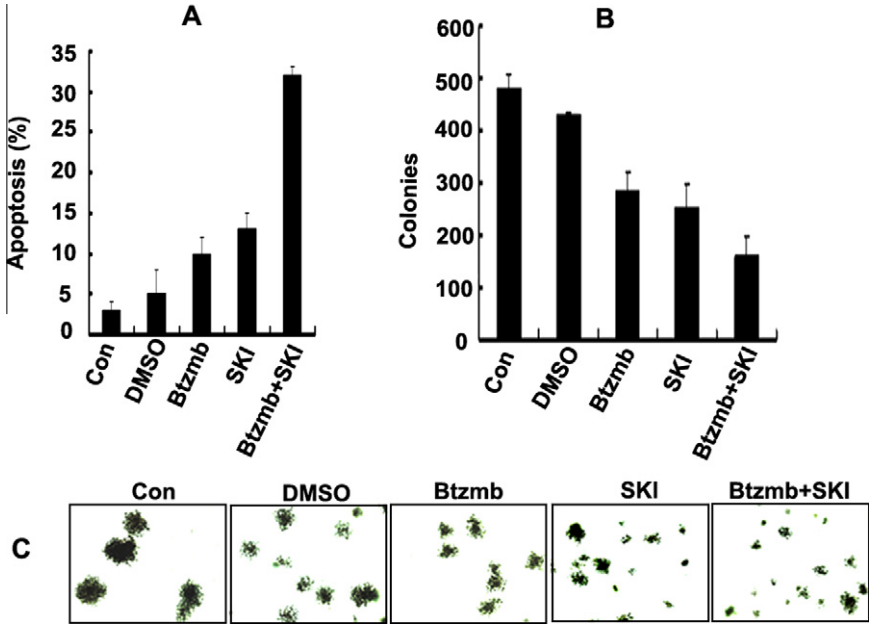


**Fig. 3.** Cotreatment with bortezomib/SKI synergistically induces apoptosis and inhibits colony-formation accompanied in BCR–ABL-independent imatinib-resistant K562 cells. (A) Cotreatment with bortezomib/SKI synergistically induced apoptosis in BCR–ABL-independent imatinib-resistant K562 cells. K562R cells were exposed to 100 nM bortezomib (Btzmb) ± 10 μM SPK inhibitor (SKI) for 24 h. Cells were collected and washed with cold PBS, then dual stained with annexin V-FITC and PI. The percentages of apoptotic cells were determined by flow cytometry analysis. Results represent the means ± SD of three separate experiments performed in triplicate. (B) Results of colony-forming assay in K562R. Colonies were counted using an inverted microscope and grid scoring dishes. Colonies with at least 50 cells were scored. Data represents means ± SD for three separate experiments performed in triplicate. (C) MTT staining K562R-clony microscope image (original magnification, 200×).

exposed to 20/100 nM bortezomib ± 5/10 μM SKI for 24 h, after which apoptosis was assessed by the annexin V/propidium iodide (PI) analysis. Treatment of cells with these agents individually for 24 h minimally increased the percentage of early and late apoptotic (Fig. 2A; lower right quadrant; annexin $V^+$, and upper right quadrant; annexin $V^+/PI^+$, respectively; <10% in both cases). In contrast, combined bortezomib/SKI treatment resulted in 26% late apoptotic cells by 24 h.

To evaluate the effect of bortezomib on the expression of SPK1, K562 cells were exposed to different concentrations of bortezomib (0, 10, 20, and 100 nM) for 24 h. As shown in Fig. 2B, bortezomib can remarkably inhibit SPK1 expression in K562 cells. Furthermore, cleaved caspase-3 and PARP were detected after treatment with bortezomib/SKI. These observations suggested that cotreatment with bortezomib/SKI synergistically prompted caspase-3 activation and PARP cleavage (Fig. 2C and D). Notably, the bortezomib/SKI regimen potently triggered apoptosis in K562R cells relative to untreated cells (3 ± 1%), as shown in Fig. 3A; apoptosis was increased in the bortezomib/SKI-treated arms (bortezomib alone, 10 ± 2%; SKI alone, 13 ± 2%; combination treatment, 32 ± 1%; all $P$ values $\leqslant 0.01$). These cells also showed decreased levels of the Mcl-1 protein (Fig. 4B).

K562R were added to MethCult® or MethCult® with 100 nM bortezomib (Btzmb) ± 10 μM SKI. Colony formation was then measured after 2 weeks of culture. Cotreatment with bortezomib/SKI synergistically induced inhibition of colony formation (Fig. 3C). With colony counts shown in Fig. 3B, relative to untreated cells (481.7 ± 28.9), colony formation was reduced significantly in the bortezomib/SKI-treated arms (bortezomib alone, 287.3 ± 37.1; SKI alone, 254.3 ± 45.6; combination, 160.0 ± 40.9; all $P$ values $\leqslant 0.01$).

Considering the important role of the SPK1/S1P axis in BCR–$ABL^+$ cells [3], we investigated whether S1P could influence the expression of BCR–ABL and Mcl-1 in BCR–$ABL^+$ cells. The BCR–$ABL^+$ cell line, K562, was starved overnight in serum-free medium;

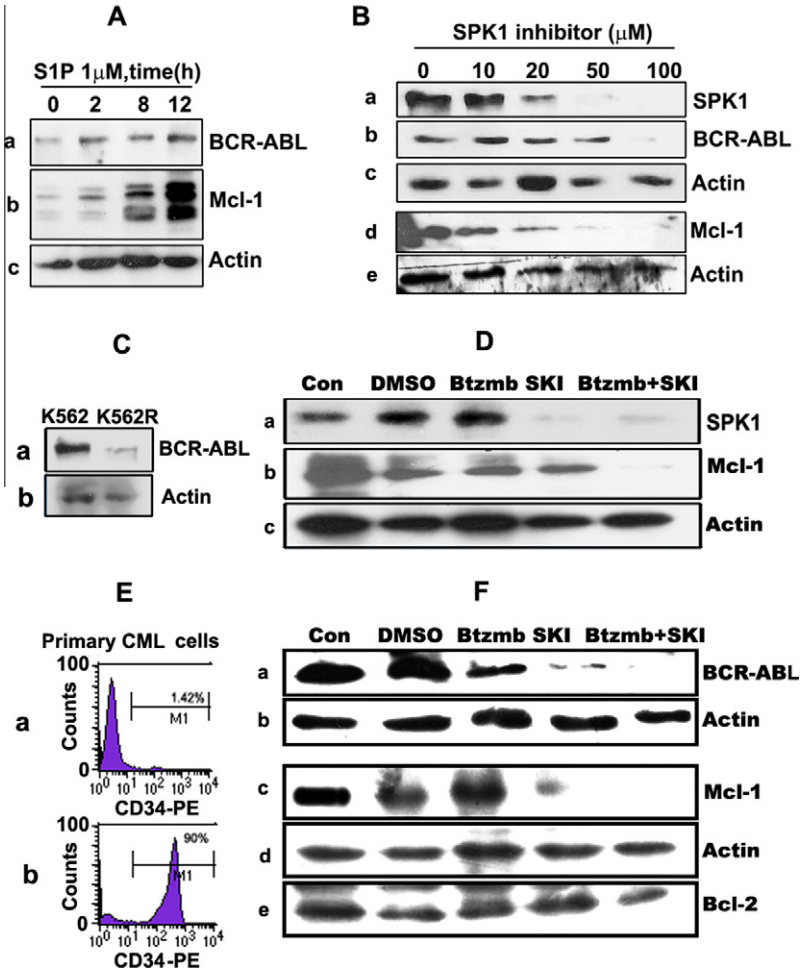


**Fig. 4.** Inhibition of SPK1 sensitizes BCR/$ABL^+$ cells to bortezomib through downregulation of Mcl-1. (A) S1P upregulates BCR–ABL and Mcl-1 expression. K562 cells were starved overnight in RPMI 1640 with 0.5% FBS, and were then cultured with 1 M S1P for different time periods (0, 2, 8, and 12 h). Western blot results are representative of three experiments. (a and b) BCR–ABL and Mcl-1 expression were upregulated at 2 h; (c) actin served as the loading control. (B) SPK1 inhibitor (SKI) diminished BCR–ABL and Mcl-1 expression. K562 cells were cultured overnight for 12 h in RPMI 1640 supplemented with different concentrations of SPKI. Cells were lysed and (a) SPK1, (b) BCR–ABL, and (d) Mcl-1 expression was determined by Western blotting. (c and e) Actin served as the loading control. Western blot results are representative of three experiments. (C) The decrease of BCR–ABL expression in imatinib-resistant K562 cells. K562 and imatinib-resistant K562R cells were collected and cell lysates obtained from these cells were subjected to Western blot analysis to monitor levels of BCR–ABL (a) and actin (b). (D) Cotreatment with bortezomib/SKI synergistically inhibited Mcl-1 expression in BCR–ABL-independent imatinib-resistant K562 cells. K562R cells were exposed to 100 nM bortezomib (Btzmb) ± 10 μM SPK inhibitor (SKI) for 24 h. Cells were collected and cell lysates were subjected to Western blot analysis to monitor levels of Mcl-1. The results are representative of three separate experiments. (E) Primary CML cells were obtained from patients in the chronic phase with the approval of the Local Research Ethics Committee. The purification of $CD34^+$ cells (a) before purification and (b) after purification. (F) Bortezomib and SKI interact synergistically to downregulate BCR–ABL and Mcl-1 expression in primary BCR/$ABL^+$ cells. Human CML CD34 + samples ($n = 3$) were exposed to 100 nM bortezomib (Btzmb) ± 10 μM SPK inhibitor (SKI) for 12 h. Cells were lysed and (a) BCR–ABL, (c) Mcl-1 and (e) Bcl-2 expression levels were determined by Western blotting. (b and d) Actin served as the loading control. The data is a representative of triplicate experiments.

1 μM S1P was added at the specified times, and the expression of BCR–ABL and Mcl-1 were assayed. As shown in Fig. 4A, S1P upregulated BCR–ABL and Mcl-1 expression in a time-dependent manner. The SPK1 inhibitor diminished BCR–ABL and Mcl-1 expression (Fig. 4B). Western blot analysis was used to characterize the molecular basis of IM resistance in K562R cells. K562R cells displayed a marked reduction in BCR/ABL expression (Fig. 4C), which was analogous to the IM-resistant K562 cell lines described by other groups [6,18]. These results reveal the involvement of SPK1 in regulating IM-induced apoptosis and establish that SPK1 is a downstream effector of the BCR–ABL/Ras/ERK pathway that is inhibited by IM but is an upstream regulator of the *Bcl-2* family members [2].

Myeloid cell leukemia-1 (Mcl-1), an anti-apoptotic member of the *Bcl-2* family, has recently been identified as a BCR/ABL-dependent survival factor in CML [3,4]. As shown in Fig. 4D, cotreatment with bortezomib/SKI synergistically decreased Mcl-1 expression in BCR/ABL-independent IM-resistant K562 cells. The finding that the bortezomib/SKI regimen effectively induced apoptosis and inhibition of colony formation in the IM-resistant K562 cell line, which also displayed decreased BCR/ABL expression levels. In this regard, the observation that the combination of bortezomib/SKI also effectively decreased BCR–ABL and Mcl-1 expression in primary $CD34^+$ CML cells is noteworthy. It will also be of interest to determine whether the bortezomib/SKI regimen spares normal $CD34^+$progenitor cells, particularly with respect to the preservation of clonogenic potential.

To determine whether synergistic interactions between bortezomib and SKI can influence the expression of BCR–ABL and Mcl-1 in CML $CD34^+$ patient samples, human CML $CD34^+$ samples (Fig. 4E, pure >90%, $n = 3$) were exposed to 100 nM bortezomib ± 10 μM SKI for 12 h. Cells were lysed and BCR–ABL and Mcl-1 expression levels were determined by Western blotting. Interestingly, the combined exposure of cells to bortezomib and SKI resulted in the downregulation of BCR–ABL and Mcl-1 expression in CML $CD34^+$ patient samples, but Bcl-2 expression remained unchanged (Fig. 4F). Notably, inhibition of SPK1 sensitized BCR/$ABL^+$ cells to bortezomib through the downregulation of Mcl-1.

Collectively, these findings suggest that the combination of SKI and bortezomib may represent a novel strategy in leukemia, including apoptosis-resistant BCR–$ABL^+$ hematological malignancies. Furthermore, this strategy may not only be specifically related to BCR/ABL-associated pathways, but may have broader applicability to leukemias in general.

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