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Triptolide induces cell death independent of cellular responses to imatinib in blast crisis CML cells including quiescent CD34+ primitive progenitor cells

Duncan H. Mak[1], **Wendy D. Schober**[1], **Wenjing Chen**[1], **Marina Konopleva**[1,2], **Jorge Cortes**[2], **Hagop M. Kantarjian**[2], **Michael Andreeff**[1,2], and **Bing Z. Carter**[1]

[1]Section of Molecular Hematology and Therapy, Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

[2]Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Abstract

The advent of Bcr-Abl tyrosine kinase inhibitors (TKIs) has revolutionized the treatment of CML. However, resistance evolves due to BCR-ABL mutations and other mechanisms. Furthermore, patients with blast crisis (BC) CML are less responsive and quiescent CML stem cells are insensitive to these inhibitors. We found that triptolide, a diterpenoid, at nM concentrations, promoted equally significant death of KBM5 cells, a cell line derived from a Bcr-Abl-bearing BC CML patient and KBM5STI571 cells, an imatinib-resistant KBM5 subline bearing the T315I mutation. Similarly, Ba/F3 cells harboring mutated BCR-ABL were as sensitive as Ba/F3Bcr-Abl^{p210wt} cells to triptolide. Importantly, triptolide induced apoptosis in primary samples from BC CML patients, who showed resistance to Bcr-Abl TKIs *in vivo*, with less toxicity to normal cells. Triptolide decreased XIAP, Mcl-1, and Bcr-Abl protein levels in K562, KBM5, KBM5STI571 cells and in cells from BC CML patients. It sensitized KBM5, but not KBM5STI571 cells to imatinib. More importantly, triptolide also induced death of quiescent CD34$^+$ CML progenitor cells, a major problem in the therapy of CML with TKIs. Collectively, these results suggest that triptolide potently induces BC CML cell death independent of the cellular responses to Bcr-Abl TKIs, suggesting that triptolide could eradicate residual quiescent CML progenitor cells in TKI-treated patients and benefit TKI-resistant BC CML patients.

Keywords

triptolide; XIAP; Mcl-1; Bcr-Abl; quiescent CD34$^+$ CML cells

Introduction

CML is a lethal myeloproliferative disorder derived from the clonal expansion of transformed hematopoietic stem cells(1) and is characterized by the formation of the BCR-ABL fusion gene coding for a constitutively active tyrosine kinase which is necessary and sufficient for malignant transformation.(2-4) Imatinib, a Bcr-Abl tyrosine kinase inhibitor (TKI), has

Correspondence: Bing Z. Carter, Ph.D., Section of Molecular Hematology and Therapy, Department of Stem Cell Transplantation and Cellular Therapy, Unit 448, The University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Telephone: 713-794-4014. Fax: 713-794-4747. bicarter@mdanderson.org.

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drastically improved the outcome of patients with CML. However, imatinib is less effective in accelerated phase and blast crisis (BC) CML patients, 45-90% of whom fail to respond or develop resistance within 3-12 months.(5;6) Resistance to TKIs develops in both chronic and advanced CML patients, which leads to the reactivation of Bcr-Abl kinase activity within leukemic cells, through either gene amplification(7-9) or mutations.(7;10-13) New Bcr-Abl TKIs such as nilotinib and dasatinib have been reported to overcome resistance of most mutants but not of the T315I mutation. Regardless, since these agents inhibit tyrosine kinase activity but do not affect protein expression, mutations will likely develop again. Clearly, alternative therapeutic strategies are needed for patients with advanced and TKI-resistant CML.

Further, Bcr-Abl was shown to induce DNA damage and contribute to genomic instability (14) which is believed to be responsible for promoting the transition from the benign chronic to blast phase and providing additional growth and survival advantages for BC CML cells. Therefore, eliminating the Bcr-Abl protein could be an alternative approach to treating CML patients and overcoming resistance to Bcr-Abl TKIs. Several groups have worked toward the development of such strategy.(15-17)

Although the majority of CML progenitors have a higher proliferative capacity than normal progenitors,(18) a subpopulation of $CD34^+$ CML progenitors is quiescent. This cell population constitutes approximately 0.5% of the total $CD34^+$ compartment and is able to engraft *NOD/SCID* mice and to initiate leukemia, which makes it a candidate CML stem cell.(19;20) An additional problem with current therapies for CML, including TKIs, other signal transduction inhibitors, and conventional chemotherapeutic agents is that they act by inhibiting cell proliferation and inducing apoptosis. Thus, complete cures are rare because TKIs are not effective against quiescent progenitor/stem cells(21;22) and these cells persist even when complete hematological and cytogenetic remissions are achieved.(20) Discontinuation of therapy frequently leads to relapse of the disease.(21) *In vitro* studies, corroborating these findings, have demonstrated that quiescent CML stem cells are insensitive to imatinib and dasatinib.(21;22) Clearly, the cure of CML depends on the eradication of quiescent stem cells.

Triptolide, a diterpenoid isolated from a Chinese herb, *Tripterygium wilfordii* Hook.f, has been shown to have anti-tumor properties by suppressing cell growth and inducing apoptosis. (23-26) We recently reported that triptolide induces cell death in AML by inhibiting the RNA and protein levels of XIAP and decreasing the protein level of Mcl-1,(27) two potent cellular antiapoptotic proteins. Mcl-1 was recently reported to be a Bcr-Abl targeted gene in CML cells. (27;28) We also showed that triptolide effectively kills KBM5 and K562 cells, two BC CML cell lines.(27) We here examined the effect of triptolide on imatinib-resistant CML cells and $CD34^+$ quiescent CML progenitor cells. The results show that triptolide potently kills BC CML cells independent of their responses to TKIs, in agreement with a recent report by Shi et al.. (29) The results also suggest that triptolide has the potential to target quiescent $CD34^+$ CML progenitor cells.

Materials and Methods

Cells and cell culture

KBM5,(30) an imatinib-sensitive BC CML cell line and KBM5STI571, an imatinib-resistant KBM5 subline harboring a T315I mutation(31) in the BCR-ABL gene were cultured in Iscove's modified Dulbecco's medium (Gibco-BRL, Gaithersburg, MD). K562 cells and Ba/F3vec, Ba/F3Bcr-Abl^{p210wt}, Ba/F3Bcr-Abl^{E255K}, and Ba/F3Bcr-Abl^{T315I} cells (kindly provided by Dr. C. Sawyers, UCLA, Los Angeles, CA) were cultured in RPMI-1640 medium. Both media were supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Medium for Ba/F3vec cells also contained 2 ng/mL mouse recombinant IL-3 (Peprotech Inc., Rocky Hill, NY). Bone marrow (BM) or

peripheral blood (PB) samples from CML patients in BC were acquired after informed consent had been obtained according to institutional guidelines and in concordance with the declaration of Helsinki. Mononuclear cells from these samples were purified by Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) density-gradient centrifugation and cultured in the same medium as K562 cells.

Cell viability assay

CML cells from either cell lines (0.2×10^6/mL) or patients with CML (0.5×10^6/mL) and Ba/F3 cells (0.05×10^6/mL) were treated with triptolide (Alexis Biochemicals, San Diego, CA) for 24 or 48 hours. KBM5 and KBM5STI571 cells were treated with imatinib (LC Laboratories, Woburn, MA) for 24 to 72 hours. For triptolide and imatinib combination, triptolide was administered 24 hours before imatinib (1 μM) was added and cell death was assessed 24 hours after imatinib treatment (total 48 hours). Apoptotic cell death was analyzed by measuring externalized phosphatidyl serine with the Annexin-V-FLUOS Staining Kit (Roche Diagnostics Corp., Indianapolis, IN) in combination with a vital dye: propidium iodide (PI) or 7-amino-actinomycin D (7-AAD), expressed as percentage of Annexin V+/PI+ or 7-ADD+ for cell lines and as percentage of survival cells (Annexin V-/PI-) for CML patient samples of triptolide treated cells compared to the untreated cells.

Cell viability of $CD34^+$ quiescent and proliferating CML cells

Mononuclear cells (5×10^6/mL) from BM or PB of patients with BC CML were labeled with 1 μM 5-(and 6-) carboxy-fluorescein diacetate succinimidyl ester (CFSE) as described elsewhere(32) and then cultured in serum-free RPMI-1640 medium supplemented with growth factors (GM-CSF, 200 pg/mL; G-CSF, 1 ng/mL; stem cell factor, 200 pg/mL; LIF, 50 pg/mL; MIP-1α, 200 pg/mL; and IL-6, 1 ng/mL) or co-cultured in RPMI-1640/10%FCS medium with MS-5 cells, a mouse mesenchymal stromal cell (MSC) line known to support primitive human progenitor and to mimic the BM microenvironment.(33-35) Cell proliferation was monitored by flow cytometric measurement of CFSE fluorescence intensity, which halves with each cell division. Quiescent cells were defined as those within a region of CFSE fluorescence of paraformaldehyde-fixed cells on day 0 ($CFSE^{bright}$). Proliferating cells were defined as those with a fluorescent intensity less than that of the initiating cell population ($CFSE^{dim}$). After culturing for 4-7 days, cells were treated with triptolide for 24 or 48 hours and then stained with CD34-PE and annexin V-Cy5. Apoptosis of quiescent primitive $CD34^+$ CML cells was defined as annexin V positivity in the $CD34^+CFSE^{bright}$ population, while apoptosis of proliferating primitive $CD34^+$ CML cells was defined as annexin V positivity in the $CD34^+CFSE^{dim}$ population.

Western blot analysis

XIAP, Mcl-1, and Bcr-Abl protein levels were determined by western blot analysis, as described previously.(36) XIAP antibody was purchased from BD-Transduction lab (San Diego, CA), Mcl-1 antibody from BD-Pharmingen, and c-Abl antibody from Cell Signaling Technology (Danvers, MA). Signals were detected using a PhosphorImager (Storm 860 Version 4.0; Molecular Dynamics, Sunnyvale, CA) and quantitated by ImageJ (NIH, Bethesda, MD). β-actin was included as a loading control.

Real-time RT-PCR

RNA was extracted using Trizol solution (Invitrogen, Carlsbad, CA) and cDNA was generated with random hexamers and AMV reverse transcriptase (Roche Applied Science, Mannheim, Germany) at 42°C for one hour. Real-time PCR was performed in Applied Biosystems 7900HT Fast RT-PCR system using SYBR Green detection method. Reaction mixture contained 0.5 μL cDNA, 0.4 μM each of the forward and reverse primers, 2× SYBR Green master mix

(Applied Biosystems, Forster City, CA) in a total of 25 μL. The reaction was initiated at 95° C for 10 minutes, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. The specificity of the PCR products was confirmed by dissociation curve analysis with ABI SDS 2.3 software. The forward/reverse primers for Mcl-1 were 5′-GAGGCTGGGATGGGTTTGT-3′/5′-AAAGCCAGCAGCACATTCCT-3′ and XIAP 5′-CCCAAATTGCAGATTTATCAACG-3′/5′-TGCATGTGTCTCAGATGGCC-3′. 18S RNA was used as an internal control. The abundance of each transcript relative to that of 18S was calculated using the $2^{-\Delta Ct}$ method, where ΔCt is the mean Ct of the transcript of interest minus the mean Ct of the transcript for 18S.

Statistical analysis

Experiments were performed three times and the results expressed as mean ± standard deviation. For experiments with patient samples, the results were expressed as mean ± standard error. Statistical significance was denoted at $p<0.05$, where applicable, using Student's t-test. Triptolide concentrations that induced annexin V positivity in 50% of cells (IC_{50}) was calculated using Calcusyn software (Biosoft, Ferguson, MO).

Results

Triptolide induces cell death independent of BCR-ABL mutations and cellular response to imatinib

We showed previously that triptolide potently induces apoptosis in various acute leukemic cells including KBM5 and K562 cells,(27) two BC CML cell lines. To evaluate whether triptolide is effective in imatinib-resistant CML cells, we treated KBM5 and KBM5STI571 cells with triptolide for 48 hours. As shown in Fig 1A, KBM5STI571 cells and KBM5 cells had similar sensitivities to triptolide. We then treated Ba/F3vec, Ba/F3Bcr-Abl^{p210wt}, Ba/F3Bcr-Abl^{E255K}, and Ba/F3Bcr-Abl^{T315I} cells with triptolide. We found that it invariably killed Ba/F3 cells harboring either the wild type (wt) or mutant BCR-ABL genes at 48 hours (Fig 1B). We concluded that triptolide induces cell death independent of BCR-ABL mutation status and cellular response to imatinib. Of note, CML cells with a T315I mutation in the BCR-ABL gene are resistant not only to imatinib but also to most other more potent Bcr-Abl TKIs.

Triptolide induces death of blasts from patients with BC CML in vitro independent of patients' responses to imatinib and other Bcr-Abl TKIs in vivo

Seven samples of mononuclear cells were obtained from six patients with BC CML and treated with triptolide *in vitro*. The characteristics of these patients and their *in vivo* responses to treatments are illustrated in Table 1. Interestingly, apart from patient 4, who had not been treated with imatinib or other TKIs, all patients were resistant to imatinib. Three of six patients (1, 3, and 5) were also resistant to nilotinib, a more potent Bcr-Abl TKI. In addition, patient 5 was also treated with dasatinib, a dual Bcr-Abl and Src inhibitor, but had failed to achieve a cytogenetic remission. As shown in Table 1, other than M351T mutation detected in BCR-ABL gene in patient 1, no mutations were found in other patients. Nevertheless, most of them were insensitive to TKIs in agreement with literature reports. Regardless of their response to TKIs, all samples were sensitive to triptolide *in vitro*. As shown in Fig 1C and Table 1, triptolide induced significant cell death in all samples studied at 24 hours, indicating that triptolide induces death of CML blast cells *in vitro* independent of patients' clinical responses to imatinib and other Bcr-Abl TKIs. It is important to point out that with 50 or 100 nM of triptolide, approximately 40% or 50% of cells, respectively, lost viability in those samples, while our previous study showed that at the same concentrations, >90% or about 75% of $CD34^+$ cells, respectively, from normal BM samples were viable.(27)

Triptolide decreases antiapoptotic XIAP and Mcl-1 as well as CML-causative Bcr-Abl levels

Next, we investigated the involvement of apoptosis regulators in triptolide-induced cell death in CML cells. Triptolide at 100 nM decreased XIAP and Mcl-1 mRNA levels in a time-dependent manner in K562 cells (Fig 2A). Lou Y et al.(37) had reported that triptolide decreased BCR-ABL RNA levels in K562 cells. We therefore analyzed XIAP, Mcl-1, and Bcr-Abl protein levels in triptolide-treated CML cells. Results showed that triptolide indeed induced dose- and time-dependent decreases in XIAP, Mcl-1, and Bcr-Abl protein levels in K562 cells (Fig 2B). To demonstrate that triptolide has a similar effect on imatinib resistant cells, we measured XIAP, Mcl-1, and Bcr-Abl protein levels in triptolide treated KBM5 and KBM5STI571 cells. As shown in Fig 2C, triptolide treatment resulted in decreases of XIAP, Mcl-1, and Bcr-Abl protein levels in both cell lines. The responses of KBM5 and KBM5STI571 cells to imatinib were also verified and shown in Fig 2C. We next determined the levels of these proteins in triptolide-treated primary blast cells from CML patients and Fig 2D shows that XIAP, Mcl-1, and Bcr-Abl protein levels decreased in a dose-dependent manner. These were the same samples that were used in the *in vitro* triptolide experiments shown in Fig 1C. Because of the limited number of cells available in some samples, XIAP protein levels were determined in four and Bcr-Abl and Mcl-1 protein levels in three of the seven samples (Table 1).

Triptolide sensitizes CML cells to imatinib-induced cell death

Based on the observation that triptolide decreases the levels not only of antiapoptotic proteins XIAP and Mcl-1 but also of the CML-causative Bcr-Abl protein, we hypothesized that the combination of triptolide and imatinib would be more effective at inducing death in CML cells than each of the agents alone. To test this hypothesis, we treated KBM5 cells with both triptolide and imatinib and did not observe any sensitization. This was probably due to the fact that imatinib primarily inhibits cell growth at low concentrations and with short treatment which diminishes the killing effect of triptolide. We then treated KBM5 cells with triptolide for 24 hours and then added 1 μM imatinib for additional 24 hours. This combination treatment was more effective in inducing cell death in KBM5 cells (Figure 3, IC_{50}=15.4 ± 0.6 nM, compared with IC_{50}=24.3 ± 2.8 nM for triptolide alone). Triptolide did not sensitize KBM5STI571 cells to imatinib (results not shown).

Triptolide induces death of quiescent $CD34^+$ primitive CML cells

To test the effect of triptolide on the viability of quiescent $CD34^+$ CML cells, which are known to be resistant to TKIs,(21;22) we first cultured CFSE-labeled cells from five CML patients (Table 2) with medium supplemented with a cocktail of growth factors (patients 1 and 2) or with MS-5 stromal cells (patients 3-5) for 4-7 days. Cell proliferation was tracked by flow cytometric analysis of CFSE fluorescence intensity and shown on the left panel of Fig 4 (gated on live cells by scattering of bulk population for patient 1 who had <2% $CD34^+$ cells and of $CD34^+$ cells for other patients). As illustrated in Fig 4, proliferating cells which represent the majority of blasts from all five patients were sensitive to triptolide, consistent with the results shown in Fig 1C. Quiescent $CD34^+$ cells were in general as sensitive as proliferating $CD34^+$ cells to triptolide: in four of the five patient samples (patients 1, 3-5), proliferating and quiescent cells responded similarly, undergoing the same degree of apoptosis. Only in cells from patient 2, quiescent $CD34^+$ cells were resistant, while proliferating $CD34^+$ cells were sensitive to triptolide. The sensitivity of $CD34^+$ cells to triptolide was not affected by pre-culturing these cells with growth factors or with MS-5 cells (Fig 4). Note as shown in Table 2, 4 out of 5 patients had mutation in BCR-ABL gene and patient 4 had T315I mutation.

Discussion

Our study demonstrated that triptolide potently induces apoptosis of BC CML cells from both cell lines and primary patient samples, independent of the BCR-ABL mutation status and response to imatinib and other TKIs, at least in part, by decreasing XIAP, Mcl-1, and Bcr-Abl proteins. The triptolide-induced decrease in these protein levels is likely part of the mechanism by which triptolide induces death of Bcr-Abl-expressing cells and overcomes TKI resistance. More importantly, triptolide promotes the death not only of proliferating but also of quiescent $CD34^+$ primitive CML cells.

Generation of the Bcr-Abl fusion gene is the causative genetic defect in early stage CML. With disease progression, additional chromosomal and molecular changes occur and blasts from patients with BC CML show enhanced proliferation and survival. Hence, it is not surprising that imatinib and other TKIs are not very effective in patients with advanced CML. Triptolide decreases not only Bcr-Abl protein level, but also the levels of XIAP and Mcl-1, two potent antiapoptotic proteins. These attributes of triptolide give it a distinct advantage over imatinib and other TKIs which affect only proliferating cells; in particular in decreasing Bcr-Abl protein levels, and by lowering the apoptotic threshold and activating the apoptotic cascade. This ability of triptolide to decrease Bcr-Abl, XIAP, and Mcl-1 levels also explains why triptolide-induced cell death is independent of cellular response to imatinib or other second-generation Bcr-Abl TKIs and occurs in quiescent cells. Mcl-1 expression was reported to be dependent on BCR-ABL in CML,(27;28) therefore the decrease in Bcr-Abl levels by triptolide may result in further decrease in Mcl-1 levels in triptolide-treated CML cells. In our experiments, Bcr-Abl protein decreased prior to Mcl-1 (Fig 2B) supporting the notion that Mcl-1 is a downstream target of Bcr-Abl.

Ba/F3vec cells, the vector control without Bcr-Abl, were also killed by triptolide. These cells were cultured in a medium supplemented with IL-3 which activates various survival pathways of the cells. Since triptolide also induces cell death by decreasing survival proteins XIAP and Mcl-1 independent of Bcr-Abl, it is not surprising that they are sensitive to triptolide.

We have previously reported that although triptolide inhibits colony formation of normal BM cells, normal $CD34^+$ cells are less sensitive than AML blasts to triptolide.(27) This is probably due to the fact that apoptotic pathways are often deregulated in leukemia cells and these cells are more than normal cells dependent on XIAP, Mcl-1, Bcr-Abl in the case of CML, and other antiapoptotic proteins for survival, as supported by the oncogene addiction theory.(38) Given the resistance of quiescent CML stem cells to most anticancer agents, the ability of triptolide to promote the death of quiescent $CD34^+$ CML cells warrants the clinical development of this agent. It was reported that PG490-88, a water-soluble derivative of triptolide, at 0.25 mg/kg markedly decreased tumor growth and at 0.5 and 0.75 mg/kg doses caused profound tumor regression without apparent toxicities in nude mouse human tumor xenograft models.(39) A decrease in white blood cells was reported in clinical trials with triptolide or extracts of Tripterygium wilfordii.(40;41) A phase I clinical trial with a water-soluble derivative of triptolide in solid tumors is presently ongoing and importantly, a clinical Phase I trial in France has determined the MTD for triptolide and reported 3 CRs out of 26 patients with AML.(42) The importance of the hematopoietic microenvironment in the maintenance and differentiation of hematopoietic progenitor cells has been recognized only in the past few years. Both *in vivo* and *in vitro* the growth, survival, and differentiation of hematopoietic cells have been found to require direct contact with MSCs, which produce various cytokines and chemokines. (43-47) MSC interacts similarly with leukemic cells *in vivo* and provide a protective microenvironment that enables leukemic cells to proliferate and survive. For these reasons, a cocktail of growth factors is commonly used for the maintenance of $CD34^+$ CML progenitor cells *in vitro*, as was done in the current study. We found that the sensitivity of $CD34^+$ cells to

triptolide is not affected by pre-culturing these cells with either the cocktail (Fig 4, patients 1 and 2) or with MS-5 cells (Fig 4, patients 3-5).

Taken together, results show that triptolide has the potential of complementing the activity of imatinib and other TKIs by effective killing bulk CML cells in blast crisis and depleting quiescent as well as proliferating $CD34^+$ primitive CML progenitor cells, independent of their sensitivity to TKIs. Thus, triptolide could be a novel agent in patients with CML in accelerated and blast crisis, in patients not responsive to TKIs including those with the T315I mutation, and in eradicating quiescent $CD34^+$ CML progenitor cells.

Our findings of effects of triptolide on CML are in agreement with Shi et al's results(29) that triptolide decreases Bcr-Abl, XIAP, and Mcl-1 protein levels in CML cell lines and our studies extend these results to primary CML samples. Importantly, we here demonstrate that triptolide sensitizes to imatinib-induced cell death and has major activity in quiescent primitive $CD34^+$ CML progenitor cells.

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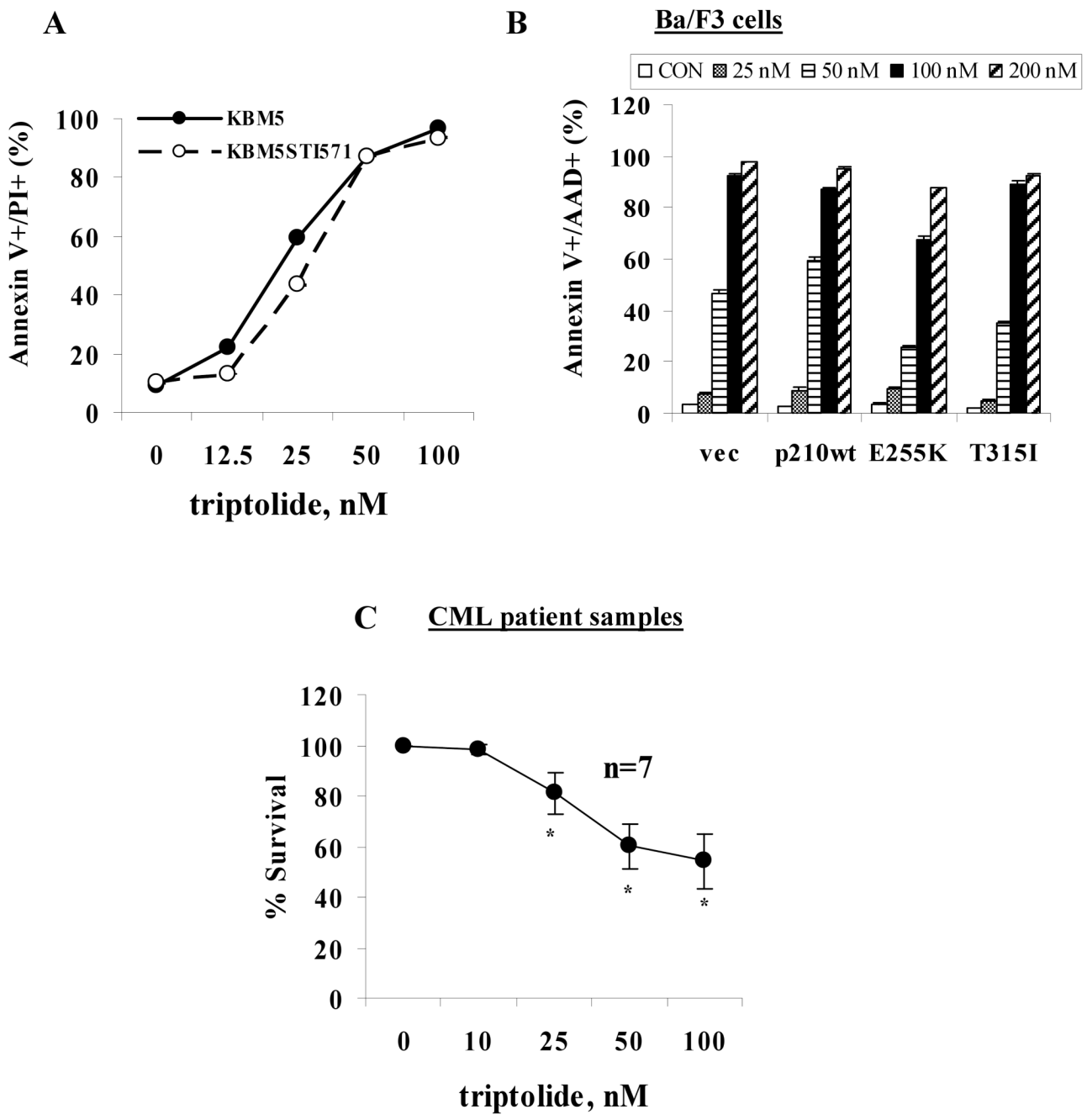

Figure 1.

Triptolide potently induces cell death in BC CML cells independent of cellular responses to imatinib. (A) KBM5 and KBM5STI571 cells and (B) Ba/F3vec, Ba/F3Bcr-Abl^{p210wt}, Ba/F3Bcr-Abl^{E255K}, and Ba/F3Bcr-Abl^{T315I} cells were treated with various concentrations of triptolide for 48 hours. (C) Blasts from patients with CML were treated for 24 hours. Cell death was determined by annexin V staining.

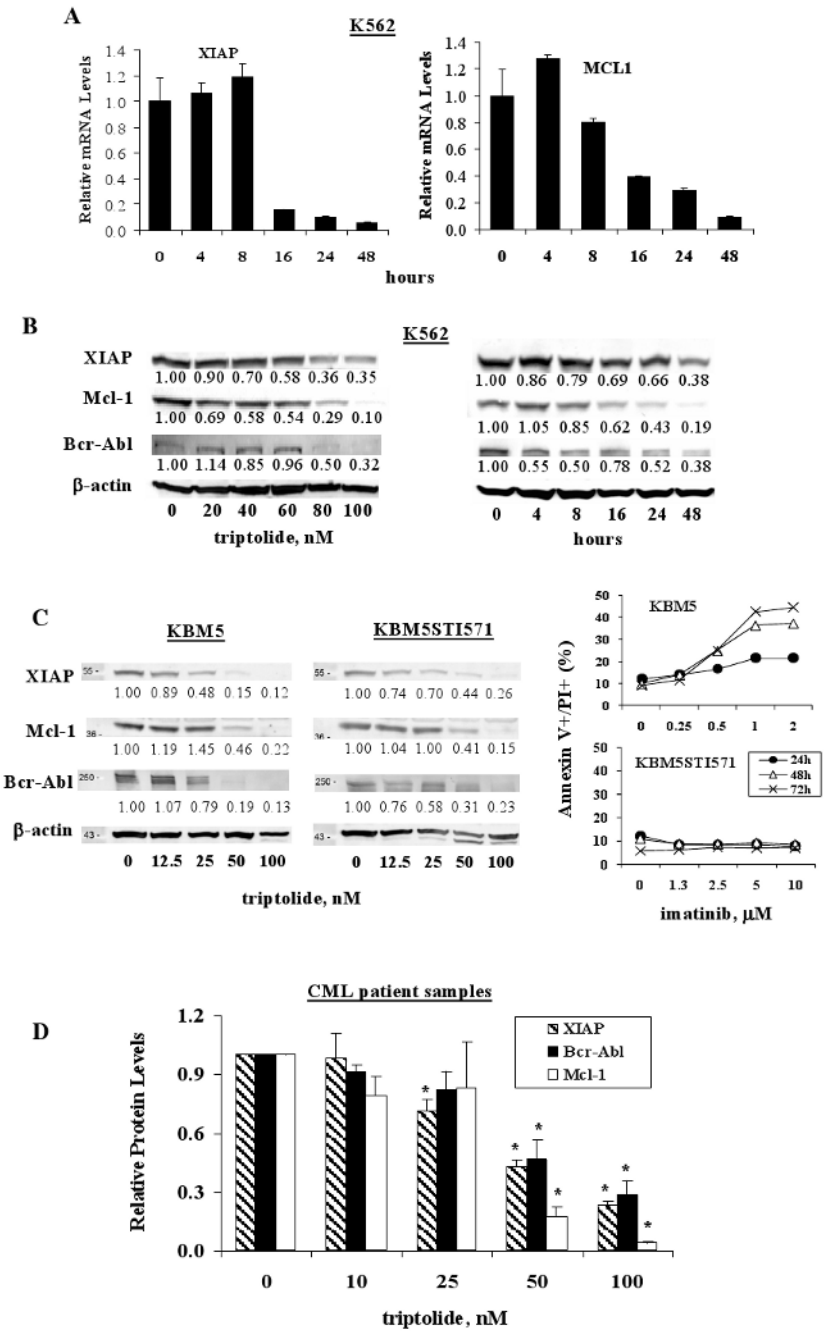

Figure 2.

Triptolide decreases XIAP, Mcl-1, and Bcr-Abl levels in CML cells. (A) K562 cells were treated with 100 nM of triptolide. XIAP and Mcl-1 mRNA levels were determined at various time points by Real-time RT-PCR. (B) K562 cells were treated with various concentrations of triptolide for 48 hours or with 100 nM of triptolide for various times. (C) KBM5 and KBM5STI571 cells were treated with triptolide for 24 hours and with imatinib for 24 to 72 hours and (D) cells from patients with CML were treated with triptolide for 24 hours. XIAP, Mcl-1, and Bcr-Abl protein levels were determined by western blot analysis and cell death was determined by annexin V staining.

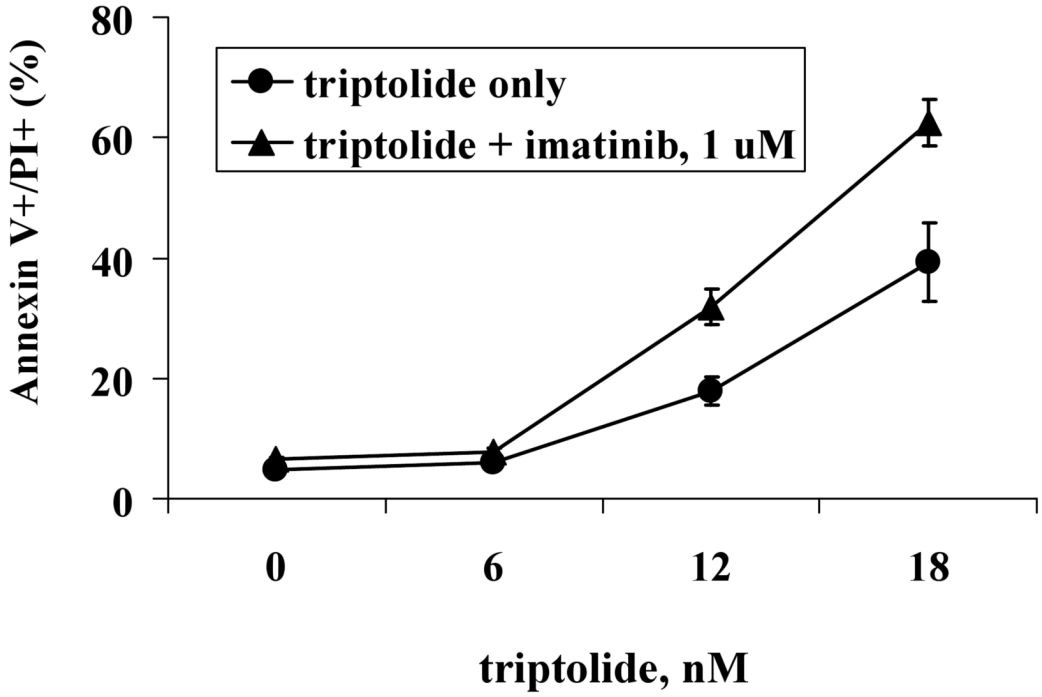

Figure 3.
Combination of triptolide and imatinib enhances death in KBM5 cells. KBM5 cells were treated with triptolide for 24 hours and then plus 1 μM imatinib for an additional 24 hours. Cell death was determined by annexin V staining.

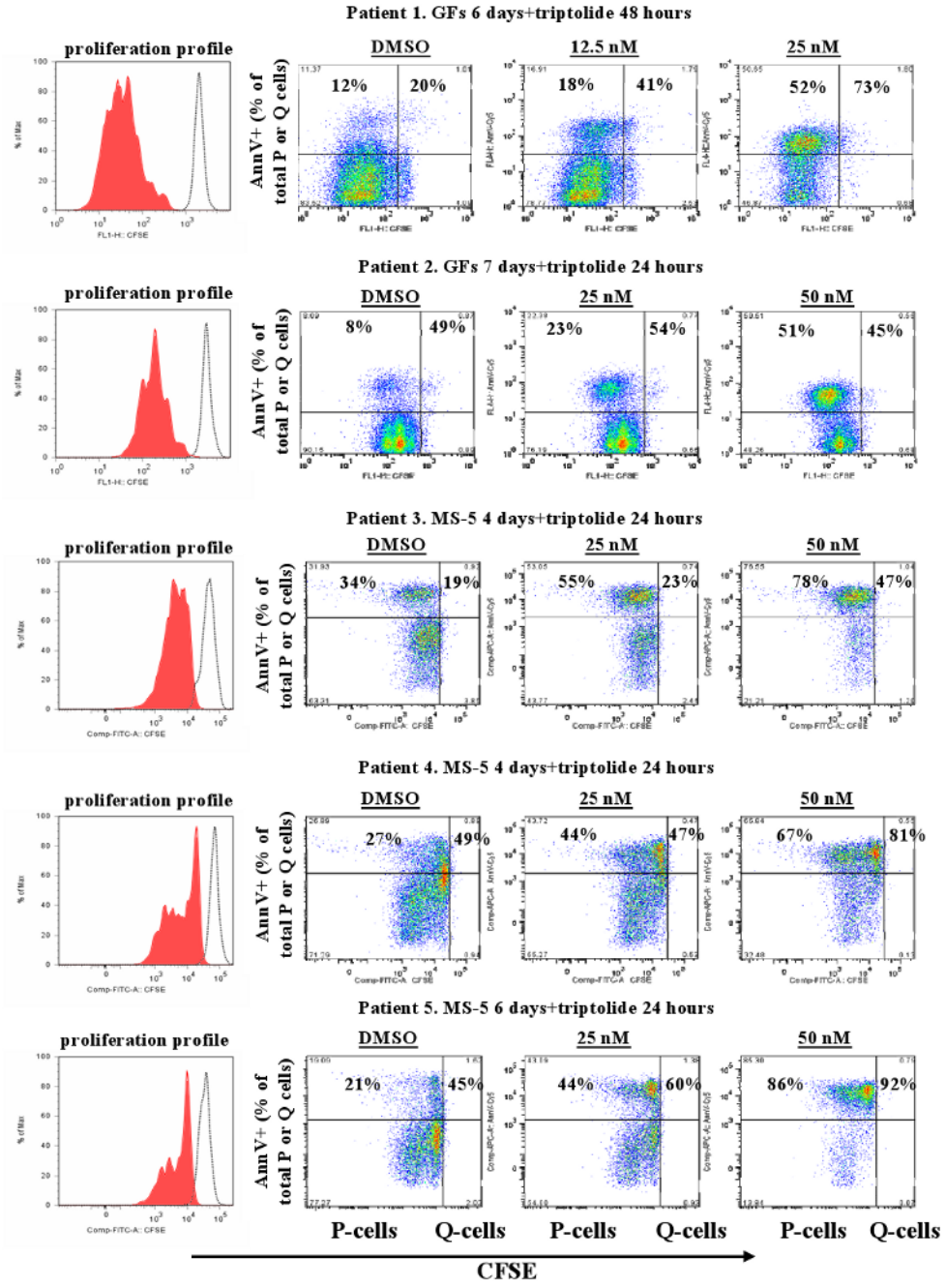

Figure 4.

Triptolide induces the death of both quiescent and proliferating $CD34^+$ cells from patients with CML. Profiles of CFSE-labeled cells from patients with CML were shown on the left panel. Shaded area represents proliferation profile at the time of annexin V assay while open area shows the fluorescence profiles of cells before starting the culture. Cells were cultured with growth factors or co-cultured with MS-5 cells for 4-7 days and then treated with triptolide for 24 or 48 hours. Apoptosis in quiescent and proliferating $CD34^+$ cells was determined using annexin V staining and shown as % positivity of the respective populations. GF, growth factor; P, proliferating; Q, quiescent.

Table 1

Characteristics of CML Patients Treated with Triptolide *in vitro*

patient	blast %	sample source	*in vivo* treatments and responses at the time of sampling	Bcr-Abl mutation status	BC lineage	*in vitro* triptolide treatment	
						IC50 (nM)	western blot
1	49	PB	Resistant to imatinib and nilotinib	M351T	myeloid	32.5	XIAP
2	62	PB	Resistant to imatinib	wt	myeloid	178.9	XIAP, Mcl-1, Bcr-Abl
3	80	PB	Resistant to imatinib, nilotinib and other therapies	wt	myeloid	84.1	-
3a	83	BM		wt		87.9	-
4	91	PB	Hydrea, later died	wt	myeloid	43.0	-
5	87	PB	no cytogenetic remission with imatinib, dasatinib, or nilotinib	wt	myeloid	111.7	XIAP, Mcl-1, Bcr-Abl
6	64	PB	failed imatinib and other chemotherapies	wt	bilineage	206.2	XIAP, Mcl-1, Bcr-Abl

Table 2

Characteristics of Patients with CML whose CD34+ Cells were Used for Testing Triptolide Sensitivity

patient	source	blast %	treatment and responses at the time of sampling	Bcr-Abl mutation status	BC lineage
1	PB	59	resistant to or relapsed from various treatment including Interferon, imatinib, dasatinib, fludarabine+Ara-C bid, and intravenous homoharringtonine + imatinib	F317L	myeloid
2	PB	17	resistant to various therapies including Interferon + low dose Ara-C, imatinib, nilotinib, and dasatinib	wt	myeloid
3	PB	58	resistant to imatinib and nilotinib	F317L	lymphoid
4	PB	58	achieved short hematological remission with Idarubicin+Ara-C / imatinib but soon after had evidence of Ph+ chromosome, resistant to dasatinib and MK-0457	T315I	myeloid
5	BM	60	achieved hematological response with imatinib and dasatinib, achieved and then lost cytogenetic response to dasatinib, was on bosutinib	V299L	myeloid