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R-(–)-gossypol (AT-101) activates programmed cell death in multiple myeloma cells

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Abstract

Objective—Bcl-2 family proteins play a critical role in malignancies by regulating the balance between cell survival and apoptosis. R-(–)-gossypol (AT-101) is a small molecule that mimics the BH3 domain of cellular Bcl-2 inhibitors and interferes with the function of prosurvival Bcl-2 proteins. We examined the cytotoxicity of AT-101 in the context of multiple myeloma, a fatal hematological malignancy.

Materials and Methods—Multiple myeloma cell lines and primary cells obtained from multiple myeloma patients were used to investigate the effects of AT-101. Cell viability, apoptosis, and apoptosis pathways were examined using conventional viability assays, flow cytometry, and immunoblots.

Results—AT-101 was not only cytotoxic to conventional multiple myeloma cell lines, but was also effective against drug-resistant cell lines and primary multiple myeloma patient cells. Furthermore, AT-101 decreased proliferation of multiple myeloma cell lines in the presence of marrow stromal cells, indicating that this drug may overcome the protective effect of the microenvironment that is important for multiple myeloma cell proliferation and survival. Apoptosis was activated via the mitochondrial pathway in multiple myeloma cell lines treated with AT-101 as demonstrated by an increased Bax to Bcl-2 ratio, mitochondrial membrane depolarization, and caspase activation. Finally, our studies demonstrated that AT-101 exhibits potent synergy with dexamethasone, a valuable therapeutic for multiple myeloma.

Conclusion—These data suggest that the activity of AT-101 may be highly relevant to multiple myeloma disease biology and may represent an option for treatment of patients with this disease.

Gossypol is a natural chemical found in cotton seed oil that has been examined as an anticancer agent [1–8]. The R-(–) enantiomer of gossypol (AT-101) is a small molecule with binding properties similar to the inhibitory BH3 domain found in cellular proteins that bind to and inhibit members of the Bcl-2 family. AT-101 interaction with the BH3 binding domain of prosurvival Bcl-2 family proteins inhibits their interaction with proapoptotic proteins and therefore promotes initiation of apoptosis, making this chemical an attractive therapeutic candidate.

Multiple myeloma is a fatal B-cell malignancy characterized by accumulation and proliferation of clonal plasma cells in the bone marrow [9,10]. New therapeutics and treatment regimens are being developed continuously, however, this disease remains incurable. As with most malignancies, the roles of cell-death and cell-survival proteins have been questioned and examined in multiple myeloma. Elevated expression of prosurvival

Bcl-2 family proteins including Bcl-2, Bcl-xL, and Mcl-1 has been found in multiple myeloma and implicated in cell survival, disease stage, and chemoresistance [11–21]. In vivo studies using transgenic mice have also revealed potential consequences of Bcl-2 family member overexpression as increased expression of Bcl-2 using the immunoglobulin heavy-chain enhancer led to increased accumulation and survival of cells in the B-cell lineage [22]. Furthermore, transgenic mice overexpressing Bcl-xL in mature B cells and plasma cells develop nonmalignant accumulation of plasma cells, whereas coexpression of Bcl-xL and c-Myc leads to development of malignant plasma cell lesions [23]. These studies suggest that interference with the function of prosurvival Bcl-2 family members may be an effective strategy for killing multiple myeloma cells.

Multiple studies have demonstrated that inhibitors of Bcl-2 promote apoptosis in multiple myeloma cells. Use of the small molecule Bcl-2 inhibitor HA14-1 in combination with proteasome inhibitors or flavopiridol promoted apoptosis in multiple myeloma cells [24,25]. Additionally, we have recently demonstrated efficacy of other small molecule Bcl-2 inhibitors to stimulate apoptosis in multiple myeloma cells (unpublished data) [31]. Finally, efficacy of Bcl-2 antisense oligonucleotides has been evaluated in vitro and in vivo (G3139) in clinical trials with promising initial results [26–30]. Based upon these findings, we evaluated AT-101 in the context of multiple myeloma to determine its utility as a potential therapeutic agent. This study demonstrates that AT-101 is cytotoxic in multiple myeloma cell lines and patient plasma cells. AT-101 stimulates programmed cell death in multiple myeloma cells via the mitochondrial pathway characterized by alterations in the Bax/Bcl-2 ratio, mitochondrial membrane depolarization, and cleavage of effector caspases. Our findings demonstrate effectiveness of AT-101 in vitro and present AT-101 as a prospective tool for multiple myeloma research and therapy. This orally active agent is already in early clinical trials and the data presented here will form the framework for rapid translation to the clinic.

Materials and methods

Cell cultures

Multiple myeloma cell lines RPMI 8226, U266, OPM-1, OPM-2, MM1.S, KAS-6/1, Dox 40, LR5, and MM1.R were used for the AT-101 studies. Multiple myeloma cell lines were cultured as described previously [31]. Bone marrow aspirates from patients with multiple myeloma were obtained after informed consent under a protocol reviewed and approved by the Mayo Foundation Institutional Review Board in accordance with the Declaration of Helsinki and the applicable federal regulations. The samples were processed and plasma cells and bone marrow stromal cells isolated as described [31].

AT-101

AT-101 [R-(–)-2,2'-bis(1,6,7-trihydroxy-3-methyl-5-isopropyl-8-aldehydonaphthalene) acetic acid] was obtained from Ascenta Therapeutics, Inc. (San Diego, CA, USA). AT-101 was dissolved in dimethyl sulfoxide at a concentration of 200 mM and stored in frozen aliquots until use. AT-101 was diluted in culture medium immediately before use with a final dimethyl sulfoxide concentration of <0.1%.

Cell viability and proliferation

Viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrasodium bromide (MTT) assay [31]. Cells were incubated with the indicated concentrations of AT-101 for 48 hours to measure dose-dependent effects upon viability. All experiments were performed in triplicate. Examination of the AT-101 (1.0–7.0 μ M)/dexamethasone (0.01–0.07 μ M) combination was performed over a period of 24 hours, and cytotoxicity was

determined using the MTT assay. DNA synthesis in coculture experiments using multiple myeloma cells and bone marrow stromal cells was measured by tritiated thymidine uptake as described [31]. Stromal cells were plated onto flat-bottomed 96-well plates at a concentration of 10,000 cells per well and allowed to adhere overnight. Multiple myeloma cells (3×10^4 cells/well) were added to the wells in the presence of medium alone or AT-101 and incubated for 48 hours at 37°C. Cells were pulsed with ^3H -TdR (0.5 μCi /well; 0.0185 MBq; Perkin-Elmer, Boston, MA, USA) during the last 8 hours of 48-hour cultures. All experiments were performed in triplicate.

Multiple myeloma cell-line apoptosis

Multiple myeloma cells were washed twice with Annexin binding buffer (10 mM HEPES pH 7.4, 140 mM NaCl, 2.5 mM CaCl_2) and resuspended at a concentration of 1×10^7 cells per mL. Annexin-V–fluorescein isothiocyanate (3 μL ; Caltag, Burlingame, CA, USA) was added to 100 μL cell suspension and cells were incubated for 15 minutes at room temperature. After a subsequent wash with Annexin binding buffer, cells were resuspended in 500 μL Annexin binding buffer containing 5 μL propidium iodide (1 mg/mL; Sigma Chemical, St Louis, MO, USA). Flow cytometry was performed using a FACSCanto (BD Biosciences, San Jose, CA, USA)

Primary patient cell apoptosis

Multiple myeloma patient bone marrow cells were washed with phosphate-buffered saline and 1 million cells were used per tube for flow cytometry. CD38–allophycocyanin (BD Biosciences), CD45–peridinin chlorophyll protein (BD Biosciences) and either IgG1–phycoerythrin (BD Biosciences), or Apo 2.7–phycoerythrin (Immunotech, Fullerton, CA, USA) antibodies were added to each tube. After 15-minute incubation at room temperature, cells were washed with phosphate-buffered saline, suspended in 1% paraformaldehyde, and stored at 4°C until analyzed using a FACSCanto. Plasma cells were identified by the characteristic CD38/CD45 staining pattern and the Apo 2.7 levels were analyzed for the entire plasma cell population.

Bax/Bcl-2 ratio determination

Cell lines were cultured as described with or without AT-101 (10 μM). Bax and Bcl-2 levels were measured and the ratio calculated using flow cytometry as described previously [31].

Mitochondrial membrane potential

The MitoCapture Apoptosis Detection Kit (Calbiochem) was used to measure disruption of mitochondrial transmembrane potential. In healthy cells, MitoCapture reagent accumulates and aggregates in the mitochondria, emitting red fluorescence. In apoptotic cells, MitoCapture reagent cannot aggregate in the mitochondria due to the altered mitochondrial membrane potential, and thus remains in the cytoplasm in its monomer form, emitting green fluorescence. Cell lines were incubated for varying durations with or without AT-101 (10 μM). Cells were prepared following manufacturer's instructions and analyzed using a FACSCanto.

Immunoblotting

Multiple myeloma cell lines ($5\text{--}10 \times 10^6$ cells per well of a six-well plate) were cultured as described and immunoblots prepared as described previously [31].

Isobologram analysis

The interaction between AT-101 and dexamethasone was analyzed using the CalcuSyn software program (Biosoft, Ferguson, MO, USA) to determine whether the drug

combination was additive or synergistic. This program is based upon the Chou-Talalay method, which calculates a combination index (CI), and analysis is performed based on the following equation: $CI = (D)_1 / (D_x)_1 + (D)_2 / (D_x)_2 + (D)_1(D)_2 / (D_x)_1(D_x)_2$, where (D)₁ and (D)₂ are the doses of drug 1 and drug 2 that have “x” effect when used in combination, and (D_x)₁ and (D_x)₂ are the doses of drug 1 and drug 2 that have the same “x” effect when used alone [32]. Data from the cell viability assay (MTT) was expressed as the fraction of cells killed by the individual drug or the combination in drug-treated cells compared with untreated cells. A CI value of 1.0 indicates an additive effect, whereas a CI value <1.0 indicates synergy.

Results

AT-101 exhibits cytotoxicity against multiple myeloma cell lines

We cultured nine multiple myeloma cell lines with various AT-101 concentrations to examine the effects upon multiple myeloma cell viability. A marked decrease in cell viability after incubation with AT-101 was observed with all multiple myeloma cell lines examined, including RPMI 8226, U266, OPM-1, OPM-2, MM1.S, and the interleukin (IL)-6-dependent cell line KAS-6/1 (Fig. 1A). The IC₅₀ values for AT-101 were very comparable for the different cell lines and ranged between 2.5 and 7.5 μM. We also examined the cytotoxicity of AT-101 in multiple myeloma cell lines that are resistant to drugs used for multiple myeloma therapy. The viability of Dox40 (doxorubicin-resistant), LR5 (melphalan-resistant), and MM1.R (dexamethasone-resistant) cell lines was decreased dramatically by AT-101, and IC₅₀ values of approximately 2.5 to 5 μM were obtained in these experiments (Fig. 1B). These studies indicate that both conventional and drug-resistant multiple myeloma cell lines are sensitive to AT-101 at similar concentrations as those found to be effective in other tumor systems [3–5,8].

AT-101 inhibits multiple myeloma cell proliferation stimulated by bone marrow stromal cells

Stromal cells residing in the bone marrow microenvironment stimulate the growth of multiple myeloma cells by producing growth factors, such as IL-6. We examined the effects of AT-101 at various concentrations upon the growth of multiple myeloma cells cultured in the presence of stromal cells to determine if AT-101 was able to overcome the stimulatory effect of bone marrow stromal cells on multiple myeloma cell line proliferation. MM1.S multiple myeloma cells were cultured in the presence or absence of bone marrow stromal cells with varying concentrations of AT-101 and ³H-thymidine incorporation was measured. Incubation of multiple myeloma cells with stromal cells increased the proliferation of multiple myeloma cells by 1.8-fold, and this increase in proliferation was inhibited in a dose-dependent manner by AT-101 (Fig. 2). These results indicate that AT-101 is able to inhibit the growth of multiple myeloma cells despite the stimulatory growth effects provided by stromal cells in the bone marrow milieu.

AT-101 triggers apoptosis in multiple myeloma cells

We examined the induction of apoptosis in multiple myeloma cells treated with AT-101 to determine if drug toxicity was associated with activation of the programmed cell death pathways. The MM1.S multiple myeloma cell line was cultured in media containing AT-101 (10 μM) for the indicated time periods, and the percentage of apoptotic cells was determined using Annexin-V/propidium iodide staining and flow cytometry measurement. A progressive decrease in the viable cell population and an increase in Annexin V-positive cell population were observed as time progressed (Fig. 3A). We also examined the activation of caspase cleavage cascades to further understand the apoptosis pathways stimulated by AT-101. Immunoblots of extracts from cells treated with AT-101 for the indicated time

periods show that caspase 9, caspase 3, and PARP are each cleaved, and hence activated (Fig. 3B). These data indicate that AT-101 induces apoptosis in multiple myeloma cells via the activation of caspases.

AT-101 alters the myeloma cell Bax/Bcl-2 ratio and disrupts mitochondrial membrane potential

The relative level of pro and anti-apoptotic proteins, specifically the Bax/Bcl-2 ratio, is an indicator of the propensity of cells to undergo apoptosis. To investigate the impact of AT-101 upon this critical early parameter of apoptosis, MM1.S multiple myeloma cells were treated with AT-101 (10 μ M) for 8 hours and the levels of Bax (proapoptotic) and Bcl-2 (antiapoptotic) proteins were measured using flow cytometry at regular intervals to determine the Bax/Bcl-2 protein ratio. A time-dependent increase in the Bax/Bcl-2 ratio was observed upon treatment of multiple myeloma cells with AT-101 as seen in Figure 4A. Figure 4B shows that the Bax/Bcl-2 ratio also increases in a concentration-dependent manner, promoting multiple myeloma cell apoptosis. Depolarization of the mitochondrial transmembrane potential is another early cellular event that occurs during apoptosis, and this was examined using MitoCapture dye measurement by flow cytometry after treatment of cells with AT-101. MitoCapture dye normally aggregates in the mitochondria and emits bright red fluorescence, whereas loss of mitochondrial membrane potential during apoptosis eliminates aggregation in the mitochondria and the dye no longer emits red fluorescence but emits green fluorescence instead. Figure 4C shows a time-dependent increase in green fluorescence following treatment of MM1.S cells with AT-101 (10 μ M), indicating increasing loss of mitochondrial membrane potential and loss of dye aggregation in the mitochondria. Therefore, our data indicate that AT-101 promotes apoptosis in multiple myeloma cells by disrupting the Bax/Bcl-2 ratio and the mitochondrial membrane potential.

Multiple myeloma patient cells undergo apoptosis in response to AT-101

Bone marrow cells were isolated from multiple myeloma patients and cultured with AT-101 to examine the drug toxicity in primary cells. Bone marrow cells were left untreated or were treated with AT-101 at concentrations of 10 μ M or 20 μ M for a period of 48 hours, after which plasma cell apoptosis was measured by examining the Apo 2.7 staining of the CD38⁺/CD45⁻ cells. Tumor cell apoptosis was stimulated by AT-101 in all of the primary multiple myeloma samples to varied degrees, ranging from no induction of apoptosis to 56% induction with 10 μ M AT-101, and 22% to 77% induction with 20 μ M AT-101 (Fig. 5). This clearly demonstrates that AT-101 is capable of inducing programmed cell death in freshly isolated patient plasma cells.

Combination of AT-101 and dexamethasone exhibits synergistic toxicity in multiple myeloma cells

We examined the effectiveness of AT-101 when used in combination with various multiple myeloma therapeutics. As part of this examination, we evaluated the toxicities of AT-101 and the commonly used corticosteroid dexamethasone individually and in combination. As seen in Figure 6A, these compounds exhibited synergy of their toxicity to multiple myeloma cells. The Chou-Talalay CI method was used to validate that synergy was obtained (CI <1) with the use of AT-101 and dexamethasone, as shown in Figure 6B.

Discussion

Dysregulation of the programmed cell death system is an important feature of many types of malignancies and appears to provide resistance to therapeutic intervention. Expression of antiapoptotic members of the Bcl-2 family is increased in multiple myeloma. These “survival proteins” include Bcl-2, Bcl-xL, and Mcl-1, and their elevated expression has been

associated with prolonged cell survival, clinical disease stage, and resistance to chemotherapy. The mechanism by which these proteins are expressed at higher levels is not currently understood, however, the bone marrow microenvironment is known to be critical for myeloma cell survival and cells residing in the marrow produce a multitude of factors that regulate the production of Bcl-2 family proteins in myeloma tumor cells [33–35]. Blocking the function of antiapoptotic proteins may promote cell death in multiple myeloma cells and other tumor types as well. We are in dire need of effective therapeutic strategies for multiple myeloma, and we believe that disruption of the programmed cell death mechanism represents a plausible target for chemotherapy.

Inhibition of Bcl-2 function in multiple myeloma has been evaluated in multiple investigations. The effectiveness of antisense oligonucleotides corresponding to Bcl-2 has been studied in vitro and in vivo. G3139 (Genasense), an 18-base oligonucleotide complementary to the first six codons of the Bcl-2 mRNA, decreases Bcl-2 levels in multiple myeloma cells and decreases their resistance to dexamethasone, doxorubicin, and paclitaxel [26,27]. Encouraging clinical trials using G3139 in combination with other chemotherapeutic agents have been completed and a number of trials are ongoing [30]. Small molecule inhibitors are also attractive candidates for therapy because of the specificity that they may exhibit and their potential ease of delivery. An example is HA14-1, a small molecule organic ligand of Bcl-2 that was discovered using structure-based methods [36]. Studies evaluating the efficacy of HA14-1 in multiple myeloma cells indicate that this inhibitor promotes apoptosis in combination with flavopiridol or proteasome inhibitors [24,25]. Another small molecule inhibitor of Bcl-2 under study is ABT-737, which has high affinity and specificity for the BH3-binding groove on Bcl-2 [37]. We have demonstrated that ABT-737 effectively induces apoptosis in multiple myeloma cell lines and primary patient cells and interferes with signaling pathways critical for myeloma cell survival [31]. These observations with Bcl-2 inhibitors indicate that this therapeutic strategy is feasible, and therefore we have evaluated AT-101, another inhibitor of Bcl-2 function.

Gossypol is present in cotton seed oil and several studies have confirmed its effectiveness as an anti-cancer agent. AT-101 is the R-(–) enantiomer of gossypol and binds and inhibits prosurvival members of the Bcl-2 family. We have found that AT-101 is cytotoxic to multiple myeloma cell lines and also primary multiple myeloma cells isolated from patients. Multiple cell lines were examined, including multiple lines that are resistant to particular chemotherapeutic agents, and we found that 48-hour incubation of these lines with AT-101 led to substantial toxicity in all cell lines with IC₅₀ values between 2.5 and 7.5 μ M. Clearly, AT-101 circumvents the mechanisms of drug resistance present in the cell lines tested.

The bone marrow microenvironment is critical for the growth and survival of tumor plasma cells in multiple myeloma. Stromal cells present in the marrow respond to signals from plasma cells and other cell types by producing factors, including IL-6 and vascular endothelial growth factor, which appear to impact a multitude of growth and survival signaling pathways in multiple myeloma cells. We cultured multiple myeloma cells together with stromal cells to verify that AT-101 is effective under these conditions. AT-101 exhibited potent toxicity in multiple myeloma cells, despite the presence of stromal cells and inhibited the boost in myeloma cell proliferation provided by the stromal cells in a dose-dependent manner.

We examined the effect of AT-101 upon stimulation of apoptosis in multiple myeloma cells as this was the expected mechanism of action. We observed both a time- and concentration-dependent induction of apoptosis in multiple myeloma cells as measured using conventional Annexin-V/propidium iodide staining. Closer examination of apoptosis pathways revealed that caspase cleavage and hence activation occurred upon exposure of cells to AT-101.

Looking at events farther upstream in the apoptosis cascade, evaluation of cells after AT-101 treatment revealed both time- and dose-dependent increases in the Bax/Bcl-2 ratio in multiple myeloma cells. This ratio is an indicator that the death/survival balance within the cell has tilted in favor of programmed cell death, which is consistent with other studies of apoptosis and the effects of AT-101 that we describe here [38,39]. We also investigated effects of AT-101 upon the integrity of the mitochondrial membrane potential, which is characteristically disrupted during apoptosis and represents an early event in programmed cell death. Using the fluorescent dye MitoCapture we demonstrated that the mitochondrial membrane is depolarized in cells treated with AT-101, as the accumulation and aggregation of the reagent in the mitochondria lessens from that in untreated cells.

Primary patient multiple myeloma cells were isolated and examined for their susceptibility to treatment with AT-101. We measured the percentage of apoptotic cells using Apo2.7 staining and flow cytometry before and after treatment and found that potent cytotoxic effects, including a rate of cellular apoptosis >50% were observed in the majority of patient samples. The response to AT-101 was variable among patient samples, however, these data indicate that AT-101 is effective in inducing apoptosis in primary cells and targeting the majority of multiple patient cells at the concentrations evaluated in this preliminary study. Drug combinations are commonly used for treatment of multiple myeloma and other malignancies. This approach allows the attack of multiple cellular targets and often affords the use of lower drug concentrations to achieve synergy of drug action in killing tumor cells. We examined the combination of dexamethasone, a commonly prescribed drug for treatment of multiple myeloma, and AT-101 at range of concentrations to evaluate whether this drug tandem would demonstrate increased potency against multiple myeloma cells. Synergy was clearly obtained when this combination of agents was used to kill multiple myeloma cells, suggesting that AT-101 toxicity may be enhanced significantly when used with other agents.

In summary, this study demonstrates that AT-101, an inhibitor of prosurvival Bcl-2 family members, is toxic to multiple myeloma cell lines and patient cells. As expected, the mechanism of action appears to be induction of apoptosis in the multiple myeloma cells as demonstrated using conventional means of evaluating the execution of programmed cell death. These data suggest that the small molecule Bcl-2 inhibitor AT-101 is a potential therapeutic agent for use against multiple myeloma and further in vitro and in vivo investigation into the use of this molecule by itself and in tandem with other agents is warranted.

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Figure 1.

AT-101 kills multiple myeloma cell lines. AT-101 is cytotoxic in a dose-dependent manner in multiple myeloma cell lines. The MTT assay was used to determine cell viability. **(A)** Viability measurement of multiple myeloma cells incubated with AT-101 for a period of 48 hours. IC₅₀ values of 2.5 to 7.5 μM were obtained. **(B)** Examination of AT-101 toxicity in drug-resistant multiple myeloma cell lines including Dox40 (resistant to doxorubicin), LR5 (resistant to melphalan), and MM1.R (resistant to dexamethasone). IC₅₀ values of 2.5–5.0 μM were obtained with these drug resistant lines. AT-101 concentrations (μM) are indicated on the x-axis and viability (as a percentage of the untreated control) is indicated on the y-axis. Error bars represent one standard deviation.

Figure 2.

Multiple myeloma cell proliferation is blocked by AT-101, despite the presence of bone marrow stromal cells. MM1.S multiple myeloma cells were cultured with normal bone marrow stromal cells to simulate the bone marrow environment. As expected, stromal cells stimulated the proliferation of MM1.S cells, and this stimulation was inhibited by the addition of AT-101 at the indicated concentrations for a period of 48 hours. AT-101 concentrations (μM) are indicated on the x-axis and ^3H -thymidine incorporation (counts per minute [CPM]) is indicated on the y-axis. Error bars represent one standard deviation.

Figure 3.

AT-101 induces apoptosis in multiple myeloma cell lines. **(A)** MM1.S multiple myeloma cells were treated with AT-101 (10 μ M) for the indicated time periods, and a time-dependent increase in apoptosis was observed as cells transitioned to staining with Annexin-V. Viable cells were defined as those cells that did not stain for Annexin-V or propidium iodide as measured using flow cytometry. Levels of viable and Annexin-V staining cells are indicated. **(B)** Cleavage of caspase 9, caspase 3, and PARP demonstrated by immunoblotting after treatment of MM1.S multiple myeloma cells with AT-101 (10 μ M) for the indicated time periods.

Figure 4.

AT-101 disrupts the Bax/Bcl-2 ratio and mitochondrial membrane potential in multiple myeloma cells. **(A)** MM1.S cells were cultured with AT-101 (10 μ M) for the times indicated and the Bax/Bcl-2 ratio increased with the duration of treatment. Bax and Bcl-2 levels were measured using flow cytometry and the ratio was calculated from these values. **(B)** Dose-dependent increase in the Bax/Bcl-2 ratio upon treatment of MM1.S cells for 12 hours. **(C)** Loss of mitochondrial membrane potential in MM1.S cells treated with AT-101. MM1.S cells were cultured with AT-101 (10 μ M) for the indicated times. Loss of mitochondrial membrane potential was measured as increasing green fluorescence due to loss of MitoCapture reagent aggregation in the mitochondria.

Figure 5.

Primary multiple myeloma cells are susceptible to AT-101. Multiple myeloma patient cells were cultured with the indicated AT-101 concentrations for 48 hours and apoptosis was measured using Apo 2.7 staining and flow cytometry. The percentage of apoptotic cells (cells expressing Apo 2.7) is indicated on the y-axis.

Figure 6.

The combination of AT-101 and dexamethasone is a potent treatment for inducing apoptosis in multiple myeloma cells. (A) MM1.S multiple myeloma cells were incubated with the indicated concentrations of AT-101, or dexamethasone, or a combination of the drugs for a period of 24 hours, and viability was measured using the MTT assay. Synergy was clearly observed with the combination treatment of AT-101 and dexamethasone. Drug concentrations are indicated on the x-axis and viability is indicated on the y-axis. Error bars represent one standard deviation. (B) Chou-Talalay test results for the AT-101 and dexamethasone combination as calculated using CalcuSyn software. The combination indices (CI) and fraction affected (Fa) values are as shown in the table. CI values <1 indicate synergy.