ABT-737 Induces Apoptosis in Mantle Cell Lymphoma Cells with a Bcl-2^{high}/Mcl-1^{low} Profile and Synergizes with Other Antineoplastic Agents

Cyrille Touzeau¹,², Christelle Dousset¹,⁴, Linda Bodei¹,⁵, Patricia Gomez-Bougie¹,⁴,⁵, Stéphanie Bonnaud¹, Anne Moreau³, Philippe Moreau¹,², Catherine Pellat-Deceunynk¹, Martine Amiot¹, and Steven Le Gouill¹,²,⁴

Abstract

Purpose: Mantle cell lymphoma (MCL) is considered to be incurable. ABT-737 is a BH3 mimic that targets Bcl-2, which is overexpressed in MCL and implicated in drug resistance. The present work investigated the antitumor effect of ABT-737.

Experimental Design: Six MCL cell lines and primary MCL cells (n = 13) were used. Sensitivity to ABT-737 was assessed, and expression levels of Bcl-2 and Mcl-1 were analyzed. Finally, ABT-737 was combined with other cytotoxic agents to promote tailored therapy.

Results: MINO and GRANTA-519 cell lines were highly sensitive to ABT-737 [the median lethal dose (LD₅₀) = 20 and 80 nmol/L, respectively], whereas other cell lines were resistant. In primary MCL cells, 46% of patients’ samples were sensitive to ABT-737. The analysis of protein expression levels revealed that both sensitive cell lines and primary MCL cells could be characterized by a Bcl-2^{high}/Mcl-1^{low} profile, whereas resistant MCL cells contained high levels of Mcl-1. ABT-737 induced a rapid disruption of both Bcl-2/Bax and Bcl-2/Bik complexes. In addition, silencing of Mcl-1 by siRNA sensitized MCL cell lines to ABT-737. Similarly, flavopiridol, which induces Mcl-1 downregulation, in combination with ABT-737 led to a synergistic anti-MCL effect in ABT-737-resistant cell lines. This synergy was also observed when ABT-737 was combined with either bortezomib or cytarabine.

Conclusions: The present work shows that ABT-737 induces strong apoptosis in MCL cells expressing a Bcl-2^{high}/Mcl-1^{low} profile. In ABT-737-resistant MCL cells, downregulation of Mcl-1 overcomes Mcl-1-induced resistance and synergizes ABT-737 effects. Our results strongly support the use of ABT-737 according to the Bcl-2/Mcl-1 tumor cell profiles in the treatment of MCL.

Introduction

Mantle cell lymphoma (MCL) is an aggressive non-Hodgkin’s lymphoma (NHL) that accounts for approximately 5% of all NHLs (1). Despite recent improvements in therapy, most patients experience relapses and survive an average of approximately 5 years from the time of diagnosis (2). Intensive chemotherapy regimens combined with anti-CD20 antibodies with or without autologous stem cell transplantation are widely used but not well-tolerated in elderly or unfit patients. Indeed, there is an urgent need for new targeted molecular approaches using novel compounds. New insights into the pathogenesis of MCL tumor cells have recently opened windows of opportunity for innovative targeted therapies (3, 4). One of these new therapeutic approaches is to induce apoptosis via the intrinsic pathway and/or to overcome MCL cell resistance by antagonizing the antiapoptotic Bcl-2 family proteins. This approach is prompted by several investigations showing that the antiapoptotic protein Bcl-2 is constitutively overexpressed in MCL and plays a major role in drug resistance (3, 6). These findings highlight the potential of Bcl-2-targeted therapy in MCL.

Members of the Bcl-2 family are critical regulators of apoptosis, and the interactions between prosurvival and proapoptotic members are major determinant of cell fate
Translational Relevance

Prognosis of mantle cell lymphoma (MCL) is considered to be inferior to most of the other non–Hodgkin’s lymphomas (NHL) and new therapeutic approaches are warranted. The rationale to target Bcl-2 proteins in MCL has been investigated using ABT-737, a BH3 mimic that targets Bcl-2 and Bcl-xL, but not Mcl-1. We show that ABT-737 alone induces apoptosis only in Bcl-2– and Bcl-xL–deficient MCL cells. The use of siMcl-1 transfection, we show the key role of Mcl-1 in ABT-737 resistance. To overcome Mcl-1 resistance in clinical practice, we investigated two different strategies using three different drugs in combination with ABT-737 as follows: (i) to downregulate Mcl-1 expression using flavopiridol or 1-β-D-arabinofuranosylcytosine and (ii) to neutralize Mcl-1 antiapoptotic function by increasing Noxa with bortezomib. Our investigations confirm the synergistic effect of these combinations in ABT-737–resistant cell lines. In conclusion, the present work brings the rationale for the use of ABT-737 alone or in combination according to the apoptotic profile of MCL cells.

(7). Structural and functional characteristics divide them into 3 subgroups as follows: multidomain antiapoptotic members (Mcl-1, Bcl-2, Bcl-xL, Bcl-W, and A1), multidomain proapoptotic members (Bax and Bak), and BH3-only members (Bim, Bid, Bad, Bik, Puma, and Noxa). In response to a wide range of derangement signals, BH3-only members are activated (8). Certain BH3-only proteins, including Bim and Bid, are called activators because of their ability to activate Bax and Bak (9). In contrast, the antiapoptotic molecules antagonize cell death by sequestering either BH3-only proteins or multidomain proapoptotic members. The presence of Bax or Bak is required to mediate mitochondrial damage. First, these multidomain proapoptotic family members have to be activated to oligomerize; subsequently, oligomers form pores in the mitochondrial outer membrane. The apoptotic cascade is frequently, if not always, altered in tumor cells including MCL cells (3).

ABT-737 is a new compound that selectively binds with high affinity to Bcl-2 and Bcl-xL (10). ABT-737 displaces proapoptotic BH3-only from Bcl-2 or Bcl-xL, leading to activation of Bax and Bak and downstream caspases (9). Because of the low affinity of ABT-737 for Mcl-1, high basal levels of Mcl-1 have been associated with ABT-737 resistance (11–14). Previous studies have also shown that ABT-737 is effective as a single agent against some leukemia/lymphoma cell lines both in vitro and in vivo (11, 15–17).

This present study was focused on the antitumoral effect of ABT-737 in MCL. We also aimed to identify prognostic biomarkers that may predict MCL tumor cell responses to ABT-737. Using this approach, we have developed a rationale for ABT-737–based tailored therapeutic strategies in MCL.

Materials and Methods

MCL cell lines and primary MCL cells

JEKO-1, MINO, REC-1, GRANTA-519, and JVM-2 MCL cell lines were purchased from DSMZ. The UPM-1 MCL cell line was kindly provided by Dr. V. Ribrag (Institut Gustave-Roussy, Villejuif, France). JEKO-1, MINO, REC-1, GRANTA-519, and JVM-2 cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and 2 mmol/L glutamine. UPM-1 was maintained in MEM supplemented with 10% FCS and 2 mmol/L glutamine. Primary MCL cells were obtained from patients treated in our institution who had provided their written informed consent and been diagnosed with de novo or relapsed MCL; diagnosis was confirmed according to the WHO classification (18). MCL cells from blood or ascites were obtained following gradient density centrifugation using Ficoll-Hypaque. For Western blot analysis, MCL cells were purified with CD19 immunomagnetic beads.

Antibodies (monoclonal antibodies) and reagents

Antibodies used in this study were as follows: anti-Bcl-2 (clone 124; Dako), anti-Mcl-1 (S19; Santa Cruz Biotechnology), anti-caspase 3 (E-8; Santa Cruz Biotechnology), anti-Bik (N19; Santa Cruz Biotechnology), anti-Bcl-xL (BD Biosciences), anti-actin (Chemicon), anti-Noxa (Alexis Coger), anti-Bax (clone 4F11; ImmunoTech), anti-Bim (Millipore), and anti-Puma (Ab-1; Calbiochem). ABT-737 was kindly provided by Abbott Laboratories. Flavopiridol and 1-β-D-arabinofuranosylcytosine (ara-C) were obtained from Sigma Aldrich. Bortezomib was kindly provided by Millenium Laboratory.

Flow cytometric analysis of apoptosis and Bax/Bak activation

Cell death in MCL cell lines was assessed by Apo 2.7 staining. Cell death in primary MCL cells was assessed by both Apo 2.7 and CD19 staining combined with an analysis of altered cellular morphology [lower forward scattering (FSC)]. Flow cytometric analysis was carried out on a FACScalibur flow cytometer using CellQuest software (Becton Dickinson). For Bax and Bak immunofluorescence staining, 5 × 10^5 cells were fixed using the IntraPrep Permeabilization Reagent Kit (ImmunoTech) following the manufacturer’s recommendations. The cells were incubated with anti-Bax (clone 6A7) monoclonal antibody, anti-Bak (BD Biosciences, Pharmigen), or IgG1 isotype control for 20 minutes. The cells were then incubated with anti-mouse- or anti-rabbit–FITC antibodies (ImmunoTech) for 20 minutes, washed once in PBS, and resuspended in PBS–1% formaldehyde. The flow cytometric analysis was carried out as previously.

Immunoprecipitation and immunoblotting

Cells (10 × 10^6) were lysed in 1% CHAPS containing lysis buffer. Whole-cell lysates were obtained, preclarred with Protein A Sepharose, and incubated overnight with 10 μg of the specific antibody. Immunocomplexes were
captured with Protein A Sepharose. The beads were pelleted, washed 3 times, and boiled in SDS sample buffer. The presence of immunocomplexes was determined by Western blot analyses, which were carried out as described previously (19).

RNA isolation and quantitative real-time PCR
RNA was isolated using the NucleoSpin RNA II Kit (Macherey-Nagel). Two micrograms of total RNA was reverse transcribed using the Moloney murine leukemia virus reverse transcriptase (Invitrogen) and random hexamers (Amersham Biosciences). Quantitative PCR was carried out using the TaqMan Universal PCR Master Mix (Applied Biosystems) in a MX4000 instrument (Stratagene). Bcl-2 (Hs00608023_m1), Mcl-1 (Hs00172036_m1), and RPL37a (Hs01102345_m1) TaqMan gene expression assays were purchased from Applied Biosystems. The thermal cycling parameters used consisted of 50°C for 2 minutes for optimal AmpErase UNG activity and then 40 cycles of 95°C for 30 seconds and 60°C for 1 minute. To control specificity of the amplified product, a melting curve analysis was carried out. No amplification of nonspecific product was observed. Amplification of the housekeeping gene RPL37a was conducted for each sample as an endogenous control.

siRNA
Mcl-1 and control siRNAs duplexes used were ON-TARGET plus siRNA pools of 4 oligos purchased from Dharmacon. One hundred picomoles of siRNA was mixed with 10^6 cells and electroporated using the Amaxa Nucleofector Apparatus (Amaxa). Two days after transfection, the cells were incubated for 24 hours with ABT-737. The gene-silencing effect was evaluated by Western blot analysis.

Results

Sensitivity of MCL cell lines to ABT-737
The sensitivity of MCL cell lines to ABT-737 was investigated in normal culture conditions. After a 24-hour treatment protocol, cell death was assessed using Apo 2.7 staining (Fig. 1A). MINO and GRANTA-519 were highly sensitive to ABT-737 with a lethal dose (LD)_{50} of 25 and 80 nmol/L, respectively. In contrast, the 4 other MCL cell lines (JEKO-1, REC-1, UPN-1, and JVM-2) showed a higher LD_{50} ranging from 15 to 150 nmol/L, whereas 7 samples were classified as resistant to ABT-737 (median LD_{50} not reached). In 8 samples, the number of MCL cells was sufficient to evaluate Bcl-2 and Mcl-1 expression by Western blotting (Fig. 2B). As observed earlier in MCL cell lines, only Bcl-2 and Mcl-1 mRNA levels for the 6 MCL cell lines were measured. No amplification of nonspecific product was observed. Amplification of the housekeeping gene RPL37a was conducted for each sample as an endogenous control.

Sensitivity of primary MCL cells to ABT-737
ABT-737–induced apoptosis was also evaluated in primary MCL tumor cells from 13 patients. Characteristics of patients are summarized in Supplementary Table. After 24 hours of ABT-737 treatment, cell death was quantified in the MCL cell compartment (CD19-positive cells), and a combined analysis of altered cellular morphology (lower FSC) was also carried out (Fig. 2A). ABT-737 induced apoptosis in MCL cells in 6 samples (with LD_{50} values ranging from 15 to 150 nmol/L), whereas 7 samples were classified as resistant to ABT-737 (median LD_{50} not reached). In 8 samples, the number of MCL cells was sufficient to evaluate Bcl-2 and Mcl-1 expression by Western blotting (Fig. 2B). As observed earlier in MCL cell lines, only Bcl-2 and Mcl-1 mRNA levels for the 6 MCL cell lines were measured. No amplification of nonspecific product was observed. Amplification of the housekeeping gene RPL37a was conducted for each sample as an endogenous control.

ABT-737 triggered a rapid disruption of Bcl-2/Bax and Bcl-2/Bik complexes
To determine the mechanism of action of ABT-737, the constitution of Bcl-2 complexes and their dynamic upon short-time ABT-737 treatment were examined. For this purpose, Bcl-2 immunoprecipitations were realized after 2-hour ABT-737 treatment in the 2 sensitive cell lines. In untreated GRANTA-519 cells, Bcl-2 was associated with both the effector protein Bax and the BH3-only proteins Bim, Puma, and Bik (Fig. 3). In contrast to GRANTA-519, MINO does not express Bim. In MINO, endogenous Bcl-2 was mainly associated with Bax, Puma, and Bik. Of note, in both cells, no interaction between Bcl-2 and Bak was found (result not shown). In GRANTA-519 cells, an examination of Bcl-2 heterodimers after a 2-hour ABT-737 treatment showed a strong decrease of Bcl-2/Bax complexes and a very weak decrease of both Bcl-2/Bak and Bcl-2/Puma complexes. Consistent with the strong decrease of Bcl-2/Bax complexes, there was an increase of Bak in the immunoprecipitate supernatant (Fig. 3). In ABT-737-treated MINO, an important decrease of both Bcl-2/Bax and Bcl-2/Bik complexes were observed. Similarly to GRANTA-519, a weak decrease of Bcl-2/Puma complex was found. Finally, although no disruption of the Bcl-2/Bim complex was observed after short-time ABT-737 treatment in both cell lines, a longer treatment of 8 hours led to a partial...
disruption of Bcl-2/Bim complexes (data not shown). This last result is in agreement with the fact that Bim displayed the highest affinity for Bcl-2.

**Mcl-1 protects MCL cells against ABT-737–induced apoptosis**

In MCL cells, a high level of antiapoptotic protein Mcl-1 was observed in resistant cells, suggesting that Mcl-1 expression level might be associated with ABT-737 resistance. To investigate the specific role of Mcl-1 in ABT-737 resistance, Mcl-1 was silenced using RNA interference technology. After 48 hours of transfection with siMcl-1, a marked reduction of Mcl-1 level was observed (Fig. 4A). The knockdown of Mcl-1 sensitized both JEKO and JVM2 cells to ABT-737, showing that Mcl-1 level plays a major role in resistance to this drug (Fig. 4B). Thus, this result underscores the interest of drug combinations that downregulate Mcl-1 expression.

**Combination of ABT-737 and flavopiridol overcomes the Mcl-1–associated resistance of MCL cells to ABT-737**

Flavopiridol, a semisynthetic flavonoid, is known to inhibit mRNA transcription by inhibiting kinases that activate RNA polymerase II (20). Inhibition of Mcl-1 transcription by flavopiridol has been reported in chronic lymphoid leukemia (CLL) and multiple myeloma (MM) cells (21, 22). ABT-737–resistant MCL cell lines were cultured with flavopiridol (250 nmol/L) for 24 hours. A drastic diminution of Mcl-1 protein level was observed, whereas neither Bcl-2 nor Bcl-xL levels were affected by flavopiridol exposure (Fig. 4C). Interestingly, the decrease
of Mcl-1 level occurred rapidly, after 2 hours, and became undetectable after 4 hours of treatment with flavopiridol. This decrease was observed prior to caspase 3 cleavage, which was detected only after 6 hours of treatment (Fig. 4D). Following 2 hours of flavopiridol exposure, qRT-PCR analysis confirmed a decrease in Mcl-1 mRNA levels for up to 90% (Fig. 4E). Taken together, these results show that Mcl-1 protein downregulation in flavopiridol-treated cells is not due to a caspase-dependant cleavage but occurs at the mRNA level. Because flavopiridol induces a rapid and strong caspase-independent downregulation of Mcl-1, we hypothesized that flavopiridol might overcome the putative Mcl-1-associated resistance to ABT-737. The 4 ABT-737–resistant MCL cell lines were then treated with suboptimal doses of ABT-737 or flavopiridol or a combination of both drugs. Cell viability was assessed after a 24-hour exposure (Fig. 4F). The measurement of induced apoptosis by the combination of ABT-737 plus flavopiridol shows a major synergistic effect with suboptimal doses of each drug, as shown by a combination index (CI) < 1 according to the Chou–Talalay method (23).

**ABT-737 synergizes with ara-C or bortezomib in ABT-737–resistant MCL cell lines**

ara-C and, more recently, bortezomib have been shown to be 2 important compounds in the treatment of MCL patients (24–26). ara-C exposure can induce a rapid decrease of Mcl-1 that occurs independently of caspase 3 (Fig. 5A). In contrast, bortezomib exposure does not modify Mcl-1 but induces a strong increase of the BH3-only Noxa protein (Fig. 5B). Interestingly, Noxa binds selectively to Mcl-1 and thus counteracts its antiapoptotic function (27). The same results were observed in JVM-2 cell line (data not shown). As observed with flavopiridol, a strong synergistic effect was measured (CI < 1 according to the Chou–Talalay method) when both compounds were combined with ABT-737 in resistant JEKO-1 and JVM-2 cell lines (Fig. 5C).

**Discussion**

The present study evaluated the antineoplastic activity of ABT-737 in MCL cells. It shows that MCL cells are quite heterogeneous with regard to their sensitivity to ABT-737 (with LD_{50} values ranging >1,000 fold). Indeed, the less sensitive MCL cell lines show only limited induced apoptosis when exposed to high doses of ABT-737 (in the micromolar range), whereas the most sensitive lines undertake massive apoptosis after exposure to extremely low concentrations of ABT-737 (in the nanomolar range). Investigations conducted with fresh tumor cells from MCL patients have confirmed this finding and revealed that about half of the patient samples are highly sensitive to ABT-737 alone. Interestingly, the level of sensitivity in these samples is comparable with the one observed in sensitive MCL cell lines. Indeed, our results not only support the use of ABT-737 in MCL but also highlight the need for biological markers that could predict ABT-737 responsiveness.

The analysis of protein expression level in MCL cells reveals that the subset of highly sensitive MCL cells is consistently characterized by a Bcl-2^{low}/Mcl-1^{high} profile. This is in accordance not only with the molecular mechanism of action of ABT-737 but also with previous studies conducted in other hematologic malignancies. Indeed, CLL cells have been reported to be extremely sensitive to ABT-737 and, like ABT-737–sensitive MCLs, express high levels of Bcl-2 but always low levels of Mcl-1 (11, 28). It is also interesting to note that ABT-737 induces apoptosis at a higher drug concentration (5–15 μmol/L) in MM cell lines, which are known to express high levels of Mcl-1 (29). Therefore, Mcl-1 expression level seems as a valuable
biomarker regardless of both the level of Bcl-2 (12–14) and the nature of the tumor cell. Indeed, several studies and ours have identified a uniform profile (Bcl-2<sup>high</sup>/Mcl-1<sup>low</sup>) for ABT-737–sensitive cells. Therefore, both biomarker profiling (Bcl-2<sup>high</sup>/Mcl-1<sup>low</sup> and Mcl-1 levels) could be used to predict responses to ABT-737 exposure. These biomarkers are of major interest for future targeted therapies using ABT-737 alone in MCL.

Because half of the MCL samples did not undergo apoptosis in the presence of ABT-737 alone and because our findings show that the silencing of Mcl-1 overcomes ABT-737 resistance, the second part of the present work was devoted to investigate strategies that may overcome MCL resistance to ABT-737 by downregulating Mcl-1 expression. We observe that the level of Mcl-1 protein is highly variable from one patient to another and from one MCL cell line to

Figure 4. Flavopiridol (Flavo) induces a rapid downregulation of Mcl-1 at both the protein and mRNA levels and synergizes with ABT-737. A and B, Mcl-1 silencing sensitizes JEKO and JVM2 to ABT-737–induced apoptosis. MCL cells were transfected with either siControl (siCtrl) or siMcl-1. At 48 hours after transfection, cells were used to prepare cell lysates and Mcl-1 level was determined by immunoblotting (A). Transfected cells were treated with ABT-737 for 24 hours before being stained by Apo 2.7 and analyzed by flow cytometry (B). Results are means ± SD from at least 3 independent siRNA transfections. C, MCL cell lines resistant to ABT-737 were cultured for 24 hours with or without 250 nmol/L flavopiridol. Western blot analysis of Mcl-1, Bcl-2, and Bcl-xL protein expression is shown. D, the MCL cell line JEKO-1 was cultured for 2, 4, 6, and 24 hours with 500 nmol/L flavopiridol. Western blot analysis of Mcl-1 and caspase 3 proteins is shown. E, Mcl-1 mRNA level was measured by qRT-PCR at baseline and after 2, 4, and 6 hours of treatment with flavopiridol (500 nmol/L) in JEKO-1. F, effect of combining ABT-737 and flavopiridol in ABT-737–resistant cell lines. ABT-737–resistant MCL cell lines were cultured for 24 hours with the indicated drugs. In all experiments, drugs were added simultaneously. Cell death was quantified using Apo 2.7 staining. Suboptimal concentrations of ABT-737 (150 nmol/L) and flavopiridol (250 nmol/L for JEKO-1, REC-1, and JVM-2 and 150 nmol/L for UPN-1) were used. Synergy was confirmed by CI < 1 according to the Chou–Talalay method. Data represent means ± SD from 3 independent experiments.
another. As reported by Khoury and colleagues, high Mcl-1 expression level has been associated with a more aggressive biological behavior of the MCL cells (30). More recently, Perez-Galan and colleagues reported that Mcl-1 is also involved in bortezomib resistance in MCL cells (27). Flavopiridol is known to effectively inhibit Mcl-1 transcription in CLL and MM (21, 22). In MCL, flavopiridol was reported to induce apoptosis (31). Therefore, flavopiridol is currently under investigation for treatment of several hematologic malignancies including both CLL and MCL (32–34). Our investigation confirms that flavopiridol downregulates Mcl-1 at both the mRNA and protein levels in MCL cells and shows a highly synergistic apoptotic effect when combined with ABT-737 in ABT-737–resistant cell lines. The effectiveness of combining ABT-737 with compounds that inactivate Mcl-1 firmly establishes the validity of such a therapeutic approach in the treatment of MCL (12, 35–37). A similar synergistic effect is measured when ABT-737 is combined with ara-C, which is a heavily prescribed drug in the treatment of MCL (24, 38, 39). As reported with flavopiridol, ara-C induces Mcl-1 downregulation at the mRNA level (40). Of note, the regulation of Noxa protein expression level was not significantly modified under both combinations (data not shown). Thus, both flavopiridol and ara-C, which decrease Mcl-1 expression, seem to be promising supplements in ABT-737 therapy of patients presenting high Mcl-1 levels. A second investigated strategy to overcome Mcl-1 is to modify Mcl-1 complexes and its protein network. Bortezomib is among the most promising compounds in the treatment of MCL (25). Interestingly, the mixture of ABT-737 with bortezomib shows a synergistic effect, although no decrease of Mcl-1 protein expression is observed in the presence of bortezomib for up to 24 hours. Among the various proapoptotic effects of bortezomib, one involves the drastic increase of Noxa, a BH-3–only protein that is known to specifically interact with Mcl-1 (41). The upregulation of Noxa leads to an increase of Noxa/Mcl-1 heterodimers. Thus, it counteracts the antiapoptotic role of Mcl-1 and then Mcl-1 fails to protect cells from apoptosis (27). Taken together, our results show that several strategies might be used to overcome ABT-737 resistance. One would be to include compounds that decrease Mcl-1 expression (such as ABT-737 treatment in combination with flavopiridol or ara-C) and another approach would be to inhibit Mcl-1 antiapoptotic action by increasing Noxa expression using bortezomib. Recent publications suggested that ABT-737 exposure might enhance Mcl-1 upregulation, leading to ABT-737 resistance (36, 42). This putative role played by ABT-737 as a Mcl-1 upregulator further enhances the potential of Mcl-1–targeted therapy used in combination with ABT-737.

In MCL-sensitive cells, our findings show that short exposure to ABT-737 induces a rapid disruption of both Bcl-2/Bax and Bcl-2/Bik complexes. Bax is a multidomain proapoptotic protein that permeabilizes the outer mitochondria membrane leading to cytochrome C release.
whereas Bik is a sensitizer BH3-only protein. The major impact of the dissociation between Bcl-2 and Bax in ABT-737–induced apoptosis in MCL is in agreement with the work from van Delft and colleagues (14). In contrast, Bcl-2/Bim and Bcl-2/Puma complexes are weakly affected by ABT-737, which could be explained by their high affinity to Bcl-2 (9). In contrast, we did not observe a correlation between Bcl-xl expression level and response to ABT-737 in MCL.

The present work strongly supports the use of ABT-737 in MCL and opens the window for selected ABT-737-tailored therapies that use biomarkers that are able to predict patient responses. Indeed, MCL patients presenting a Bcl-2-high/Mcl-1-low profile may be good candidates for ABT-737 monotherapy, whereas ABT-737 combined with a Mcl-1–targeted therapy (such as flavopiridol, ara-C, or bortezomib) would be more appropriate for MCL patients with high Mcl-1 levels. These biomarkers are easily assessed prior to treatment induction. In our experiments, Bcl-2 and Mcl-1 protein level assessment is a powerful tool to determine Bcl-2/Mcl-1 patient profiles, but other methods such as flow cytometric or quantitative PCR analysis could also evaluate these markers effectively. The unique mechanism of action of ABT-737 offers the opportunity to treat patients by using a targeted therapy based on their Bcl-2/Mcl-1 profile in tumor cells. The present investigation provides the biological rationale for these future clinical trials to evaluate ABT-737 alone or in combination with Mcl-1–reducing agents in MCL patients. Indeed, ABT-263 (navitoclax; Abbott), which is an orally bioavailable BH3-mimetic compound of the same class as ABT-737 and currently under investigation in hematologic and solid malignancies, could also be used as ABT-737 (13, 43–46).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

C. Touzeau, M. Amiot, and S. Le Gouill designed the research and wrote the manuscript. C. Touzeau and S. Le Gouill carried out the experiments. C. Touzeau, M. Amiot, S. Le Gouill, C. Doussset, L. Bodet, P. Gomez-Bougie, S. Bonnard, and C. Pellat-Decuerynk analyzed the data. S. Le Gouill, P. Moreau, and A. Moreau provided patient samples and clinical data.

Grant Support

This study is supported by Région Pays de la Loire (France), the INCa PAIR lymphoma grant, and the Ligue Régionale Grand-Ouest contre le Cancer. C. Touzeau is supported by Association pour la Recherche contre le Cancer (ARC).

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Received April 13, 2011; revised July 12, 2011; accepted July 26, 2011; published OnlineFirst August 5, 2011.

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