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The Bcl-2 specific BH3 mimetic ABT-199: a promising targeted therapy for t(11;14) multiple myeloma

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Despite recent advances in the treatment of multiple myeloma (MM), including proteasome inhibitors and immunomodulatory agents, patients invariably relapse and alternative treatment strategies are urgently needed. Bcl-2 inhibitors such as ABT-737 and ABT-263 (navitoclax), which target specifically anti-apoptotic proteins Bcl-2 and Bcl-x_L, bring new hope for therapy.[1] We recently demonstrated that ABT-737 induces apoptotic cell death in a sub-group of MM at low nanomolar concentration (<150 nM).[2] This sub-group of MM can be identified by its high Bcl-2/Mcl-1 Affymetrix gene expression ratio indicating that the expression level of Bcl-2 versus Mcl-1 mainly determines the susceptibility of myeloma cells to ABT-737 as previously demonstrated in other hematopoietic cells.[3] In addition, we have shown that Mcl-1 silencing strongly increases the sensitivity to ABT-737 of MM cells, highlighting the essential role of Mcl-1 in ABT-737 resistance. Although the orally active navitoclax has entered clinical trials and shown activity in hematological malignancies that are believed to be Bcl-2 dependent, the efficacy of this agent is limited by the thrombocytopenia caused by Bcl-x_L inhibition.[4] To overcome this dose-limiting toxicity, ABT-199, the first-in-class orally bioavailable Bcl-2-selective BH3 mimetic, has been developed. Notably, it was reported that selective inhibition of Bcl-2 by ABT-199 demonstrated weak activity *ex vivo* on platelets.[5] These data were further extended to clinical observations. Indeed, ABT-199 showed promising antitumor activity while sparing platelets in chronic lymphoid leukemia patients.[5] Moreover, in a mouse

Table 1. ABT-199 sensitivity of primary myeloma cells

Patient	*Patients characteristics*				*ABT-199 sensitivity*		*FISH analysis*			*Bcl-2/Mcl-1 mRNA ratio*
	Age	*Disease*	*Isotype*	*Disease Status*	*% apoptotic cells (100 nM)*	*LD_{50} < 100 nM*	*t(11;14)*	*t(4;14)*	*del 17*	
#1	40	MM	l	Diag	21	No	+	−	−	0.10
#2	80	MM	A k	Rel	12	No	−	+	−	NA
#3	65	pPCL	l	Diag	<5	No	−	−	−	1.63
#4	49	pPCL	NA	Diag	40	No	−	+	−	0.62
#5	57	MM	G k	Rel	64	Yes	+	−	−	NA
#6	64	MM	G k	Diag	16	No	−	+	−	0.87
#7	38	MM	k	Diag	70	Yes	+	−	−	1.91
#8	80	MM	NA	Diag	15	No	−	−	−	0.46
#9	52	MM	G k	Rel	12	No	−	−	+	0.90
#10	45	MM	G k	Diag	91	Yes	+	−	−	6.69
#11	76	MM	NA	Rel	35	No	−	+	−	0.15
#12	70	sPCL	A k	Rel	<5	No	−	−	−	NA
#13	71	pPCL	NA	Diag	87	Yes	+	−	+	NA
#14	27	pPCL	A l	Diag	<5	No	−	−	−	NA
#15	74	MM	A l	Diag	<5	No	−	−	−	NA

Abbreviations: Diag, diagnosis; F, female; k, kappa; l, lambda, LD: lethal dose; M, male; MM, multiple; NA, data not available; PCL, plasma cell leukemia; pPCL, primary plasma cell leukemia; Rel, relapse. Plasma cells were obtained after gradient density centrifugation on Ficoll Hypaque and purification with CD138-immunomagnetic beads. Blood or bone marrow samples were obtained after informed consent from 15 patients with a diagnosis of de novo or relapsed MM according to standard criteria. Plasma cells were cultured with increasing doses of ABT-199 during 18 h. Cell death was assessed by APO 2.7 staining. The relative expression of Bcl-2 and Mcl-1 mRNA was defined on purified CD138-positive plasma cells as described above for HMCLs and Bcl-2/Mcl-1 mRNA ratio was indicated.

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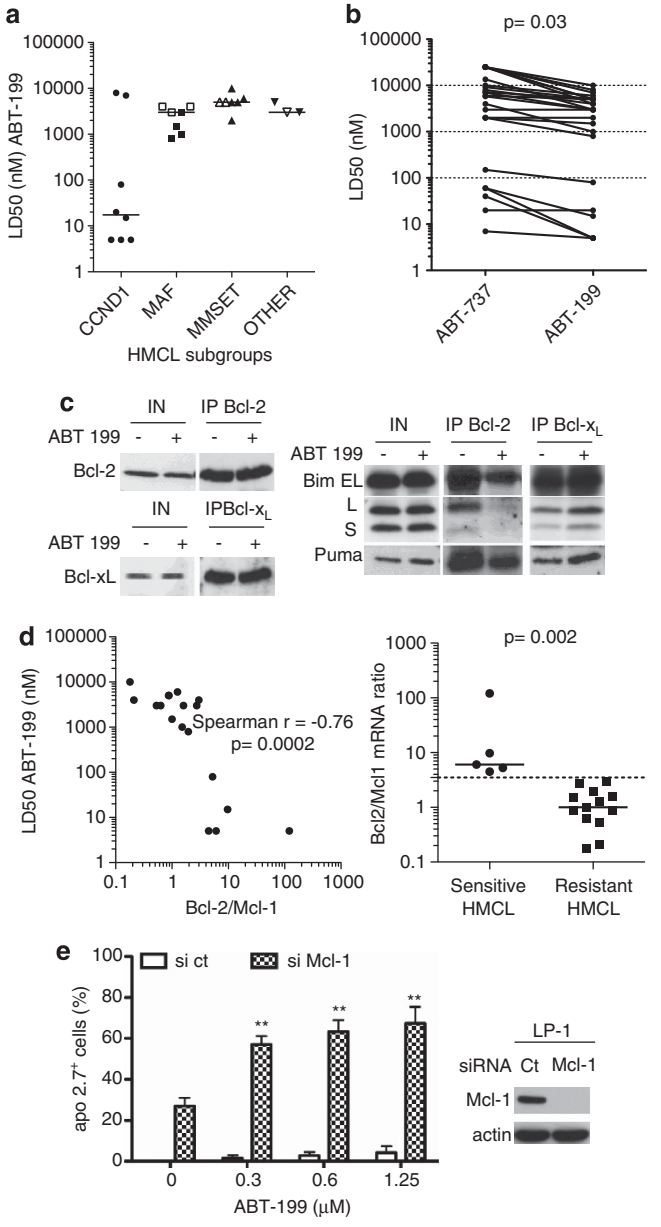
model, ABT-199 also demonstrated *in vivo* efficacy against aggressive lymphoma without provoking thrombocytopenia.[6]

In the present study, the apoptotic efficiency of ABT-199 was evaluated in both human multiple myeloma cell lines (HMCLs) and primary myeloma cells.

Sensitivity of ABT-199 was first analyzed across a collection of 25 HMCLs reflecting the molecular heterogeneity found in MM patients. These included non-14q32 translocated or non-recurrent translocated lines along with those harboring a translocation in MMSET, c-MAF or MAFB, or CCND1.[7] We found that high sensitivity to ABT-199 was restricted to HMCLs with the CCND1 translocation (Figure 1a). Indeed, six out of eight CCND1 cell lines were efficiently killed by ABT-199 with a median LD_{50} value of 10 nM (ranging from 5–80 nM). Comparison of CCND1 cell lines sensitivity targeted by ABT-737[2] and ABT-199 revealed a similar specificity. However, the efficiency of ABT-199-induced cell killing was always superior to that of ABT-737, as indicated by decreased LD_{50} values (Figure 1b). It is of particular interest to note that sensitive CCND1 cell lines harbor an abnormal p53 (Figure 1a), indicating that ABT-199-induced cell death is independent of the p53 status.



Furthermore, we identified two cell lines (NAN-1 and RPMI-8226) belonging to the MAF subgroup that show an intermediate sensitivity (LD_{50} between 100 nM and 1 μM) to ABT-199, whereas none have intermediate sensitivity to ABT-737 ($\leqslant$1 μM). Finally, comparison of LD_{50} values demonstrated that the cellular potency of ABT-199 is equal to or greater than that of ABT-737 in all the tested HMCLs (Figure 1b). Of note, the median ABT-199 LD_{50} of sensitive HMCLs was significantly lower than that of peripheral blood lymphocytes (PBL) from healthy donors (10 vs 200 nM, $P = 0.001$) (Supplementary Figure 1).

Characterization of the selectivity of ABT-199 for Bcl-2 has been evidenced by its affinity for Bcl-2 (Ki $<$ 0.010 nM) in contrast to that for Bcl-x_L (Ki = 0.48 nM).[5] To confirm the selective killing of ABT-199 in a MM cellular context, we analyzed the capacity of ABT-199 to disrupt BH3-only/Bcl-2 complexes versus BH3-only/Bcl-x_L complexes by immunoprecipitation (Figure 1c). For this purpose, the Karpas 620 CCND1 line was selected for its high Bcl-x_L expression. Puma and Bim were found associated with both Bcl-2 and Bcl-x_L in this cell line. After 6 h of ABT-199 treatment (25 nM), Puma/Bcl-2 and Bim/Bcl-2 complexes were disrupted in contrast to the Bcl-x_L heterodimers that were not modified (Figure 1c). Altogether, immunoprecipitation data confirm the ability of ABT-199 to only bind the Bcl-2 hydrophobic groove according to its strong affinity for Bcl-2.

Sensitivity to ABT-199 was significantly associated with higher expression of Bcl-2 ($P = 0.008$) and tended to be associated with a lower Mcl-1 expression ($P = 0.09$) (Supplementary Figure 2). However, the Bcl-2/Mcl-1 ratio was the most powerful biomarker for predicting ABT-199 sensitivity in this panel of HMCLs ($P = 0.002$) (Figure 1d). Median ratios were 6.06 (range 4.47–121) and 1 (range 0.18–3) for sensitive and resistant cell lines, respectively. Quantifying the Bcl-2/Mcl-1 ratio by RT-PCR therefore provides a strong predictive marker for ABT-199 sensitivity. This clearly suggests a strong involvement of Mcl-1 in ABT-199 resistance. To confirm the role of Mcl-1 in ABT-199 resistance, siRNA against Mcl-1 was transfected into LP1 cells, leading to a complete downregulation of Mcl-1 (Figure 1e). Mcl-1 silencing resulted in a moderate induction of apoptosis in LP1 cells but highly sensitized these cells to low doses of ABT-199 ($P = 0.0014$), indicating that Mcl-1 can confer ABT-199 resistance, as previously demonstrated for ABT-737.[2]

Figure 1. (**a**) Sensitivity to ABT-199 of HMCLs according to different molecular subgroups. (**b**) Sensitivity of HMCLs to ABT-199 compared to ABT-737. *P*-value was determined using Mann–Whitney test. For a and b, 25 HMCLs (respectively 8 CCND1 (KMS-12-PE, KMS-12-BM, SKMM-2, Karpas 620, U-266, XG-1, XG-5, NAN-7), 7 MAF (JJN-3, MM.1S, L-363, RPMI 8266, XG-6, BCN, NAN-1,) 7 MMSET (JIM-3, LP-1, OPM-2, NCI-H929, KMS-11, XG-7, NAN-3,) and 3 non recurrent translocation (XG-2, KMM-1, AMO1)) cell lines were cultured with increasing doses of ABT-199 or ABT-737 during 48 h. Cell death was assessed by APO 2.7 staining. LD_{50} was determined for each cell line. White symbols indicate wild-type P53 HMCL and black symbols indicate abnormal P53 HMCL (**c**) ABT-199 disrupts BH3-only/Bcl-2 heterodimers but not BH3-only/Bcl-xL heterodimers. Karpas 620 were treated or not for 6 h with 25 nM ABT-199. Lysate (600 μg) and immunoprecipitation were done as previously described[2] using the indicated antibodies. The immunoprecipitates were analyzed for the presence of BH3-only. (**d**) The Bcl-2/Mcl-1 mRNA ratio discriminates cell lines sensitive or resistant to ABT-199. Quantitative PCR was performed using the following TaqMan probes (BCL2 (Hs00608023_m1), MCL1 (Hs00172036_m1) and RPL37a (Hs01102345_m1). The relative expression of Bcl-2 and Mcl-1 mRNA was calculated according to the equation of Pfaffl and normalized to JJN3 cell line. Sensitive HMCL were defined by a LD_{50} inferior to 100 nM. *P*-value was determined using Mann–Whitney test. (**e**) The LP-1 cell line was transfected with either si Control (Ct) or si Mcl-1. Following 48 h transfection, the protein level of Mcl-1 was assessed by immunoblotting, and cells were treated with ABT-199 for a further 48 h. Cell death was quantified by APO 2.7 staining. *P*-value was determined using paired Student's *t*-test. $^{**}P < 0.008$.

In order to translate our findings in cell lines to human tumor cells, ABT-199 sensitivity was evaluated against 15 primary samples from patients with MM. Patient characteristics are summarized in Table 1. After 24 h, ABT-199 treatment (10, 100 and 1000 nM) resulted in cell death that was quantified by flow cytometry using a combined analysis of the loss of CD138 and alteration of cellular morphology (lower FCS). As for HMCLs, we observed a considerable variability in the sensitivity to ABT-199 among patient samples. Four patient samples, three of which were either *de novo* MM ($n = 2$), plasma cell leukemia ($n = 1$) or relapse ($n = 1$), were found to be highly sensitive to ABT-199 with a $LD_{50} < 100$ nM (Table 1).

As ABT-199-sensitive HMCLs were restricted to the CCND1 subgroup, a FISH analysis of the t(11;14) translocation was undertaken. Analyses for t(4.14) and del(17p) were also undertaken due to their high clinical significance. Of major interest, we found that four of five patients who bore a CCND1 translocation were sensitive to ABT-199, confirming that ABT-199 sensitivity is related to specific genetic subtypes. Furthermore, the Bcl-2/Mcl-1 mRNA ratio could be determined in eight patients. The median Bcl-2/Mcl-1 mRNA ratio was 4.3 (range, 1.91–6.69) for ABT-199 sensitive patients and 0.62 (range, 0.10–1.63) for ABT-199 resistant patients. Owing to the low number of patients, this difference does not reach statistical significance. Nevertheless, the results obtained using patient samples are consistent with our initial observations made in cultured myeloma cell lines and indicate that patients with a specific molecular subtype and a high Bcl-2/Mcl-1 mRNA ratio are particularly sensitive to ABT-199. While the t(11;14) group of patients is considered neutral with regard to prognosis, t(11;14) primary plasma cell leukemia (pPCL) presents an aggressive prognosis, suggesting that innovative approaches should be a priority for these patients. ABT-199 mono-therapy could represent a very interesting opportunity for the clinical management of pPCL.

Finally, del(17p) is universally associated with poor prognosis, mainly related to a defect of the TP53 pathway, because *TP53* mutations are exclusively associated with del(17p) in MM.[8] Of interest, one t(11;14) patient who also harbored a 17p deletion was found to be highly sensitive to ABT-199, indicating that ABT-199 is able to overcome the poor prognostic impact of del(17p) of t(11;14) patients. Again, this result is in accordance with the fact that most CCND1 HMCLs, which also express abnormal TP53, are still sensitive to ABT-199. Recent studies addressing the characteristics of pPCL indicate a higher incidence of del(17p) in pPCL versus MM.[9,10] Thus, the increase of the incidence of both t(11;14) and del(17p) in pPLC makes these patients especially attractive candidates for targeted therapy with ABT-199.

Our data demonstrate that ABT-199-induced lethality is restricted to a specific MM subtype and underscores the conclusion that MM should no longer be considered a single entity. Innovative targeted therapies should be developed with the aim of treating specific molecular subtypes. The clinical development of single-agent ABT-199 therapy in chronic lymphocytic leukemia and non-Hodgkin lymphomas has thus far demonstrated a favorable toxicity profile and an impressive antitumor activity.[5,11] Based on these promising clinical data, as well as our preclinical data, a phase I trial is ongoing in relapsed MM patients. The integration of comprehensive genetic studies and Bcl-2 family gene expression profiling will hopefully allow us to validate ABT-199 monotherapy as an effective targeted therapy for both t(11;14) MM and pPLC.

CONFLICT OF INTEREST

JDL and AJS are employees of Abbvie. DS is employee of Genentech, a member of the Roche Group. The remaining authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

CT, CD and SM performed experiments and analyzed data. DS, JDL, AJS analyzed the data. MCB and PM collected patients' samples and performed FISH experiments. CT, SLG, CP, MA designed the study. CT, SLG, JDL and MA wrote the manuscript. All authors critically reviewed and edited the paper.

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