Biological rationale for sequential targeting of Bruton tyrosine kinase and Bcl-2 to overcome CD40-induced ABT-199 resistance in mantle cell lymphoma

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AIMS

The aggressive biological behavior of mantle cell lymphoma (MCL) and its short response to current treatment highlight a great need for better rational therapy. Recently it has been demonstrated that tumor microenvironment strongly influences drug resistance in MCL cells. In the present study, we investigated the apoptotic efficiency of the first-in-class orally bioavailable Bcl-2-selective BH3 mimetic ABT-199 by integrating the key role of the microenvironment.

METHODS

1- To determine sensitivity of MCL cells to ABT-199, cell lines (n=8) and primary MCL cells obtained from peripheral blood of patients at diagnosis or relapse (n=12) were treated with ABT-199. To mimic the lymph node microenvironment where CD40-CD40L interaction takes place, MCL cells were cultured on CD40L-expressing cells.

2- Ibrutinib has been shown to induce a redistribution of lymph node-resident MCL (Lymphocytosis). Thus, we investigated the lymphocyte population in the peripheral blood of two patients that received ibrutinib. Blood was collected and analyzed for the presence of CD19+ CD5+ MCL cells before treatment and at day 7 following ibrutinib monotherapy. Peripheral blood population obtained on day 7 was treated with ABT-199 to analyze the cytotoxic efficiency.

RESULTS

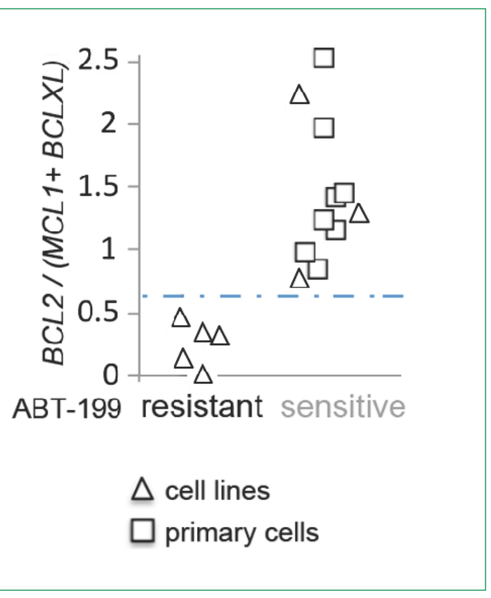

***Figure 1**. The BCL2/(MCL1+BCLXL) mRNA ratio correlates with ABT-199 sensitivity in MCL cells.*
Resistant : ABT-199 LD_{50} > 1000 nM
Sensitive : ABT-199 LD_{50} < 200 nM

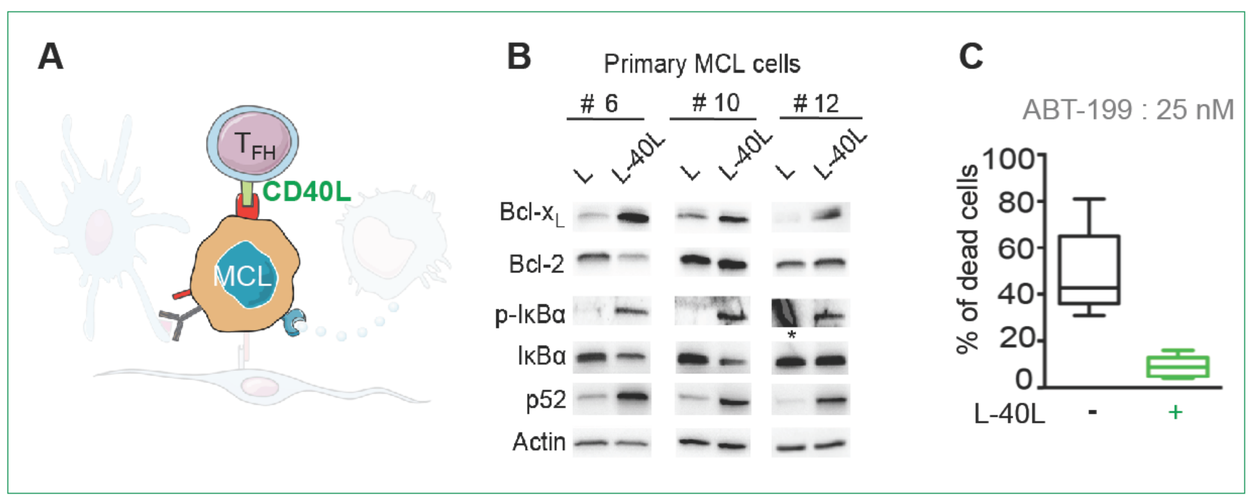

***Figure 2.** Up-regulation of Bcl-x_L by CD40 stimulation confers ABT-199 resistance to primary MCL cells. In order to mimic CD40-CD40L interaction that takes place in the lymph node microenvironment (A), primary MCL cells were cultured on CD40L-expressing fibroblast L cells (L-40L). (B) CD40 stimulation (24 hours) of primary MCL cells (n=3) demonstrated a strong Bcl-xL upregulation associated to the activation of both classical (p-IkBα) and alternative (p52) NF-kB pathways. (C) These modulations have implication in chemoresistance as illustrated with ABT-199, in contrast, to highly sensitive peripheral blood MCL cells, cells in coculture with L-40L become resistant. * non-specific staining*

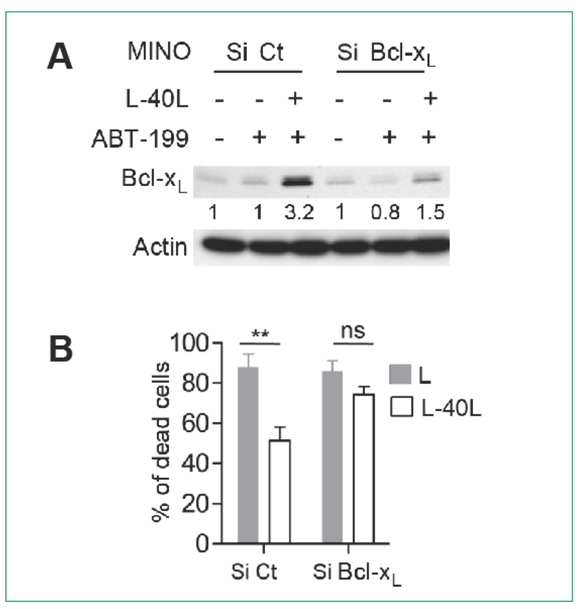

***Figure 3**. Downregulation of BclxL through its silencing by siRNA restores ABT-199 sensitivity in MINO cells cocultured with L-40L.*

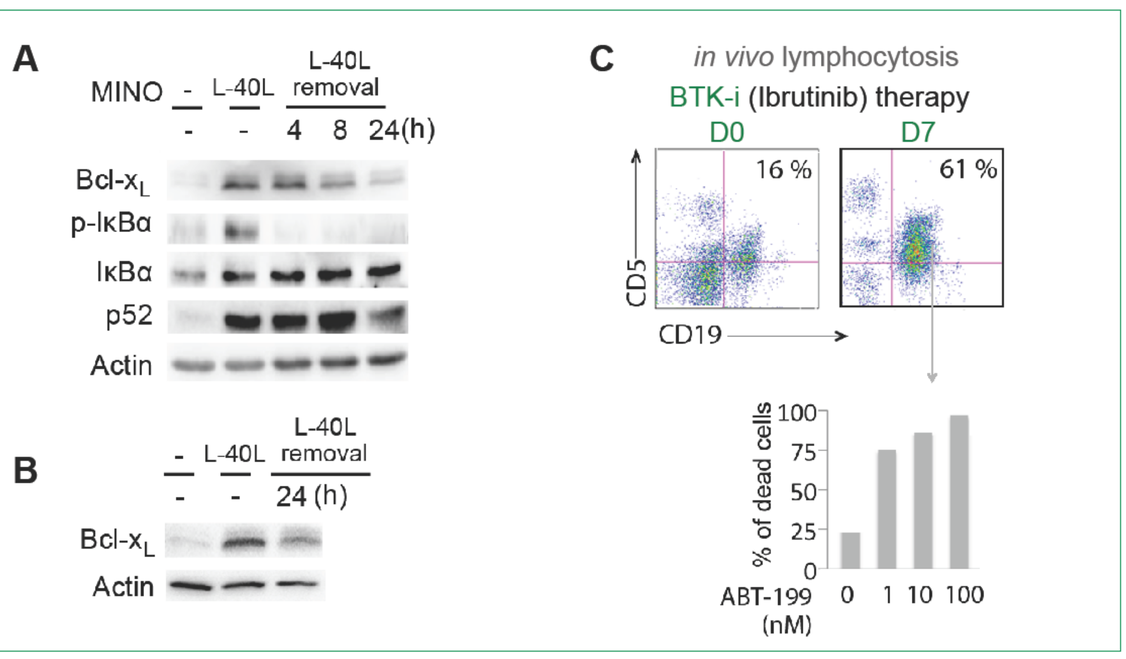

***Figure 4**. Because MCL cells frequently disseminate from lymph nodes into circulation, we mimicked this process by removing MINO (A) or primary MCL (B) cells from L40-L before assessing protein levels. We observed a rapid decrease of IkBα phosphorylation and reduction of p52 and Bcl-x_L protein level within 24 hours after detachment. (C) Sensitivity of *de novo* mobilized primary MCL cells (CD19+CD5+) to ABT-199 was addressed in a patient who displayed ibrutinib-dependent lymphocytosis.*

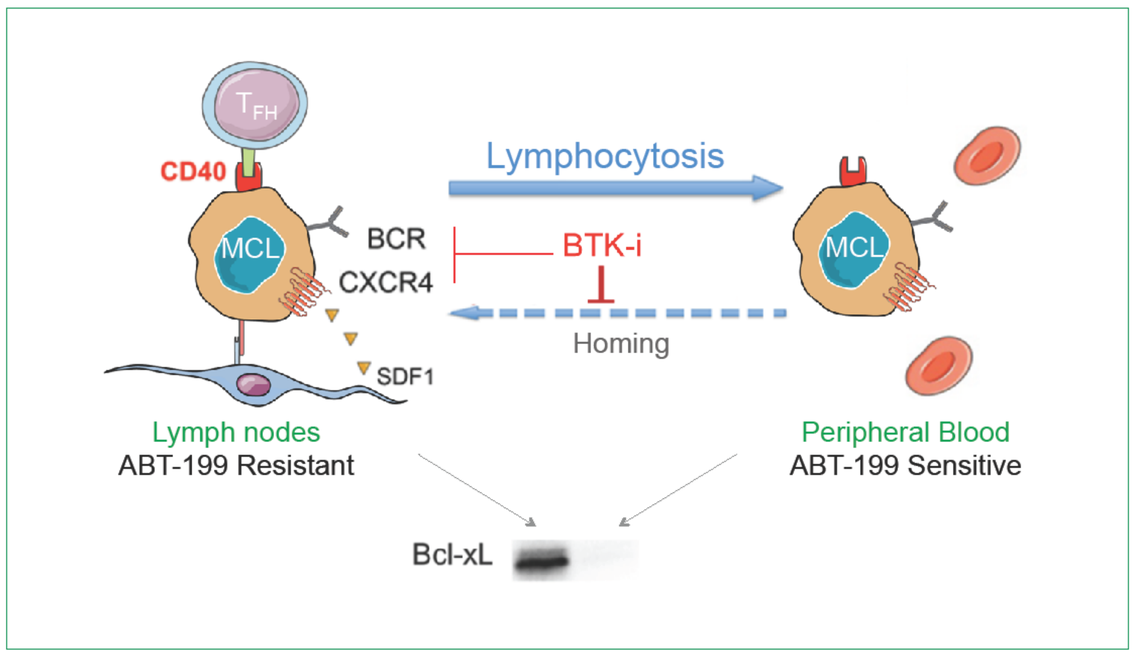

***Figure 5**. Schematic representation of ibrutinib mechanism of action in MCL cells. By inhibiting BTK, ibrutinib neutralize both BCR and CXCR4 axis, resulting in egress of MCL cells from the protective microenvironment into peripheral blood. The reduced Bcl-xL expression that characterized circulating MCL cells results in an increased activity of ABT-199.*

CONCLUSIONS

All primary MCL samples from peripheral blood were highly sensitive to low concentration of ABT-199, in contrast, among MCL cell lines tested, 5 out of 8 were resistant. Our observations revealed that *BCL2/(BCLXL+MCL1)* mRNA ratio is a strong predictor of ABT-199 sensitivity (Figure 1).

By mimicking the lymphoid microenvironment through CD40 stimulation, we show that ABT-199 sensitivity is impaired through activation of NF-kB pathway and Bcl-xL up-regulation (Figures 2 and 3). We further demonstrate that resistance is rapidly lost when MCL cells detach from CD40L-expressing fibroblasts (Figure 4A-B). Ibrutinib induces lymphocytosis *in vivo* by holding off malignant cells from their protective microenvironment and we show here for a patient undergoing ibrutinib therapy that mobilized MCL cells are highly sensitive to ABT-199 (Figures 4C).

In conclusion, the Bcl-2-selective BH3 mimetic ABT-199 is a promising agent for the treatment of B-cell malignancies including MCL and may be especially attractive in combination with BCR signaling inhibitory drugs such as ibrutinib, which can drive malignant cells out of the protective microenvironment (Figure 5).

Ref : Chiron et al., Oncotarget. 2015 Apr 20;6(11):8750-9.

13icml 261--P
Mantle cell lymphoma
David Chiron
DOI: 10.3252/pso.eu.13icml.2015
Poster presented at: 13-ICML
Printing supported by: Takeda ONCOLOGY
PosterSessionOnline.com