Resistance to venetoclax-induced apoptosis of CLL cells due to CD40 stimulation can be reversed by BCL-XL inhibition

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Abstract

Dependence on anti-apoptotic protein BCL-2 for prolonged survival, a well-known feature of circulating chronic lymphocytic leukaemia (CLL) cells, makes BCL-2 an ideal target for therapeutic intervention. Consequently, venetoclax (also known as ABT-199), a small molecule inhibitor disrupting the interaction of BCL-2 with apoptotic BH3-only proteins, has been successfully developed (Souers et al, 2013), and demonstrates impressive clinical activity in CLL (Roberts et al, 2016). However, recent studies show that venetoclax is less effective in killing CLL cells that are exposed to pro-survival stimuli present in the CLL microenvironment. Interaction of CLL cells with non-malignant T cells in the bone marrow and lymph nodes constitutes an essential part of the CLL microenvironment *in vivo* (Burger, 2011; Caligaris-Cappio et al, 2014). One of the most important molecular modulators mediating pro-survival effects is stimulation of CD40 on CLL cells by CD40 ligand (CD40L, also known as CD154) on T cells (Schattner, 2000; Granziero et al, 2001; Ghia et al, 2002). The CD40 stimulation can be replicated *in vitro* using a co-culture system where CLL cells are cultured on a monolayer of transfected murine fibroblasts expressing human CD154. We and others have shown that such co-culture protected CLL cells from both spontaneous apoptosis and apoptosis induced by cytotoxic agents that activate either mitochondrial (intrinsic) or death receptor-mediated (extrinsic) apoptosis pathways (Kitada et al, 1999; Granziero et al, 2001; Vogler et al, 2009; Pascutti et al, 2013; Zhuang et al, 2014; Chapman et al, 2017). More recently, it has been shown that co-culture of CLL cells with CD154-expressing fibroblasts renders them highly resistant to venetoclax-induced apoptosis, most likely as a result of upregulation of other anti-apoptotic members of BCL-2 family proteins including MCL-1 and BCL-XL (Thijssen et al, 2015; Bojarczuk et al, 2016). Therefore, the efficacy of venetoclax might be further enhanced by strategies to overcome the cytoprotective effect of CD40 stimulation.

This study thus set out to investigate whether pharmacological inhibition of MCL-1 or BCL-XL can restore the sensitivity of CD40-stimulated CLL cells to venetoclax and, if so, what the underlying mechanisms are. CD40 stimulation significantly reduced the sensitivity of primary CLL cells to venetoclax-induced apoptosis. This reduction correlated with increased expression of MCL-1 and BCL-XL in CD40-stimulated CLL cells. To target MCL-1 and BCL-XL, we used two novel small molecule inhibitors S63845 and A-1331852 that selectively inhibit MCL-1 (Kotschy et al, 2016) and BCL-XL (Leverson et al, 2015), respectively. We showed that neither S63845 nor A-1331852 alone was effective in killing CD40-stimulated CLL cells. Moreover, addition of the MCL-1 inhibitor did not restore sensitivity of CD40-stimulated CLL cells to venetoclax. In contrast, the BCL-XL inhibitor significantly sensitised CD40-stimulated CLL cells to cell death induced by venetoclax. Mechanistically, we showed that BCL-XL inhibitor mediated

sensitisation effect by blocking the binding of BH3-only protein BIM to BCL-XL in these cells. Finally, we showed that BIM was required for the induction of apoptosis by venetoclax as knockdown of BIM expression by siRNA reduced the level of apoptosis induced by venetoclax. Taken together, our data provide proof of concept that CD40-mediated resistance to venetoclax-induced apoptosis of CLL cells can be overcome by combining venetoclax with an inhibitor of BCL-XL.

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