To BH3 profile or not to BH3 profile

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In this issue of *Blood*, Anderson et al demonstrate that venetoclax induces apoptosis of chronic lymphocytic leukemia (CLL) cells independently of TP53 function in vitro and in vivo, and suggest a role for BH3-profiling in determining a patient's response to treatment[1].

Apoptosis, a form of controlled programmed cell death occurs following irreparable cellular damage. The Bcl-2-family of proteins consist of three main subfamilies that tightly control this apoptotic process. The first are anti-apoptotic proteins such as B-cell lymphoma 2 (BCL-2), BCL-2-related gene, long isoform (BCL-X_L), and myeloid cell leukemia 1 (MCL-1). The second are pro-apoptotic effector proteins including BCL-2 antagonist killer 1 (BAK) and BCL-2-associated x protein (BAX), and the third are pro-apoptotic BH3-only proteins such as BCL-2 associated agonist of cell death (BAD), Phorbol-12-myristate-13-acetate-induced protein 1 (*PMAIP1*/Noxa), BH3-interacting domain death agonist (BID) and BCL-2-interacting mediator of cell death (BIM) [2]. Inhibition of anti-apoptotic proteins by the pro-apoptotic BH3-only proteins results in BAX/BAK dimerization and insertion into the mitochondrial membrane where they initiate mitochondrial outer membrane permeabilization (MOMP). MOMP results in the release of cytochrome C, caspase activation and subsequently apoptosis[2] **(Figure 1A)**. CLL cells have impaired apoptosis due to overexpression of pro-survival Bcl-2 resulting in part from deletion of microRNA15/16-1 on chromosome 13 [del(13q)]. However their reliance and addiction to BCL-2 overexpression leaves them susceptible to therapeutic targeting. A number of the pro-apoptotic BCL-2 family genes including Noxa are induced by p53, therefore del(17p)/TP53 mutations can impair basal and drug induced apoptosis which require functional p53 **(Figure 1B)**. However if we mimic these p53-induced BH3-only proteins we can potentially induce cellular apoptosis regardless of p53 functionality **(Figure 1C)**. Drugs like venetoclax (ABT-199) mimic BAD and Noxa and inhibit Bcl-2 function (IC_{50} <0.01nM(Ki)), with reduced or no inhibition of Bcl-X_L (IC_{50} 48nM(Ki)) and Mcl-1 (>444nM(Ki))[3] respectively, resulting in apoptosis. Venetoclax is approved for the treatment of CLL patients with del(17p)[4] and is currently in phase Ib/II clinical trials alone (NCT02265731) and in combination with bendamustine, duvelalisib, ibrutinib and antibody therapies (NCT02640833, NCT01685892, NCT01671904 and NCT02427451).

Here in conjunction with the phase 1 clinical trial (M12-175), Anderson et al[1] show venetoclax induces rapid apoptosis of CLL cells, in vitro, in paired peripheral blood and bone marrow samples in a caspase dependent manner, at similar concentrations. However, this might be expected, since the bone marrow derived tumor cells were evaluated following density gradient separation and therefore lacked support from signals within the tissue microenvironment. Importantly they showed venetoclax kills CLL cells in the presence or absence of del(17p) using primary human CLL cells, B cells isolated from mice lacking TP53 expression ($Trp53^{-/-}$) and in a human B cell line with clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated TP53 loss. Highlighting that BH3-mimetics like venetoclax can promote apoptosis in the absence of functional p53. Moreover, the authors showed that patients with del(17p)/*TP53* mutation did not differ in their initial apoptotic response to venetoclax in vivo, compared to patients with functional p53. This is seemingly in contrast with reports which indicated that venetoclax-induced progression-free survival (PFS) was poorer in patients with del(17p) compared to patients without del(17p)[5]. Indicating that over a longer treatment period del(17p) abnormalities may adversely impact venetoclax-mediated PFS, irrespective of the depth of initial response to treatment, but may simply reflect evolution of samples with a complex karyotype, the involvement of genes other than *TP53* in patients with del(17p) or signals within the

tumor microenvironment. In the present study sensitivity of tumor cells from CLL lymph nodes to venetoclax were not evaluated. However, it is likely that microenvironmental signals within the lymph node will determine how well a patient responds to treatment, because they can protect tumor cells from therapy induced killing. CLL cells within the lymph node microenvironment express greater levels of Mcl-1 and Bcl-X_L protein compared to peripheral blood.[6] Both of these proteins are induced in vitro following treatment with anti-IgM or interleukin-4 (IL-4)/CD154, mimicking signals within the lymph node microenvironment from (auto)antigen and T-cells respectively[7-10]. Expression of these proteins and particularly Mcl-1 confer resistance to venetoclax induced killing in vitro[7,8]. Indeed, Mcl-1 levels vary between CLL samples[9] therefore its expression before and after venetoclax treatment may identify those patients who are likely to progress and warrants further investigation as a predictive marker.

Anderson et al investigated in vitro sensitivity to venetoclax-induced apoptosis with the depth of clinical response in patients; however, there was no correlation. Whereas, BH3-profiling, as a measure of mitochondrial priming/depolarization by a BIM-peptide in vitro, did correlate with a reduction in the circulating lymphocyte count and bone marrow tumor burden by venetoclax. However, there was no correlation with deeper lymph node responses. This suggests that BH3-profiling may in part be beneficial in determining a patient's response to venetoclax and perhaps other BH3-mimetics. However, it will be important to determine if the depth of response observed with venetoclax in this study correlates with improved progression free survival and overall survival with longer follow-up, before we can understand the potential benefit of using BIM BH3-profiling to better predict patient outcome. Importantly performing BH3-profiling instead on CLL cells from the lymph node or following B-cell receptor (BCR) signaling, IL-4/CD154 treatment or with stromal support, may provide better insight for BH3-profiling as a predictive biomarker in determining a patient's sensitivity to venetoclax. Furthermore, these data support a strategy for simultaneous treatment with brutons tyrosine kinase (BTK) or Spleen tyrosine kinase (SYK) inhibitors, which inhibit ingress into and/or promote efflux out of the lymph nodes into the blood, and venetoclax.

Despite these unresolved queries, Anderson et al provides compelling new data and insight into the biology of venetoclax for the treatment of CLL. Since a proportion of patient's are already developing resistance to BCR-kinase inhibitors and that venetoclax as a single agent has been shown to induce minimum residual disease negativity in 5% of patients studied[5], venetoclax alone or in combination with BCR-kinase inhibitors may provide an important therapeutic option for this currently incurable disease.

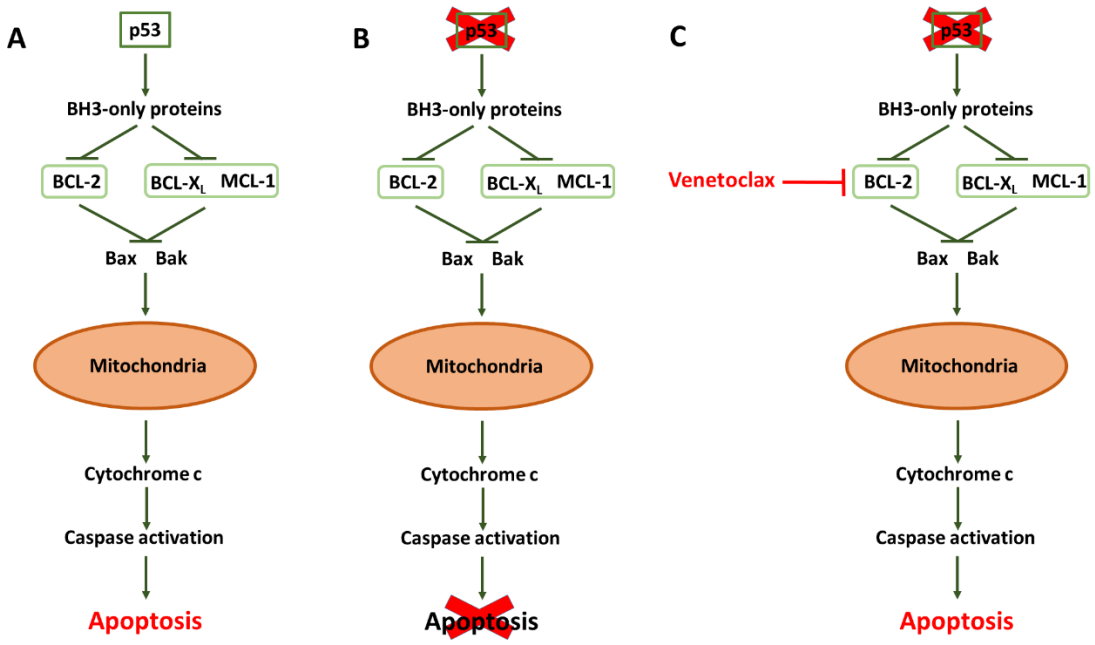

Figure 1. Venetoclax induces apoptosis of CLL cells independently of p53. A) In response to DNA damage the commonly mutated/deleted tumour suppressor p53 initiates apoptosis via the intrinsic apoptosis pathway. B) TP53 mutation and/or deletion results in defective apoptosis in response to DNA damage and contributes to chemotherapy resistance. C) Venetoclax inhibits BCL2 thereby inducing apoptosis of CLL cells in a mechanism which Anderson et al demonstrate to be independent of p53 expression/function in vitro and in vivo.

Conflict of interest disclosure: The authors declare no competing financial interests

1. Anderson MA, Deng J, Seymour JF, et al. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53 independent mechanism. *Blood*. 2016.
2. Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. The BCL-2 family reunion. *Mol Cell*. 2010;37(3):299-310.
3. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med*. 2013;19(2):202-208.
4. Venetoclax Approved for CLL. *Cancer Discov*. 2016.
5. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med*. 2016;374(4):311-322.
6. Smit LA, Hallaert DY, Spijker R, et al. Differential Noxa/Mcl-1 balance in peripheral versus lymph node chronic lymphocytic leukemia cells correlates with survival capacity. *Blood*. 2007;109(4):1660-1668.
7. Bojarczuk K, Sasi BK, Gobessi S, et al. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. *Blood*. 2016.
8. Aguilar-Hernandez MM, Blunt MD, Dobson R, et al. IL-4 enhances expression and function of surface IgM in CLL cells. *Blood*. 2016.

9. Larrayoz M, Blakemore SJ, Dobson RC, et al. The SF3B1 inhibitor spliceostatin A (SSA) elicits apoptosis in chronic lymphocytic leukaemia cells through downregulation of Mcl-1. *Leukemia*. 2016;30(2):351-360.
10. Thijssen R, Slinger E, Weller K, et al. Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors. *Haematologica*. 2015;100(8):e302-306.