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Toward a cure for chronic lymphocytic leukemia: an attack on multiple fronts

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"…a high risk of infectious complications, emergence of neoplastic clones resistant to chemoimmunotherapy, and lack of a curative strategy establish a need for novel therapies for chronic lymphocytic leukemia patients."

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the USA with approximately 100,000 patients living with the disease. It is estimated that 4580 patients will die from CLL and its complications in 2013. CLL is primarily a disease of the elderly, with two-third of patients diagnosed at an age of at least 65 years and accounts for a disease incidence of 22.3/100,000 in that age group [1]. Advances in purine analog-based chemotherapy and immunotherapy over the past two decades have led to improved survival of patients with CLL [2]. However, a high risk of infectious complications, emergence of neoplastic clones resistant to chemo-immunotherapy, and lack of a curative strategy establish a need for novel therapies for CLL patients.

Inefficient apoptosis is considered the dominant defect in CLL [3]. In peripheral circulating CLL cells, apoptosis is primarily repressed by expression of the anti-apoptotic protein BCL2. However, CLL cells also reside in a stromal niche in the bone marrow and lymph nodes where they express additional anti-apoptotic BCL2 family members, including BCLX, MCL1 and BFL1/BCL2A1 [4–6]. These pro-survival BCL2 family members bind the proapoptotic family members BAX and BAK, thereby preventing their oligomerization to pore formation through which cytochrome c is released to initiate the caspase cascade of apoptosis [7]. However, the major determinants of apoptosis are the BH3-only members of

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the BCL2 family. The proapoptotic BH3-only proteins BIM and BID are considered direct activators of BAX and BAK, hence their sequestration by anti-apoptotic BCL2 family members prevents apoptosis. Other BH3-only proteins such as NOXA, PUMA and BAD are considered sensitizers in that they compete for binding to the anti-apoptotic members, thereby releasing BIM or BID to activate BAX and BAK [8].

Upsetting the fine balance between the competing proteins of the BCL2 family is a promising tactic that may revolutionize therapy of CLL. Considering that various BCL2 family members are more prevalent in different physiological compartments, a different therapeutic approach may be required to kill these distinct cell populations. Early attempts to skew this balance toward proapoptotic signaling involved an antisense oligonucleotide oblimersen (Genasense, Genta Inc.) which targets BCL2 mRNA. Oblimersen had little efficacy as a single agent in CLL and despite successful performance in a combination trial with fludarabine and cyclophosphamide [9], it will probably cede its potential place in therapy of CLL to alternative strategies.

Recent efforts have seen the emergence of small molecule ‘BH3-mimetics’ intended to directly disrupt the interaction between proapoptotic and anti-apoptotic proteins [7]. Unfortunately, the majority of these putative BH3-mimetics fail to inhibit BCL2 proteins in cells [10]. ABT-737 and its clinical congener ABT-263/navitoclax bind to the anti-apoptotic proteins BCL2, BCLX and BCL-w with high affinity, but do not target MCL1 or BFL1 [11]. Owing to its inhibition of BCLX, navitoclax also induces thrombocytopenia in patients, which led to the synthesis of ABT-199 that selectively inhibits only BCL2 and avoids this undesirable toxicity [12,13].

Expression of anti-apoptotic proteins is also highly variable between patients. Consequently, CLL cells exhibit variable sensitivity to ABT-737 *in vitro* that correlates inversely with expression of MCL1 and BFL1 transcript levels [14]. Furthermore, while navitoclax is efficient at killing CLL cells circulating in the periphery, its ability to target cells resident in the lymph node microenvironment may be lower due to higher levels of MCL1 and BFL1 [14]. BCLX is also upregulated in the microenvironment, so the current focus on using ABT-199 to avoid thrombocytopenia appears counter-intuitive as it will fail to kill these $BCLX_L$-expressing CLL cells.

The above observations carry important implications. First, while the BH3-mimetics demonstrate single agent efficacy, their value will probably be greatest when used in drug combinations that together target a broader spectrum of BCL2 family proteins. Second, because CLL cells exist in different compartments, an improved response may require different therapeutic strategies targeting each compartment. Here, we propose a rationally designed approach using a multidrug regimen to dramatically improve the outcome for CLL patients.

Kill them in the open

Prior to the emergence of BCL2 inhibitors, other approaches had been used to reduce the peripheral CLL cells. For example, rituximab can reduce the peripheral CLL cell count, but responses are transient. Initially, the introduction of BCL2 inhibitors appeared very promising, but grade 4 tumor lysis syndrome (TLS) was observed in a patient receiving navitoclax as part of a Phase I study [13]. The switch to ABT-199 resulted in additional cases of TLS, thereby halting clinical trials. Moving forward, clinical trials will employ a stepwise dose escalation of ABT-199 to slowly reduce the peripheral cell count. A better strategy might be to initially administer an alternative drug such as rituximab to decrease the

peripheral tumor load (or ofatumumab in rituximab-refractory patients) and then to administer full dose ABT-199 which may reduce the potential for drug resistance.

Other drugs might also be effectively used to kill peripheral CLL cells. For example, dinaciclib targets CDK9, inhibits global transcription and induces rapid apoptosis in CLL cells [15]. Vinca alkaloids also induce apoptosis in CLL cells *ex vivo*, and this effect can be enhanced by combination with dinaciclib, perhaps due to inhibition of MCL1 transcription[16]. Hence many strategies, both established and novel, could be effectively used in combination with BCL2 inhibitors.

Get out of town

In the past decade, several B-cell receptor-targeting agents have shown significant efficacy in CLL patients. Of these, the Bruton tyrosine kinase inhibitor ibrutinib and the PI3-kinase delta inhibitor idelalisib have been most extensively studied in both preclinical and clinic settings [17]. Through disruption of cell homing, these inhibitors force CLL cells to leave the protective microenvironment [18]. Alternatively, egress of the CLL cells into the periphery may be promoted via disruption of the chemokine network, for example, by pharmacologically antagonizing the α-chemokine receptor CXCR4 with plerixafor. Once deprived of the microenvironmental stimuli, CLL cells should be more sensitive to rituximab or the BCL2-targeting agents discussed above. These approaches also reduce the size of the lymph nodes which will further reduce the potential for TLS on administration of ABT199 or navitoclax.

Coming to your house

The currently available BH3-mimetics do not target MCL1 or BFL1 accounting for resistance of CLL samples in the lymph node niche. Much effort is currently being expended in many laboratories to develop such BH3-mimetics. Meanwhile, two other approaches have been taken to overcome the drug resistance elicited within the lymph node microenvironment: decreasing the expression of anti-apoptotic proteins or increasing the expression of proapoptotic proteins. For experimental purposes, it is fortunate that the lymph node microenvironment can be at least partially recapitulated *ex vivo.* For example, coculture of peripheral blood CLL cells with CD40L-expressing fibroblasts results in upregulation of MCL1, BFL1 and/or BCLX and resistance to many commonly used drugs [4,19,20]. Upregulation of these BCL2 proteins can be mediated via activation of the NFκB pathway; hence, inhibition of NFκB may circumvent the resistance [5,21]. As discussed above, ibrutinib through its inhibition of B-cell receptor signaling also inhibits NFκB and may sensitize cells in the lymph node niche. Importantly, both inhibition of NFκB or siRNA-mediated suppression of MCL1 resensitized CLL cells to BH3-mimetics [19]. Finally, treatment with ABT-737 has also been observed to induce MCL1 via activation of extracellular signal-regulated kinase. Interference with extracellular signal-regulated kinase activation via pharmacological inhibition of MEK prevented upregulation of MCL1 and led to dramatic synergy between ABT-737 and MEK inhibitor CI-1040 in a mouse xenograft model of leukemia [22].

Many chemotherapeutic agents have been demonstrated to upregulate NOXA and have been gaining greater interest as a means to target CLL cells hiding in the microenvironment. For example, we have demonstrated that microtubule disrupting agents (Vinca alkaloids and combretastatin A) induce NOXA and sensitize CLL cells to ABT-737 in the stromal niche as well as in the periphery [16]. Alternately, the proteasome inhibitor bortezomib leads to accumulation of misfolded proteins that activate the unfolded protein response through upregulation of ATF4/ATF3 and thereby induce NOXA. We recently reported that treatment

of CLL cells with gossypol, a natural phenol derived from the cotton plant (*Gossypium*), completely reversed stroma-mediated resistance to ABT-737 *ex vivo* [20]. Gossypol was originally considered a BH3-mimetic, although we have found no evidence for this in cells [10]. However, by increasing intracellular calcium and the unfolded protein response, gossypol is a strong inducer of NOXA which we have implicated in the sensitization. The importance of this observation is that racemically pure gossypol (AT-101) is currently in clinical trials and thus could readily be investigated in combination with BCL2 antagonists.

Summary

Recent developments in molecular therapeutics have armed us with powerful tools to mount a multifaceted offensive against CLL, of which BCL2-targeting agents are a critical element. A strategy consisting of initial debulking of leukemia cells in the periphery (chemoimmunotherapy), fostering exit of neoplastic B cells from the protective environment (B-cell receptor-targeting agents) and killing cells in the protective niche (drug combinations) is poised to dramatically reduce residual disease, prolong progression-free survival, increase overall survival and ultimately improve the lives of millions of CLL patients. We believe it is time to start using all these drugs in rationally designed combination trials.

References

1. Zent CS, Kyasa MJ, Evans R, Schichman SA. Chronic lymphocytic leukemia incidence is substantially higher than estimated from tumor registry data. Cancer. 2001; 92(5):1325–1330. [PubMed: 11571749]
2. Brenner H, Gondos A, Pulte D. Trends in long-term survival of patients with chronic lymphocytic leukemia from the 1980s to the early 21st century. Blood. 2008; 111(10):4916–4921. [PubMed: 18309034]
3. Danilov AV, Danilova OV, Klein AK, Huber BT. Molecular pathogenesis of chronic lymphocytic leukemia. Curr Mol Med. 2006; 6(6):665–675. [PubMed: 17022736]
4•. Vogler M, Butterworth M, Majid A, et al. Concurrent up-regulation of BCL-XL and BCL2A1 induces approximately 1000-fold resistance to ABT-737 in chronic lymphocytic leukemia. Blood. 2009; 113(18):4403–4413. Describes lymph node-mimicking coculture system. Implicates upregulation of BCLX and BFL1/BCL2A1 in resistance to ABT-737. [PubMed: 19008458]
5•. Tromp JM, Tonino SH, Elias JA, et al. Dichotomy in NF-kappaB signaling and chemoresistance in immunoglobulin variable heavy-chain-mutated versus unmutated CLL cells upon CD40/TLR9 triggering. Oncogene. 2010; 29(36):5071–5082. Describes a dichotomy in NF.B signaling under CD40/TLR9 signaling in CLL. [PubMed: 20581863]
6. Cheng EH, Wei MC, Weiler S, et al. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell. 2001; 8(3):705–711. [PubMed: 11583631]
7. Billard C. Design of novel BH3 mimetics for the treatment of chronic lymphocytic leukemia. Leukemia. 2012; 26(9):2032–2038. [PubMed: 22453662]
8. Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell. 2002; 2(3):183–192. [PubMed: 12242151]
9. O'Brien S, Moore JO, Boyd TE, et al. 5-year survival in patients with relapsed or refractory chronic lymphocytic leukemia in a randomized, Phase III trial of fludarabine plus cyclophosphamide with or without oblimersen. J Clin Oncol. 2009; 27(31):5208–5212. [PubMed: 19738118]
10. Albershardt TC, Salerni BL, Soderquist RS, et al. Multiple BH3 mimetics antagonize antiapoptotic MCL1 protein by inducing the endoplasmic reticulum stress response and up-regulating BH3-only protein NOXA. J Biol Chem. 2011; 286(28):24882–24895. [PubMed: 21628457]

11. Konopleva M, Contractor R, Tsao T, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell. 2006; 10(5):375–388. [PubMed: 17097560]

12••. 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. Develpoment of a novel BH3-mimetic that selectively targets BCL2 but not BCLX, thereby avoiding thrombocyopenia. [PubMed: 23291630]

13••. Roberts AW, Seymour JF, Brown JR, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol. 2012; 30(5):488–496. Provides evidence that navitoclax has significant therapeutic activity against CLL, but induces thrombocytopenia due to the reliance of platelets on BCLX. [PubMed: 22184378]

14. Al-Harbi S, Hill BT, Mazumder S, et al. An antiapoptotic BCL-2 family expression index predicts the response of chronic lymphocytic leukemia to ABT-737. Blood. 2011; 118(13):3579–3590. [PubMed: 21772052]

15. Bates DJ, Salerni BL, Lowrey CH, Eastman A. Vinblastine sensitizes leukemia cells to cyclin-dependent kinase inhibitors, inducing acute cell cycle phase-independent apoptosis. Cancer Biol Therapy. 2011; 12(4):314–325.

16. Bates DJ, Danilov AV, Lowrey CH, Eastman A. Vinblastine rapidly induces Noxa and acutely sensitizes primary chronic lymphocytic leukemia cells to ABT-737. Mol Cancer Therap. 2013; 12(8):1504–1514. [PubMed: 23723123]

17. Woyach JA, Johnson AJ, Byrd JC. The B-cell receptor signaling pathway as a therapeutic target in CLL. Blood. 2012; 120(6):1175–1184. [PubMed: 22715122]

18••. Hoellenriegel J, Meadows SA, Sivina M, et al. The phosphoinositide 3'-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood. 2011; 118(13):3603–3612. Demonstrates the ability of the PI3-kinase inhibitor CAL-101 to drive CLL cells out of the protective lymph node niche. [PubMed: 21803855]

19. Tromp JM, Geest CR, Breij EC, et al. Tipping the Noxa/Mcl-1 balance overcomes ABT-737 resistance in chronic lymphocytic leukemia. Clin Cancer Res. 2012; 18(2):487–498. [PubMed: 22128299]

20••. Soderquist R, Bates DJ, Danilov AV, Eastman A. Gossypol overcomes stroma-mediated resistance to the BCL2 inhibitor ABT-737 in chronic lymphocytic leukemia cells *ex vivo*. Leukemia. 2013 (In Press) Shows that gossypol induces NOXA and completely reverses stroma-mediated resistance to ABT-737 in CLL.

21. Herishanu Y, Perez-Galan P, Liu D, et al. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood. 2011; 117(2):563–574. [PubMed: 20940416]

22. Konopleva M, Milella M, Ruvolo P, et al. MEK inhibition enhances ABT-737-induced leukemia cell apoptosis via prevention of ERK-activated MCL-1 induction and modulation of MCL-1/BIM complex. Leukemia. 2012; 26(4):778–787. [PubMed: 22064351]