Apoptosis as a Therapeutic Target in Chronic Lymphocytic Leukemia

Christian Billard[1,2]

[1]INSERM Unité 965, Hôpital Lariboisière, Paris, France. [2]Université Paris Diderot-Paris 7, Paris, France.

ABSTRACT: Despite significant advances in chemoimmunotherapy, chronic lymphocytic leukemia (CLL) is still incurable. This has prompted the development of new drugs and therapeutic strategies that include reactivating the impaired apoptosis (programed cell death). Several approaches to target apoptosis-regulating proteins have attracted attention. Among them, the approach to inhibit the activity of prosurvival members of the BCL-2 family with small-molecule BH3 mimetics (navitoclax, ABT-199) has proved to be most promising in clinical trials with CLL patients. Recently, the first BH3 mimetics targeting selectively the particular prosurvival protein MCL-1 (whose overexpression is involved in therapeutic resistance) have been identified. Furthermore, small molecules capable of directly activating proapoptotic proteins of the BCL-2 family have been characterized, indicating that novel BH3-mimetic drugs displaying this property may be designed. These discoveries are opening a new era in the development of BH3 mimetics for improving CLL therapy.

KEYWORDS: chronic lymphocytic leukemia, apoptosis-targeted therapy, BCL-2 family, BH3 mimetics

CITATION: Billard. Apoptosis as a Therapeutic Target in Chronic Lymphocytic Leukemia. *Lymphoma and Chronic Lymphocytic Leukemias* 2015:5 11–15 doi:10.4137/LCLL.S13718.

TYPE: Perspective

RECEIVED: May 12, 2015. **RESUBMITTED:** June 30, 2015. **ACCEPTED FOR PUBLICATION:** July 2, 2015.

ACADEMIC EDITOR: Mitchell Smith, Editor in Chief

PEER REVIEW: Three peer reviewers contributed to the peer review report. Reviewers' reports totaled 624 words, excluding any confidential comments to the academic editor.

FUNDING: Author discloses no funding sources.

COMPETING INTERESTS: Author discloses no potential conflicts of interest.

CORRESPONDENCE: christian.billard@inserm.fr

Paper subject to independent expert blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE). Provenance: the author was invited to submit this paper.

Published by Libertas Academica. Learn more about this journal.

Introduction

The development of chemoimmunotherapy protocols combining purine analogs, alkylating agents, and monoclonal antibodies has led to clear improvements in treating patients with chronic lymphocytic leukemia (CLL). The FCR (fludarabine, cyclophosphamide, and rituximab) regimen or allied agents has enabled significant increases in the rate and duration of complete remissions and a prolongation of overall survival.[1] In spite of these advances, CLL remains incurable without allogenic transplantation. Novel therapeutic strategies are therefore needed to improve the treatments of patients. New monoclonal antibodies (eg, ofatumumab, obinutuzumab) and chemotherapeutics such as bendamustine are currently in clinical evaluation.[2,3] Furthermore, several signals involved in the activation of the B-cell receptor and other signaling pathways such as the phosphatidylinositol-3 kinase (PI3K) are constitutively activated in CLL. Strategies to inhibit these signals are being developed especially with ibrutinib (an inhibitor of the Bruton's tyrosine kinase) and idelalisib (an inhibitor of the PI3K isoform). Both agents have proved promising in clinical trials in CLL and have been approved by the U.S. Food and Drug Administration.[2–4]

Another attractive strategy is to target proteins that control apoptosis (programed cell death). Actually, CLL is thought to result from an imbalance between proliferation and apoptosis. Moreover, many prosurvival factors are overexpressed in CLL cells such as proteins of the BCL-2 and IAP families.[5] The BCL-2 family proteins regulate the mitochondrial outer membrane permeabilization (MOMP) responsible for the activation of caspases (proteolytic enzymes that are the executioners of the apoptotic program). Proteins of the IAP family (inhibitor of apoptosis protein) are caspase antagonists. Attempts to directly inhibit the expression of IAP proteins and prosurvival members of the BCL-2 family with specific antisense oligonucleotides did not provide convincing results in clinical trials.[6] The indirect and non-specific transcriptional inhibition of both families of proteins with cyclin-dependent inhibitors appeared somewhat disappointing.[6] No therapeutic benefit was recorded with the approach of inhibiting IAP activities using agents designed to mimic the endogenous IAP antagonist SMAC (second mitochondria-derived activator of caspases).[6] By contrast, significant therapeutic effects were observed with BH3 mimetics, which can antagonize the activity of prosurvival BCL-2 family proteins.[6] The aim of the present article is to highlight the great potential of these agents and the latest developments in BH3-mimetic research, which undoubtedly constitute a new avenue in improving CLL therapy.

BCL-2 Family Proteins

Proteins of the BCL-2 family[7] are characterized by their domains of homology to BCL-2 (BH1–BH4) and their functional activities. This family includes prosurvival members (BCL-2, BCL-XL, BCL-W, MCL-1, A1) and proapoptotic members (Fig. 1). The latter comprise the MOMP effectors

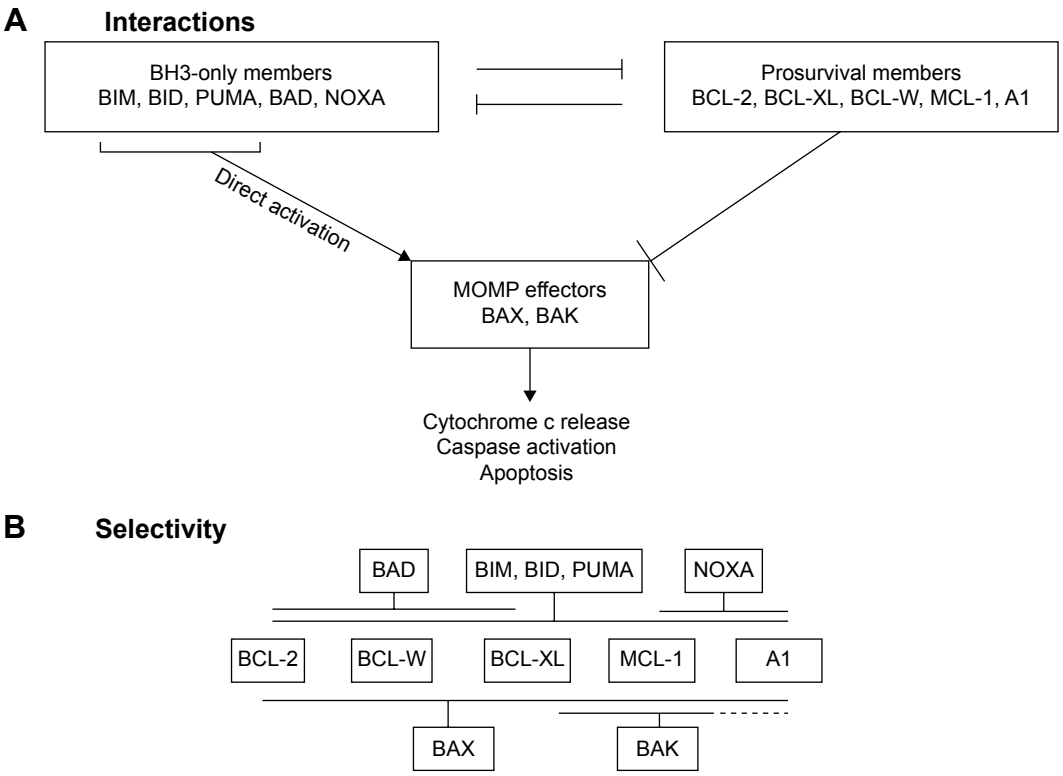

Figure 1. Schematic representation of the interactions between the three types of BCL-2 family proteins regulating the MOMP and thus apoptosis induction. **A**: the prosurvival members of the family sequester and inactivate the proapoptotic members (MOMP effectors and BH3-only proteins). Apoptotic signals increase the expression of BH3-only proteins that antagonize their prosurvival partners, leading to the release and activation of the MOMP effectors BAX and BAK. The latter can also be directly activated by some BH3-only proteins. **B**: these interactions are selective—the BH3-only protein BAD is able to bind only to BCL-2, BCL-XL, and BCL-W and NOXA can bind only to MCL-1 and A1, whereas BIM, BID, and PUMA can bind to all prosurvival proteins. The latter can interact with BAX, whereas only BCL-XL, MCL-1, and possibly A1 can interact with BAK.

BAX and BAK and the BH3-only members (so-called because they have only the BH3 domain) such as BIM, PUMA, BID, BAD, and NOXA. The activity of the prosurvival members is to bind and sequester their proapoptotic counterparts, in particular BAX and BAK, which are thus inactive. All prosurvivals can bind to BAX, whereas only BCL-XL and MCL-1 can bind to BAK. The activity of BH3-only members is to antagonize the prosurvival proteins: by inserting their BH3 domain into the binding groove of prosurvival proteins, they inhibit the activity of the latter, which results in the release and conformational activation of BAX and BAK. Whereas BIM, PUMA, and BID can antagonize all prosurvival proteins, BAD inhibits only BCL-2, BCL-XL, and BCL-W and NOXA inhibits only MCL-1 and A1. Some BH3-only proteins (BIM, BID, PUMA) can directly activate BAX and BAK. Therefore, the specific interactions between the BCL-2 family proteins govern the balance between cell death and survival.[7]

BH3 Mimetics

This biological context has spawned the concept of the BH3 mimetic: a small molecule capable of mimicking the BH3 domain of BH3-only proteins should be able to bind to prosurvival proteins with high affinity and inhibit their activity, leading to BAX/BAK activation and thus to caspase activation and apoptosis.[7] The BH3-mimetic concept has prompted the design of numerous small BH3 peptides or organic molecules.[8,9] However, many of the generated compounds turned out to act through off-target mechanisms. Only a few of them were found to behave as authentic BH3 mimetics with the ability to bind prosurvival proteins with high affinity and to induce BAX/BAK-dependent apoptosis in malignant cells.[8,9]

The prototype ABT-737 and its orally bioavailable derivative navitoclax (formerly, ABT-263) bind and antagonize BCL-2, BCL-XL, and BCL-W (so they are referred to as BAD-like BH3 mimetics).[10,11] Navitoclax has shown significant therapeutic activity in cancer patients but with a dose-limiting thrombocytopenia because of inhibition of BCL-XL (that is a prosurvival factor for platelets).[12] To avoid this toxic side-effect, the ABT-199 derivative, which is specific for BCL-2 and does not bind to BCL-XL, was then designed.[13] The first clinical trials with ABT-199 have yielded impressive results without thrombocytopenia.[13,14]

Preclinical studies have recently enabled to characterize other bona fide BH3 mimetics such as BM-1197 (a BAD-like inhibitor as navitoclax), A-1155463 (specific for BCL-XL), and BIM SAHB (a stapled peptide derived from the BH3 domain of BIM and targeting a broad range of prosurvival

BCL-2 proteins).[15–17] Several MCL-1-selective inhibitors were identified (eg, MIM-1, small-molecule Mcl-1 inhibitor, TW-37), but none of them fully meet the main criteria that define an authentic BH3 mimetic.[18–22]

BCL-2 Family Proteins in CLL

Most prosurvival members of the BCL-2 family are overexpressed in CLL cells.[23,24] The upregulated BCL-2 protein and the expression ratio relative to its proapoptotic ligand BAX are undoubtedly involved in the survival advantage of the leukemic cells. The BCL-2 overexpression results from losses of micro-interfering RNAs miR-15a and miR-16 in some cases.[25] The unique prosurvival protein MCL-1, which is known to play a crucial role in malignant development and resistance to chemotherapy in many cancers,[26] is also upregulated in CLL cells. High MCL-1 levels were inversely correlated with in vitro and in vivo responses to fludarabine.[27] They were found to be correlated with prognostic markers (disease stage, mutation status, ZAP-70 positivity, and CD38 expression) and to be predictive of patients' clinical outcome.[28,29] High MCL-1 levels have also been observed in CLL lymph node cells, which are thought to be involved in patients' drug resistance and relapse.[30] In addition, MCL-1 silencing has been shown to result in apoptosis induction in CLL cells in vitro.[31] Lastly, convergent data have highlighted that the MCL-1/NOXA axis represents an attractive target for CLL therapy.[32]

BH3 Mimetics in CLL Therapy

Navitoclax. After the first published phase I study of navitoclax in non-Hodgkin's lymphoma, which had shown 22% partial responses (PRs),[33] Roberts et al reported encouraging data of a phase I trial in patients with relapsed or refractory CLL: 35% of PRs were recorded, albeit without complete responses (CRs) and with 18% of grade 4 thrombocytopenia.[34]

ABT-199. An initial observation had indicated a reduction in tumor burden in the first three CLL patients recruited into a clinical trial of ABT-199.[13] These promising data were followed by a preliminary report of a phase I study of ABT-199 in high-risk relapsed/refractory CLL showing 23% of CR and 84% of overall responses.[14] This has been further confirmed by the latest results[35]: 22% of the 84 patients recruited for two years have shown CR with no evidence of cancer in five of nine evaluated patients, 57% have achieved PR; and the objective response rate was 79% with a median duration of response of 20.5 months. Overall, 59% of the patients have survived for two years without leukemia progression. Interestingly, the CR rate was similar in high-risk patients, including those having p53 deficiency because of the deletion in chromosome 17.[35] Although the occurrence of tumor lysis syndrome has slowed clinical development, phase III trials combining ABT-199 with chemoimmunotherapy are now ongoing.[36]

Discovery of Novel BH3 Mimetics

The latest developments in BH3-mimetic research have revealed several novel types of small molecules targeting BCL-2 family proteins that are of potential interest for CLL therapy. These compounds are described below (recapitulated in Table 1, which includes the comparison with previous authentic and putative BH3 mimetics).

First MCL-1-selective BH3 mimetics. Because the survival of malignant cells depends at least partly on MCL-1 in many cancers, including CLL, efforts focused on the identification of small molecules targeting selectively MCL-1. Despite the difficulties of this challenge, a series of MCL-1 inhibitors derived from indole-2-carboxilic acid has been obtained by high-throughput screening and structure-guided design.[37] The compounds bind to MCL-1 with excellent high affinity (0.45 nM) and selectivity over other prosurvival BCL-2 family proteins. A mechanistic study has shown that the lead compound A-1210477 and related analogs can disrupt the interactions of MCL-1 with BIM and NOXA, penetrate living cells, and act via an on-target mechanism.[38]

Table 1. Small molecules targeting BCL-2 family proteins of potential interest for CLL therapy and comparison with navitoclax and ABT-199.

SMALL MOLECULE	ACTIVITY	BH3 MIMICRY	CLINICAL DATA IN CLL
Navitoclax[11]	BCL-2, BCL-XL and BCL-W inhibitor	Authentic	35% PR[34]
ABT-199[13]	BCL-2 inhibitor	Authentic	22% CR and 57% PR[35]
MIM-1[18]	MCL-1 inhibitor	Putative	
Small molecule Mcl-1 inhibitor[19]	MCL-1 inhibitor	Putative	
TW-37[20,21]	MCL-1 inhibitor	Putative	
A-1210477[37,38]	MCL-1 inhibitor	Authentic	
BIM SAHB[17]	Pan-prosurvival protein inhibitor	Authentic*	
BAM-7[41]	BAX activator	Putative	
PUMA-BH3 peptide[42,43]	BAK activator	Putative	
PUMA SAHB analog[44]	BAX activator and BCL-2/MCL-1 inhibitor	Putative	

Note: *Subject of controversy.[46–48]
Abbreviations: PR, partial responses; CR, complete responses.

They induce the main hallmarks of the caspase-dependent mitochondrial apoptosis (including BAX/BAK activation) in multiple myeloma and non-small cell lung cancer cell lines that have been validated to be MCL-1 dependent. These compounds are therefore the first BH3 mimetics targeting selectively MCL-1. Lastly, the fact that A-1210477 synergizes with navitoclax to trigger apoptosis is of interest given that MCL-1 is a key factor in the resistance of malignant cells to ABT-737 and navitoclax.[39]

Small-molecule direct activators of BAX and BAK. The BH3-mimetic concept has recently been used to generate small molecules that can directly activate the MOMP effectors BAX and BAK. The first example was provided by Walensky and colleagues who had characterized a stapled peptide (BIM SAHB) derived from the BIM BH3 domain that is capable of binding BAX at a site that directly triggers its activation.[40] By computational screening of small molecules displacing BIM SAHB from BAX, they have further identified the substituted pirazolone molecule BAM-7, which can engage BAX and trigger its activation and subsequent apoptosis.[41] Although its binding affinity has not been determined, BAM-7 might be used to design BH3 mimetics capable of directly activating BAX. Moreover, a short peptide modeled on the PUMA BH3 domain has been reported to bind BAK with high affinity and to induce its activation and apoptosis as well.[42,43]

The identification of these two small molecules therefore indicates that it is possible to obtain BH3 mimetics having the ability to directly activate proapoptotic proteins of the BCL-2 family.

Dual proapoptotic activator and prosurvival inhibitor. The BH3-mimetic strategy has lastly allowed the discovery of a small molecule with particular properties.[44] A stapled peptide derived from the PUMA BH3 region (called PUMA SAHB) has been found to not only bind and inhibit BCL-2 and MCL-1 but also directly bind and activate BAX. A cell permeable analog of PUMA SAHB (and exhibiting the same properties) has been shown to elicit mitochondrial apoptosis in neuroblastoma cells.[44] These data suggest for the first time the possibility to design a BH3 mimetic possessing the dual property of proapoptotic activator and prosurvival inhibitor.

Conclusions and Perspectives

Targeted therapy is one of the current modalities to improve cancer treatments. Targeting apoptosis appeared as a new paradigm to assist chemoimmunotherapy particularly in CLL, a disease characterized by apoptosis deficiency. Unlike some disappointing apoptosis-based approaches, the strategy to inhibit the activity of prosurvival BCL-2 family proteins with selective BH3 mimetics is hopeful. These small molecules display the advantage to be highly specific for their target, and the clinical results observed with ABT-199 as a single agent in CLL are impressive. However, this drug is specific for BCL-2 and does not antagonize MCL-1. The latter is a unique, crucial prosurvival protein, and its overexpression is frequently involved in therapeutic resistance. It is also a cause of resistance of CLL cells to navitoclax and ABT-199 in vitro. The recent discovery of A-1210477 and its related analogs, which are the first authentic BH3 mimetics targeting selectively MCL-1, are therefore a great step forward in both BH3-mimetic research and treating CLL patients. Such MCL-1 inhibitors could induce less toxic side-effects in patients than the complete loss of MCL-1 by drugs that inhibit protein expression (such as CDK inhibitors).[45] Also of considerable interest is the identification of several small molecules derived from BH3 peptides, which are capable of directly activating the proapoptotic proteins BAX and BAK. The latter are indeed the effectors of the MOMP that is responsible for caspase activation. The discovery of these direct activators is not surprising because it is known that some BH3-only proteins can directly activate BAX and BAK (without needing the inhibition of their prosurvival ligands).[7] The clinical use of such direct activators should display reduced toxicities as compared to many inhibitors. A striking discovery is the characterization of the BH3 peptide analog that can both activate BAX and inhibit BCL-2 and MCL-1. Together, these novel types of small molecules provide proof of concept that BH3 mimetics, having the property to activate proapoptotic BCL-2 proteins or the dual property of proapoptotic activator and prosurvival inhibitor, may be designed.

Much efforts are still necessary in order to implement the clinical use of MCL-1-specific and direct-activator BH3 mimetics. Nevertheless, a new era is coming up with the latest developments in the field of BH3-mimetic research that should benefit to CLL therapy.

Author Contributions

Conceived and designed the concepts: CB. Analyzed the data: CB. Wrote the first draft of the manuscript: CB. Contributed to the writing of the manuscript: CB. Agree with manuscript results and conclusions: CB. Jointly developed the structure and arguments for the paper: CB. Made critical revisions and approved final version: CB. Author reviewed and approved of the final manuscript.

REFERENCES

1. Hallek M. Chronic lymphocytic leukemia: 2015 update on diagnosis, risk stratification, and treatment. *Am J Hematol*. 2015;90:447–460.
2. Montillo M, Frustaci AM, Picardi P, Tedeschi A. A new era is coming up in the treatment of chronic lymphocytic leukemia. *Lymphoma Chronic Lymphocytic Leuk*. 2014;4:9–19.
3. Byrd JC, Jones JJ, Woyach JA, Johnson AJ, Flynn JM. Entering the era of targeted therapy for chronic lymphocytic leukemia: impact on the practicing clinician. *J Clin Oncol*. 2014;32:3039–3047.
4. Sanford DS, Wierda WG, Burger JA, Keating MJ, O'Brien SM. Three newly approved drugs for chronic lymphocytic leukemia: incorporating ibrutinib, idelalisib, and obinutuzumab into clinical practice. *Clin Lymphoma Myeloma Leuk*. 2015;15:385–391.
5. Billard C. Apoptosis inducers in chronic lymphocytic leukemia. *Oncotarget*. 2014; 5:309–325.
6. Besbes S, Mirshahi M, Pocard M, Billard C. Strategies targeting apoptosis proteins to improve therapy of chronic lymphocytic leukemia. *Blood Rev*. 2015 Mar 31 [Epub ahead of print].

7. Czabotar PE, Lessene G, Strasser A, Adam JM. Control of apoptosis by the BCL-2protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol*. 2014;15:49–63.
8. Billard C. BH3 mimetics: status of the field and new developments. *Mol Cancer Ther*. 2013;12:1691–1700.
9. Roy MJ, Vom A, Czabotar PE, Lessene G. Cell death and the mitochondria: therapeutic targeting of the BCL-2 family-driven pathway. *Br J Pharmacol*. 2014; 171:1973–1987.
10. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumors. *Nature*. 2005;435:677–681.
11. Tse C, Shoemaker AR, Adickes J, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res*. 2008;68:3421–3428.
12. Anderson MA, Huang D, Roberts A. Targeting BCL2 for the treatment of lymphoid malignancies. *Sem Hematol*. 2014;51:219–227.
13. 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;19:202–208.
14. Seymour JF, Davids MS, Pagel JM, et al. Bcl-2 inhibitor ABT-199 (GDC-0199) monotherapy shows anti-tumor activity including complete remissions in high-risk relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). *Blood*. 2013;122:293–294. [Abstract 872].
15. Bai L, Chen J, McEachern D, et al. BM-1197: a novel and specific Bcl-2/Bcl-xL inhibitor inducing complete and long-lasting tumor regression *in vivo*. *PLoS One*. 2014;9:e99404.
16. Tao ZF, Hasvold L, Wang L, et al. Discovery of a potent and selective BCL-XL inhibitor with in vivo activity. *ACS Med Chem Lett*. 2014;5:1088–1093.
17. LaBelle JL, Katz SG, Bird GH, et al. A stapled BIM peptide overcomes apoptotic resistance in hematologic cancers. *J Clin Invest*. 2012;122:2018–2031.
18. Cohen NA, Stewart ML, Gavathiotis E, et al. A competitive stapled peptide screen identifies a selective small molecule that overcomes Mcl-1-dependent leukemia cell survival. *Chem Biol*. 2012;19:1175–1186.
19. Friberg A, Vigil D, Zhao B, et al. Discovery of potent myeloid cell leukemia 1 (Mcl-1) inhibitors using fragment-based methods and structure-based design. *J Med Chem*. 2012;56:15–30.
20. Mohammad RM, Goustin AS, Aboukameel A, et al. Preclinical studies of TW-37, a new nonpeptidic small-molecule inhibitor of Bcl-2, in diffuse large cell lymphoma xenograft model reveal drug action on both Bcl-2 and Mcl-1. *Clin Cancer Res*. 2007;13:2226–2235.
21. Varadarajan S, Vogler M, Butterworth M, Dinsdale D, Walensky LD, Cohen GM. Evaluation and critical assessment of putative MCL-1 inhibitors. *Cell Death Diff*. 2013;11:1475–1484.
22. Belmar J, Fesik SW. Small molecule Mcl-1 inhibitors for the treatment of cancer. *Pharmacol Ther*. 2015;145:76–84.
23. Packham G, Stevenson FK. Bodyguards and assassins: Bcl-2 family proteins and apoptosis control in chronic lymphocytic leukemia. *Immunology*. 2005;114: 441–449.
24. Chen L, Balakrishnan K, Gandhi V. Inflammation and survival pathways: chronic lymphocytic leukemia as a model system. *Biochem Phamacol*. 2010;80: 1936–1945.
25. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL-2. *Proc Natl Acad Sci U S A*. 2005;27:13944–13949.
26. Perciavalle RM, Opferman JT. Delving deeper: MCL-1's contributions to normal and cancer biology. *Trends Cell Biol*. 2013;23:22–29.
27. Kitada S, Andersen J, Akar S, et al. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with in vitro and in vivo chemoresponses. *Blood*. 1998;91:3379–3389.
28. Pepper C, Lin TT, Pratt G, et al. Mcl-1 expression has in vitro and in vivo significance in chronic lymphocytic leukemia and is associated with other poor prognostic markers. *Blood*. 2008;112:3807–3817.
29. Awan FT, Kay NE, Davis ME, et al. Mcl-1 expression predicts progression-free survival in chronic lymphocytic leukemia patients treated with pentostatin, cyclophosphamide, and rituximab. *Blood*. 2009;133:535–537.
30. 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;109:1660–1667.
31. Hussain SR, Cheney CM, Johnson AJ, et al. Mcl-1 is a relevant therapeutic target in acute and chronic lymphoid malignancies: downregulation enhances rituximab-mediated apoptosis and complement-dependent cytotoxicity. *Clin Cancer Res*. 2007;13:144–150.
32. 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*. 2011; 18:487–498.
33. Wilson WH, O'Connor OA, Czuczman MS, et al. Navitoclax, a targeted high-affinity inhibitor of Bcl-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumor activity. *Lancet Oncol*. 2010;11:1149–1159.
34. 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;30:488–496.
35. Seymour JF, Davids MS, Pagel JM, et al. ABT-199 (GDC-0199) in relapsed/refractory chronic lymphocytic leukemia and small lymphocytic lymphoma: high complete-response rate and durable disease control. *J Clin Oncol*. 2014;32:5s. [Abstract 7015].
36. Ng SY, Davids MS. Selective Bcl-2 inhibition to treat chronic lymphocytic leukemia and non-Hodgkin lymphoma. *Clin Adv Hematol Oncol*. 2014;12:224–229.
37. Bruncko M, Wang L, Sheppard GS, et al. Structure-guided design of a series of MCL-1 inhibitors with high affinity and selectivity. *J Med Chem*. 2015;58: 2180–2194.
38. Leverson JD, Zhang H, Chen J, et al. Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax). *Cell Death Dis*. 2015;6:e1590.
39. van Delft MF, Wei AH, Mason KD, et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. *Cancer Cell*. 2006;10:389–399.
40. Gavathiotis E, Suzuki M, Davis ML, et al. BAX activation is initiated at a novel interaction site. *Nature*. 2008;455:1076–1082.
41. Gavathiotis E, Reyna DE, Bellairs JA, Leshchiner ES, Walensky LD. Direct and selective small-molecule activation of proapoptotic BAX. *Nat Chem Biol*. 2012;8:639–645.
42. Dai H, Smith A, Meng W, Schneider PA, Pang YP, Kaufmann SH. Transient binding of an activator BH3 domain to the Bak BH3-binding groove initiates Bak oligomerization. *J Cell Biol*. 2011;194:39–48.
43. Dai H, Pang YP, Ramirez-Alvarado M, Kaufmann SH. Evaluation of the BH3-only protein Puma as a direct Bak activator. *J Biol Chem*. 2014;289:89–99.
44. Edwards AL, Gavathiotis E, LaBelle JL, et al. Multimodal interaction with BCL-2 family proteins underlies the proapoptotic activity of PUMA BH3. *Chem Biol*. 2013;20:888–902.
45. Blachy JS, Byrd JC. Emerging drug profile: cyclin-dependent kinase inhibitors. *Leuk Lymphoma*. 2013;54:2133–2143.
46. Okamoto T, Zobel K, Fedorova A, et al. Stabilizing the pro-apoptotic BimBH3 helix (BimSAHB) does not necessarily enhance affinity or biological activity. *ACS Chem Biol*. 2013;8:297–302.
47. Bird GH, Gavathiotis E, LaBelle JL, Katz SG, Walensky LD. Distinct BimBH3 (BimSAHB) stapled peptides for structural and cellular studies. *ACS Chem Biol*. 2014;9:831–837.
48. Okamoto T, Segal D, Zobel K, et al. Further insights into the effects of pre-organizing the BimBH3 helix. *ACS Chem Biol*. 2014;9:838–839.