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Molecular Cancer Research

Perspective

Development of Noxa-like BH3 Mimetics for Apoptosis-Based Therapeutic Strategy in Chronic Lymphocytic Leukemia

Christian Billard

Abstract

Despite real advances made in chemoimmunotherapy, chronic lymphocytic leukemia (CLL) is still an incurable disease. New therapeutic strategies based on the restoration of the cell death program seemed relevant. Some members of the Bcl-2 family are critical players in the defective apoptotic program in CLL cells and/or targets of apoptosis inducers *in vitro*. The concept of BH3 mimetics has led to the characterization of small molecules mimicking proapoptotic BH3-only members of the Bcl-2 family by their ability to bind and antagonize the prosurvival members. Some putative or actual BH3 mimetics are already being tested in clinical trials with somewhat promising results. However, none of them has a high enough interaction affinity with Mcl-1, a crucial antiapoptotic factor in CLL. It has been suggested that resistance to BH3 mimetics can be overcome by using inhibitors of Mcl-1 expression. An alternative and more direct strategy is to design mimetics of the Noxa BH3 domain, which is a specific antagonistic Mcl-1 ligand. The development of such Noxa-like BH3 mimetics, capable of directly interacting with Mcl-1 and efficiently neutralizing its antiapoptotic activity, is extremely important to evaluate their impact on the clinical outcome of patients with CLL. *Mol Cancer Res; 10(6); 673–6. ©2012 AACR.*

Chronic lymphocytic leukemia (CLL) is characterized by clonal expansions of CD5-positive B lymphocytes accumulating in the blood. This accumulation of leukemic cells, which are mostly quiescent, results mainly from the inability to develop their apoptotic program (1) and an excess of survival signals delivered by the tumoral microenvironment (2). Despite recent therapeutic advances with the combination of purine analogs, alkylating agents, and monoclonal antibodies [fludarabine, cyclophosphamide, rituximab, (FCR), or allied agents; ref. 3], nearly all patients relapse, and CLL remains an incurable disease (2, 3). The need to develop new strategies on the basis of apoptosis reactivation has become evident to improve treatment options for patients with CLL (1, 2).

During the last decade, convergent data contributed to highlight the pivotal role of Bcl-2 family proteins in CLL (4). Some prosurvival members of this family are overexpressed by CLL cells, and, moreover, both prosurvival and proapoptotic members are targets of various inducers of the intrinsic apoptosis pathway in CLL cells *ex vivo*.

Among the prosurvival Bcl-2 proteins, Mcl-1 now seems to be a crucial player in defective programmed cell death in CLL (5). The excess of survival signals provided by the bone marrow and/or lymph node microenvironment results in activation of several signaling pathways (such as c-Myc, NF-κB, or phosphoinositide 3-kinase/Akt), leading to the induction of *MCL-1* gene transcription and enhanced expression of Mcl-1 (5). Initially, high expression levels of Mcl-1 were inversely correlated with the susceptibility of CLL cells to apoptosis *in vitro* and with the responsiveness of patients to chemotherapy. More recently, it was shown that Mcl-1 levels are also correlated with prognostic markers and are predictive of patients' clinical outcome (6, 7). In addition, high Mcl-1 levels have been observed in CLL cells originating from the lymph nodes (8), which are thought to play an important role in drug resistance and relapse. Lastly, inhibition of Mcl-1 protein expression is sufficient to trigger apoptosis (9). The strategy targeting inhibition of Mcl-1 expression has been intensely investigated, and a number of apoptosis inducers were found to act through Mcl-1 downregulation. This is exemplified by RNA polymerase II inhibitors (by their ability to block Cdk7 and Cdk9), such as flavopiridol, roscovitine, or SNS-032, as well as translational inhibitors (homoharringtonine, silvestrol), which, respectively, reduce transcription and translation of short-lived proteins, including Mcl-1 (10).

On the other hand, proapoptotic BH3-only members of the Bcl-2 family (Puma, Bim, or Noxa) are upregulated by other types of apoptosis inducers in CLL cells. Noxa has proved to be a particularly relevant therapeutic target for CLL, because it interacts specifically with Mcl-1, thus neutralizing its antiapoptotic activity (11). Noxa upregulation is involved in the mechanism of action of several

Author's Affiliation: Centre de Recherche des Cordeliers, Equipe 18, INSERM U 872; Université Pierre et Marie Curie and Université Paris-Descartes, UMRS 872, Paris, France

Corresponding Author: Centre de Recherche des Cordeliers, UMRS 872, Equipe 18, 15 rue de l'Ecole de Médecine, 75006 Paris, France. Phone: 33 144278161; Fax: 33 144278161; E-mail: christian.billard@crc.jussieu.fr

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apoptosis inducers in CLL cells: inhibitors of histone deacetylase (HDAC) through epigenetic derepression; acadesine via increase in mRNA levels; proteasome inhibitors by enhancing the stability and accumulation of the protein; and hyperforin, a phloroglucinol of vegetal origin, possibly through proteasomal inhibition (12–15). The other BH3-only proteins Bim and Puma are also upregulated by various CLL cell apoptosis inducers such as HDAC inhibitors, acadesine, fludarabine, and glucocorticoids (12, 13, 16, 17). However, the fact that the differential Noxa/Mcl-1 balance in peripheral compared with lymph node CLL cells correlates with survival capacity (8) clearly indicates that the Noxa/Mcl-1 axis is an attractive target for apoptosis-based therapeutic strategies in CLL.

Clinical trials carried out in CLL with a few apoptosis inducers were disappointing. Flavopiridol has shown toxicity for patients, although a new schedule of administration was recently used more successfully (18). HDAC inhibitors displayed strong adverse effects (19), and no objective responses were achieved with the proteasome inhibitor bortezomib (20). These data are not surprising because the targets of these drugs are not specific; transcription inhibition by flavopiridol affects a broad range of short-lived proteins (10). Both proteasome and HDAC inhibitors enhance the expression of not only Noxa, but also other proteins including Mcl-1 (12, 21). This is a major disadvantage of the critical antiapoptotic role of Mcl-1 in CLL, and a strategy of combination with Mcl-1 downregulators was suggested (12, 14, 21).

Rather than using pharmacologic inducers of Noxa in more and more complex therapeutic protocols, the use of agents capable of mimicking this BH3-only protein and efficiently antagonizing Mcl-1 seems a more direct strategy. This corresponds to the concept of BH3 mimetics (22), which is based on the fact that BH3-only members of the Bcl-2 family are antagonistic ligands of the prosurvival members according to specific interactions, which thus neutralize their antiapoptotic activities (sequestration of the proapoptotic multi-BH members Bax and/or Bak; refs. 11, 22). This concept emerged after previous studies on Bcl-2 inhibitors, such as HA14-1, antimycin A, and others, and led to the development of novel small molecules having proapoptotic and therapeutic potential (23). A first approach was to design peptidomimetics of the BH3 domain that insert within the hydrophobic groove of the prosurvival proteins, but these peptides turned out to be toxic, not stable enough, or cell permeant (22). Another approach has been to generate hydrocarbon-stapled peptides to stabilize the α-helix of the BH domain (SAHB); these organic compounds were found to be protease resistant, cell permeable, and capable of binding with high affinity to the hydrophobic groove of Bcl-2 proteins. A SAHB of the BH3 domain of Bid (a ligand for Bcl-2 and Bcl-xL) induced apoptosis *in vitro* and antitumoral activity in human leukemia xenografts *in vivo* (23). Other small organic compounds were developed (23), such as ABT-737 and its oral version, ABT-263 (navitoclax), which bind to Bcl-2, Bcl-xL, and Bcl-w but not Mcl-1 or A1 (Bad-like BH3 mimetic structure) with high affinity (nanomolar range). ABT-737 exhibits apoptotic effects in tumor cell lines and tumor cells *ex vivo*, potentiates efficiency of therapeutic agents, and counteracts growth of human tumors xenografted in animals, but only when Mcl-1 expression is absent or impaired, due to its Bad-like structure (24). Indeed, a number of malignant cells with elevated levels of Mcl-1 are resistant to ABT-737, and yet, it has been proposed to overcome this resistance by the combination with Mcl-1 downregulators (24). In CLL cells cultured on feeder cells expressing CD40 ligand to mimic the lymph node microenvironment, decreasing Mcl-1 levels with dasatinib (a BCR-Abl tyrosine kinase inhibitor) or roscovitine overcomes resistance to ABT-737; interestingly, increasing Noxa levels with fludarabine, bortezomib, or upon NF-κB inhibition results in enhancement of the sensitivity to ABT-737 (25). These data further underscore the therapeutic role of the Noxa/Mcl-1 axis and, thus, the need for developing Noxa-like BH3 mimetics with the ability to specifically bind to and antagonize Mcl-1.

Other putative BH3 mimetics with proapoptotic activities were identified, such as obatoclax (GX15-070), a synthetic indol bipyrrol interacting with Bcl-2, Bcl-xL, and Mcl-1, but with low affinity (micromolar range; refs. 23, 26). The plant-derived gossypol (polyphenolic aldehyde), already known to induce apoptosis, proved to display BH3 mimetic properties, being able to interact with Bcl-2, Bcl-xL, Bcl-w, Bcl-B, as well as Mcl-1 and A1 with modest affinity (23, 26). Its isomer AT-101 and its derivative ApoG2, which is more stable and less toxic, and another gossypol-derived compound, TW37, are also under study (23, 26). Although AT-101 and obatoclax resemble pan-BH3 mimetics with even Noxa-like behavior, these agents seem to bind to Mcl-1 with an affinity that is not high enough to efficiently block its antiapoptotic activity (26). In addition, AT-101 and obatoclax are now thought to exert antitumoral effects independently of their BH3 mimetic properties (26). Similarly, both compounds, as well as the other putative BH3 mimetic, S1, targeting Bcl-2 and Mcl-1, were found to antagonize Mcl-1 indirectly by upregulating Noxa, but not through their direct binding (27). It was also revealed in this study that among 7 inhibitors of prosurvival Bcl-2 members, only ABT-737 acts effectively through its BH3 mimetic property, whereas the others do not (gossypol, ApoG2, obatoclax, S1, HA14-1, antimycin A3). This finding needs to be addressed with the novel BH3 peptidomimetic 072RB derived from Bim (pan-BH3–only protein), which was reported to be active in CLL cells by downregulating both Bcl-xL and Mcl-1, because it has not been elucidated whether 072RB interacts with these antiapoptotic proteins (28).

Therefore, it can be claimed that no Noxa-specific BH3 mimetic with a high Mcl-1–binding affinity has been identified at the present time. Among the various small organic molecules that were characterized in preclinical studies, only ABT-263, AT-101, and obatoclax are currently under clinical evaluation in hematologic malignancies, including CLL. As recently reviewed (26), the most encouraging preliminary data are observed with ABT-263, whereas AT-101 and obatoclax seem to display limited therapeutic activity as

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single agents. Whatever the final results of the ongoing clinical trials, the development of Noxa-like BH3 mimetics seems to be an important priority (29), particularly in the context of CLL. The fact that this priority has not yet been achieved probably results from the conformational rigidity of the hydrophobic groove of Mcl-1, which prevents the adaptation required for the insertion of designed BH3 ligands, compared with the flexible nature of the Bcl-xL groove (29). Another difficulty relates to the fact that all current BH3 mimetics were designed for interacting with soluble forms of antiapoptotic proteins, whereas it is clear that Bcl-2 family proteins change their conformation when embedded into mitochondrial and other cellular membranes (29).

Nevertheless, the way forward may be provided by 2 interesting novel BH3 ligands. One, Bim_S2A, has been unexpectedly identified in the course of a structure and function study on various mutants of the Bim BH3 domain; it selectively binds and antagonizes Mcl-1 and promotes cell death only when Bcl-xL is absent or neutralized (30). The other, a SAHB of the BH3 domain of Mcl-1, is itself a potent and exclusive Mcl-1 inhibitor capable of inducing caspase-dependent apoptosis (31). Structural analyses confirm that both compounds insert within the groove of Mcl-1 (30, 31). They, thus, seem to be true Mcl-1 antagonists of high affinity, deserving rapid preclinical studies and, eventually, clinical evaluation. As an additional perspective, the peptidic determinants of Bim_S2A and Mcl-1 SAHB, which were identified (30, 31), may contribute to novel rationales for designing other actual Noxa-like BH3 mimetics capable of inducing apoptosis of CLL cells. We do need to know whether Bim_S2A, Mcl-1 SAHB, and allied compounds to be characterized (including possibly 072RB) might affect the clinical outcome of patients with CLL. This information will undoubtedly be extremely important for further advances in CLL treatment protocols.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Christian Billard

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