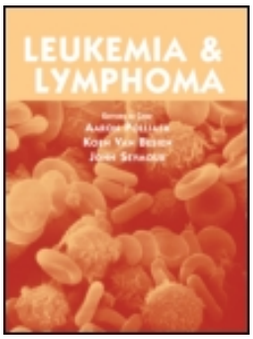

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COMMENTARY

BH3 mimetic therapy: an emerging and promising approach to treating chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is a disease characterized by inappropriate survival, and consequent accumulation *in vivo*, of genetically aberrant mature B lymphocytes. Central to maintaining the normal balance between life and death in most mammalian cells, including B lymphocytes, are intracellular proteins belonging to the Bcl-2 family. It is now 25 years since the discovery that Bcl-2 functions to protect cells from undergoing apoptosis [1]. Overexpression of Bcl-2 is associated with the development of various malignancies including follicular lymphoma and CLL. Moreover, high Bcl-2 expression confers resistance to traditional chemotherapeutics [2] and is associated with poor prognosis in selected diseases. These features have made Bcl-2 an attractive therapeutic target especially in patients for whom cytotoxic treatment is either ineffective or poorly tolerated.

Homology with Bcl-2 defines a broad family of proteins that are key regulators of apoptosis. While some members of this family function similarly to Bcl-2 in blocking apoptosis (Bcl-x_L, Bcl-W, Mcl-1, A1), others (Bax, Bak) mediate apoptosis by disrupting the integrity of the mitochondrial membrane, leading to caspase activation, which commits the cell to apoptotic death [3]. Still other Bcl-2-family members, the BH3-only proteins (including Bim, Bid, Bad, Noxa, Puma), are natural antagonists of Bcl-2 and its pro-survival relatives. The BH3-only proteins function as the physiological triggers for the induction of apoptosis [3]. As is the case for most critical cellular processes, whether a cell undergoes apoptosis is tightly regulated by the balance between Bcl-2-like pro-survival proteins and the activity of BH3-only proteins.

In CLL, overexpression of Bcl-2 is universal, sequestering and neutralizing the activity of the BH3-only proteins, such as Bim. Bcl-2 thereby prevents the malignant cells from undergoing apoptosis despite endogenous and exogenous stresses, which would normally trigger cell death. This creates a scenario whereby the CLL cells remain alive inappropriately, but are paradoxically "primed for death" if the delicate balance between pro-survival and BH3-only proteins is perturbed [4].

Over the last decade, much interest has focused on the development of drugs that kill cells by mimicking the activity of BH3-only proteins. Discovery of such BH3 mimetic drugs has been accelerated by the realization that most standard anti-cancer cytotoxics induce cell death through activation of endogenous BH3-only proteins, and by the accurate elucidation of which BH3-only proteins bind to, and inhibit, specific Bcl-2-like pro-survival proteins [5]. Further impetus has been provided by the failure of alternative approaches to target Bcl-2, by reducing its expression (e.g. Oblimerson [6]), to have significant clinical efficacy. Since 2000, many natural and synthetic molecules that both bind Bcl-2 (and/or related pro-survival proteins) and display cytotoxic properties *in vitro* have been purported to be BH3 mimetics. However, for many, the mechanism by which they induce cytotoxicity is multifactorial, and whether their activity is dependent upon inhibition of pro-survival proteins is highly uncertain [7].

Lessene *et al.* proposed that drugs should meet four key criteria to be defined as true BH3 mimetics [8]. First, cell death in response to the drug must be via the mitochondrial apoptotic pathway. As such, anti-cancer effects should be dependent on Bax and/or Bak, with double knockout cell lines proving resistant to relatively high concentrations of the agent. Second, the compound must bind to at least one of the pro-survival members of the Bcl-2 family (the target for inhibition) with high affinity in the low nanomolar range. Third, the activity of the drug should reflect the cellular levels of the relevant Bcl-2 family members, i.e. only induce apoptosis where there is significant expression of the target pro-survival protein within the cell. Finally, when the agent is used *in vivo*, pharmacodynamic markers of inhibition of relevant Bcl-2 family proteins should be demonstrable, i.e. there should be reductions in cell populations that are dependent on the inhibited pro-survival protein's function.

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In this issue of ***Leukemia and Lymphoma***, Balakrishnan *et al.* [9] demonstrate that apogossypolone (ApoG2) induces CLL cells to undergo apoptotic cell death *in vitro*, and induces apoptosis in wild-type fibroblasts but not those that are Bax and Bak deficient. However, Bax and Bak are not required for the drug's growth inhibitory effects, and its affinity for Bcl-2 and Mcl-1 is low, suggesting that any anti-cancer activity of ApoG2 can only be partially accounted for by BH3-mimickry. ApoG2 is one of a series of synthetic analogs of gossypol, which displays both BH3 mimetic and other anti-cancer activities. Gossypol, and its enantiomer AT-101, kill CLL cells *in vitro*, and AT-101 demonstrated modest anti-CLL activity in patients in early phase clinical trials [10]. Other derivatives, including apogossypol, have been tested using *in vivo* murine models, and demonstrated less toxicity and superior efficacy when compared with gossypol [11].

In parallel to the generation of gossypol derivatives, a number of other putative BH3 mimetics have been developed for treatment of hematological malignancies, and several of these have entered clinical trials. Obatoclax, a modest inhibitor of multiple Bcl-2 family proteins, demonstrated minimal activity in a phase I clinical trial in patients with advanced CLL [12].

ABT-737 is the first small molecule that is a true BH3 mimetic [13]. It meets all the criteria defining BH3 mimetic activity [8]. Generated through structure guided design, it binds Bcl-2, Bcl-x_L and Bcl-W with very high affinity, filling the hydrophobic grooves used for binding by the physiological partner BH3-only proteins, thereby inhibiting this interaction. Its anti-cancer activity is dependent on Bax and Bak [7]; it induces apoptosis of CLL cells at low nanomolar concentrations *in vitro* [4,13]; and it demonstrates significant synergy when combined with DNA-damaging cytotoxics such as fludarabine [14].

Navitoclax (ABT-263), an orally bioavailable compound in the same class as ABT-737, entered clinical development in 2007. Also a Bcl-2, Bcl-x_L and Bcl-W antagonist, it demonstrated a 35% partial response rate in patients with heavily pretreated relapsed and refractory CLL, including those with bulky, fludarabine-refractory and del17p disease [15]. Navitoclax also induced predictable, acute reductions in platelet counts within days due to its inhibition of Bcl-x_L, the key survival protein for platelets [16], and its dose-limiting toxicity proved to be thrombocytopenia [15]. To overcome this problem, a Bcl-2 specific BH3 mimetic, ABT-199 (GDC-199), has been developed with sub-nanomolar affinity for Bcl-2 ($K_i < 0.010$ nM) and three orders of magnitude less avidity for Bcl-x_L and Bcl-W ($K_i = 48$ nM and 245 nM, respectively) [17]. Phase I clinical trials are now under way, and preliminary data indicate major reductions in CLL burden in peripheral blood within 24 h, and also in lymph nodes and bone marrow [17].

These clinical trial data indicate that Bcl-2 is an important therapeutic target in CLL, validating longstanding expectations. Mcl-1 is the other pro-survival protein for which there is clinical trial evidence indicating its importance as a therapeutic target in CLL. As reviewed elsewhere, the acute activity of the cyclin-dependent kinase inhibitor flavopiridol in CLL is considered due to its inhibition of Mcl-1 translation [18]. Intriguingly, neither navitoclax nor ABT-199 inhibits Mcl-1, and flavopiridol does not reduce Bcl-2, yet inactivation of either appears sufficient to trigger apoptosis of CLL cells. This apparent paradox can be explained by considering the role of Bim, the BH3-only protein expressed at high levels in CLL, but held in check by Bcl-2 and Mcl-1 in these cells [4]. Displacement of Bim is sufficient to initiate apoptosis in CLL [19] and seemingly can be achieved by either route.

A potent BH3 mimetic inhibitor of Mcl-1 would therefore also be expected to induce rapid clinical reductions in CLL disease burden. As yet, no putative BH3 mimetic inhibitor of Mcl-1 (e.g. Obatoclax, AT-101) has demonstrated such clinical activity. However, the natural product marinopyrrole has recently been described as a BH3 mimetic inducing selective killing of Mcl-1 dependent cell lines, but not Bcl-2 or Bcl-xl dependent cells [20], and new clinical candidates are expected to emerge.

Over the next few years, the results of clinical trials with navitoclax and ABT-199 will define the effectiveness of Bcl-2-inhibitory BH3 mimetic therapy for CLL, as well as the long-term consequences of Bcl-2 inhibition for normal tissues. Initial data with navitoclax suggest durable control for many patients with relapsed, refractory disease, as judged by a median progression-free survival of 25 months [15], but this requires confirmation. Whether more potent and specific Bcl-2 inhibition with ABT-199 can result in complete remissions, and whether such selective molecular targeting is more prone to the development of treatment resistance when compared to dual Bcl-2 and Bcl-x_L inhibition, are two questions of particular interest.

For drugs such as ApoG2 and other gossypol derivatives, CLL remains a logical clinical testing ground to explore single agent biological activity. Their *bona fides* as clinically active BH3 mimetic inhibitors of either Bcl-2 or Mcl-1 can be efficiently judged by observing whether rapid reduction in circulating disease occurs.

Finally, preclinical data indicate that the combination of BH3 mimetics with cytotoxic therapy demonstrates synergy, and can theoretically overcome resistance to standard therapies [13,14,17]. To date, there are no published clinical data addressing the effectiveness of combination therapy with BH3 mimetics in CLL or other hematological malignancies. The results of combination trials are awaited with interest.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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