Targeting BCL2 for the Treatment of Lymphoid Malignancies

Mary Ann Anderson,[a,b,c] David Huang,[c,d] and Andrew Roberts[a,b,c,d]

The failure of apoptosis (programmed cell death) underpins the development of many tumors and often renders them resistant to cytotoxic therapies. In hematologic malignancies, this impairment of apoptosis is often caused by overexpression of the pro-survival protein BCL2. Because abnormally high levels of BCL2 sustain these tumors, there has been much interest in targeting BCL2 as a novel approach to treating various hematologic malignancies. One such approach is the development of BH3 mimetic compounds, small molecules that mimic the action of the BH3-only proteins, natural antagonists of BCL2 and its pro-survival relatives. These compounds act by restoring the ability of a cell to undergo apoptotic cell death. Some of them have shown very encouraging results in early-phase clinical trials that are currently underway, particularly in patients with chronic lymphocytic leukemia and some non-Hodgkin lymphomas, diseases marked by BCL2 overexpression. In this review, we discuss the rationale behind targeting BCL2, highlight the recent findings from clinical trials, and pinpoint the next steps in the clinical development of this interesting and promising class of targeted agents, particularly for the treatment of lymphoid malignancies.
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The last 50 years have seen remarkable advances in the medical management of many varieties of hematologic malignancy. Among these are treatments for chronic myeloid leukemia (CML) and acute promyelocytic leukemia (APML), diseases in which molecularly targeted agents developed to specifically correct the key aberrant features of those diseases (dysregulated ABL kinase activity in CML or differentiation arrest in APML) have radically altered both the management and the prognosis in these diseases. In both instances, therapy has been well tolerated with minimal adverse effects because normal tissues are relatively spared.

These successes serve as paradigms for developing other targeted anticancer therapies, the goal being to selectively target the key molecular drivers that sustain a specific tumor. Optimally, this therapy should maximize the antitumor effect with minimal toxicities due to on- or off-target activities. However, this goal has proven to be very challenging for most hematologic malignancies because the underlying genetic drivers are usually heterogeneous and complex. This outcome is in contrast with CML or APML, diseases driven and sustained largely by single molecular abnormalities.

Ever since resistance to apoptosis was identified as a hallmark of many cancers,[1,2] it has been recognized that therapies aimed at restoring the ability of malignant cells to undergo apoptosis might result in their killing with relative sparing of normal tissues. In the present review, we explore the background to newer therapies designed to overcome the blocks in apoptosis, focusing on small-molecule inhibitors of BCL2 that are now in clinical development, especially for treating patients with lymphoid malignancies.

[a]The Department of Medicine, The University of Melbourne, Parkville, Victoria, Australia.
[b]The Department of Clinical Haematology and Bone Marrow Transplantation, The Royal Melbourne Hospital, Parkville, Victoria, Australia.
[c]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia.
[d]The Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia.
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Address correspondence to Andrew Roberts, MBBS, PhD, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, VIC 3052, Australia. E-mail: roberts@wehi.edu.au
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CONTROL OF APOPTOSIS BY THE BCL-2 PROTEIN FAMILY

Apoptosis is a stereotypical process of cell death intrinsic to all multicellular eukaryotic organisms and is critical for the elimination of unwanted, infected, or otherwise damaged cells.[3] The effectors of this process are caspases, proteolytic enzymes that drive cellular demolition from within. Apoptosis is initiated by two major signaling pathways: (1) the extrinsic or death receptor pathway; and (2) the intrinsic or mitochondrial pathway. The former is activated when extracellular death ligands of the tumor necrosis factor family (eg, Fas/CD95) bind to specific cell surface receptors to trigger intra-cellular signals, culminating in the activation of caspases.

However, it is the intrinsic pathway that is more commonly perturbed in lymphoid malignancies. Cell death mediated through this pathway is regulated by members of a family of proteins related to BCL2. This family is considered to contain three subfamilies: pro-survival (BCL2 and the closely related proteins $BCLx_L$, BCLw, MCL1, and BFL1/A1); and subfamilies that promote cell death, the initiator BH3-only proteins (BIM, PUMA, BAD, or NOXA), and the cell death mediators, BAX and BAK. The interactions between these intra-cellular proteins determine whether a cell lives or dies.[4,5]

Under normal conditions in healthy lymphoid cells, the pro-survival members of the BCL2 family constrain the essential cell death mediators, BAX and BAK, thus maintaining cellular viability (Figure 1A). Stress signals such as DNA damage (eg, those induced by chemotherapy or radiation) or lack of growth factors trigger the activation of the BH3-only proteins such as BIM (Figure 1B). These BH3-only proteins bind to and inactivate the pro-survival family members, such as BCL2, to allow activation of BAX and BAK. Some BH3-only proteins can also directly activate BAX and BAK. In any individual cell, the relative activity of the BH3-only proteins and pro-survival BCL2 proteins determine the level of BAX and BAK activation and thus whether it lives or dies by apoptosis. Once activated, BAX and BAK permeabilize the outer mitochondrial membrane, triggering the release of factors such as cytochrome c, which functions as a co-factor for the activation of caspases, as well as damaging the mitochondria, the cell's major energy source.[6]

IMPAIRMENT OF APOPTOSIS IN LYMPHOID MALIGNANCIES

Signaling for apoptosis through the intrinsic pathway could fail by reduction in the activity of the BH3-only proteins, overactivity of the pro-survival BCL2 proteins, or loss of BAX and/or BAK (Figure 2). In lymphoid malignancies, the most common mutations are those affecting the tumor suppressor TP53, which normally acts to activate certain BH3-only proteins (eg, PUMA, NOXA) and the overexpression of BCL2. The latter is

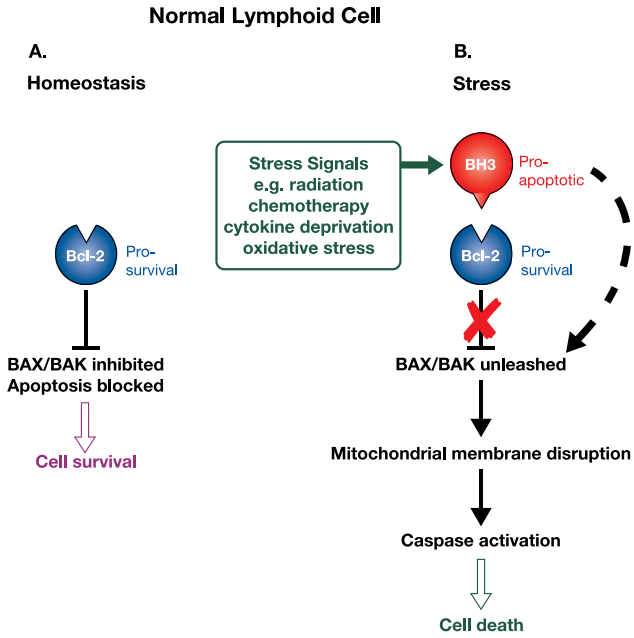

Figure 1. Control of apoptosis by the BCL2 protein family.[4] (A) In normal, untransformed mature B lymphocytes, BCL2 maintains cellular viability by blocking apoptosis. (B) When a significant stress signal is applied (eg, DNA or microtubular damage, cytokine deprivation, oxidative stress), the BH3-only proteins are activated. By binding to and inactivating BCL2 and related pro-survival proteins, these BH3-only proteins allow apoptosis to proceed. Some BH3-only proteins can directly activate BAX and/or BAK.

characteristic of follicular lymphoma[7,8] and is uniformly present in chronic lymphocytic leukemia (CLL),[9] whereby the impairment of apoptosis caused by BCL2 overactivity results in the accumulation of mature $CD5^+CD19^+$ lymphocytes in the blood and other lymphoid organs. Overexpression of BCL2 is also a feature of multiple myeloma and other plasma cell dyscrasias,[10] mantle cell lymphoma (MCL),[11] diffuse large B-cell lymphoma,[12] acute lymphoblastic leukemia,[13] and some T-cell lymphomas.[14]

Cells in which there is an excess of BCL2 are inappropriately long-lived despite endogenous or exogenous death stimuli. Moreover, because many cytotoxic therapies trigger apoptosis by activating the BH3-only proteins, the elevated levels of BCL2 found in many lymphoid malignancies can contribute to therapy resistance by blocking apoptosis.[15] Paradoxically, BCL2-overexpressing cells have been described as "primed for death"[16,17]; that is, if minor changes in the ratio of activity of pro-survival BCL2 family proteins to the activity of BH3-only proteins can be achieved, then apoptosis will be induced.

EARLY ATTEMPTS TO TARGET BCL2

These considerations led to BCL2 being identified as an attractive therapeutic target for >2 decades, especially

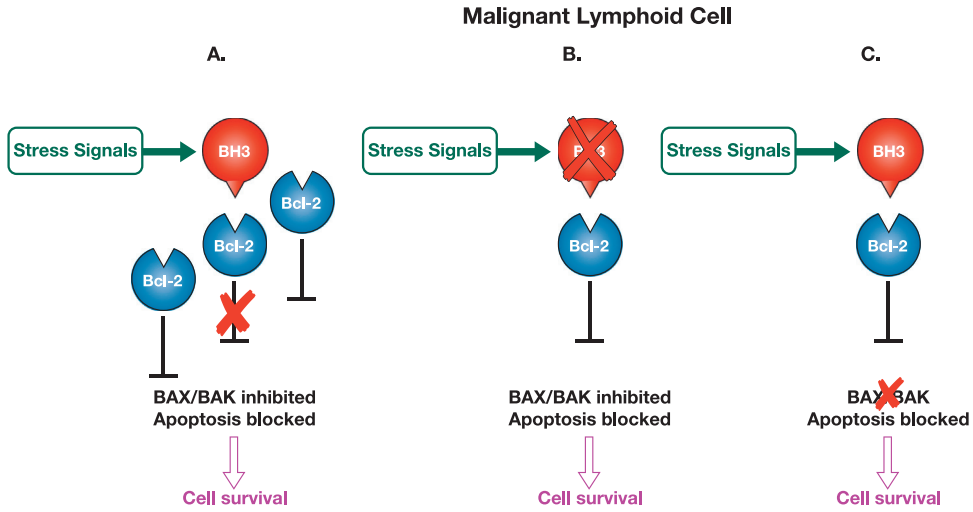

Figure 2. In malignant lymphoid cells, stress-induced apoptosis may be impaired through a number of different mechanisms, including: (A) overactivity of the pro-survival BCL2 proteins; (B) reduced BH3-only protein expression or activation; and (C) loss of BAX and/or BAK. BCL2 overexpression prevents apoptosis when the cell is stressed, despite activation of BH3-only proteins to a level that would be sufficient to induce apoptosis in a normal lymphocyte. BCL2-overexpressing cells are therefore protected from apoptosis and accumulate inappropriately in vivo.

for lymphoid malignancies. Earlier attempts focused on interfering with *BCL2* gene expression, thereby reducing the level of BCL2 protein synthesized. Oblimersen (Genasense, Genta, NJ), an antisense oligonucleotide that binds to BCL2 mRNA, thus interfering with its translation, was considered to have significant potential during early development[18] but ultimately proved in Phase III studies to have insufficient clinical efficacy.[19] Although the drug could induce apoptosis in vitro, it also induced tumor cell death by other pathways[18] (eg, induction of antiviral responses). It also seems likely that the failure of oblimersen reflects a failure to sufficiently reduce BCL2 levels, and hence BCL2 activity, within malignant cells. An alternative approach to directly inhibit the function of BCL2 has been pursued, with some success.

BH3 MIMETIC AGENTS TO INHIBIT BCL2 AND ITS RELATIVES

As discussed earlier, BCL2 and its closest relatives constrain BAX and BAK, thereby keeping cells alive (Figure 1). They do so directly by binding to the cell death mediators or indirectly by sequestering BH3-only proteins such as BIM, which can activate BAX and BAK.[4] Importantly, the BH3-only proteins antagonize pro-survival BCL2 and its relatives by binding with high affinity to a groove on their surfaces. When a cell is stressed, the elevated levels of the BH3-only proteins titrate out the amount of the pro-survival BCL2 proteins available to keep BAX and BAK in check, thereby allowing apoptosis to ensue.

Although some BH3-only proteins (eg, BIM) bind to all the pro-survival proteins, others are much more selective. BAD, for example, has high affinity for BCL2, $BCLx_L$, and BCLw, whereas NOXA binds preferentially to MCL1[20] (Figure 3). Conceptually, these findings suggest that selective targeting of BCL2 and its relatives is feasible and explains in part the biological consequences of activating one BH3-only protein (eg, BAD) compared with activating another (eg, NOXA).

The detailed molecular and structural understanding of how the BH3-only proteins act to initiate apoptosis was crucial to attempts to functionally target BCL2 and its relatives. Although it is relatively straightforward to develop inhibitors for enzymes such as kinases (eg, ABL, B-RAF), targeting the protein–protein interaction (eg, that between BCL2 protein family members) has long been considered to be highly challenging—the "holy grail" of drug discovery. However, the deployment of novel drug discovery methods, including technical innovations such

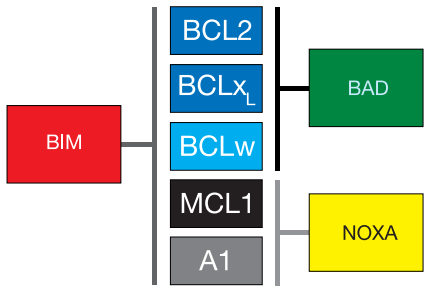

Figure 3. Selectivity of the BH3-only proteins for the pro-survival BCL2 proteins. Some BH3-only proteins (eg, BIM) bind all the pro-survival BCL2 proteins, whereas others (eg, BAD, NOXA) have preferential binding partners.[20] Within a cell, the reservoir of pro-survival BCL2 proteins determines whether a cell lives or dies, and this action is in turn controlled by whether they are inactivated by the BH3-only proteins. Hence, the amounts and binding selectivities of the BH3-only proteins determine whether apoptosis proceeds. Adapted from Chen et al.[20]

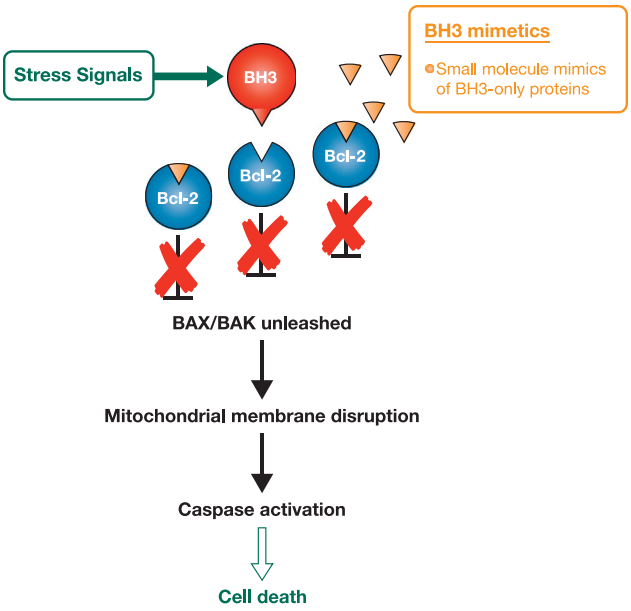

Figure 4. BH3 mimetic compounds re-sensitize BCL2-overexpressing tumor cells to apoptosis. The BH3 mimetic compounds are drugs that mimic the function of endogenous BH3-only proteins. At relevant pharmacologic concentrations, the BH3 mimetic agents bind and inhibit excess BCL2 (and related pro-survival proteins), thereby unleashing apoptosis.[23]

as SAR-by-NMR (structure–activity relationships obtained from nuclear magnetic resonance),[21,22] detailed understanding obtained from molecular and structural studies combined with state-of-the-art medicinal chemistry have enabled the discovery and development of small-molecule inhibitors of BCL2 and its relatives: the BH3 mimetic compounds.[23]

Given the early experience with oblimersen and particularly because many chemicals can induce cell killing, it is critical to clearly distinguish the BH3 mimetic agents from other drugs. In this regard, four criteria have been proposed to assess how closely these agents act like the BH3-only proteins, the natural antagonists[23] (Figure 4): (1) the biological activity of the agent must be to induce apoptosis via mitochondrial disruption by BAX and BAK; (2) the agent must bind to at least one BCL2 family member with high affinity; (3) the activity of the agent must correlate with the expression of the relevant BCL2 family members in the cell of interest; and (4) relevant biomarkers should be affected by the agent in animal models.

A detailed consideration of four putative BH3 mimetic inhibitors of BCL2 that have entered clinical trials illustrate the relevance of these criteria for clinical development.

CLINICAL EXPERIENCE WITH PUTATIVE BH3 MIMETICS

Obatoclax (GX15-070) has been described as a pan–BCL2 family inhibitor. It binds to BCL2, $BCLx_L$, BCLw, and MCL1 with low micromolar affinity and reportedly kills cells via BAX and BAK.[24] However, the response rates in clinical trials have been low,[25,26] and its development has ceased.

The natural product gossypol and its synthetic derivative AT-101 binds BCL2, $BCLx_L$, and MCL1.[27–29] However, the cell killing induced is not entirely mediated by BAX and BAK, indicating that the drug kills through multiple mechanisms.[30] As with obatoclax, these agents have demonstrated minimal single-agent activity in clinical trials, although some efficacy was observed in patients with follicular lymphoma when combined with rituximab.[31–33]

ABT-737 is an extensively validated BH3 mimetic, discovered by using SAR-by-NMR and redefined through structure-guided medicinal chemistry efforts.[22] Its description in 2005 dramatically advanced the field. ABT-737 binds to and inhibits BCL2, $BCLx_L$, and BCLw with high affinity (K_i <1 nM for all) but has $\sim$ 500-fold weaker binding to MCL1 and BFL1/A1 (lower K_i values reflect tighter binding to the target). Although the binding assays undertaken in different laboratories cannot always be directly compared, this binding affinity probably represents much greater affinity for BCL2, $BCLx_L$, and BCLw than that reported for the BH3 mimetic agents described earlier. The in vitro killing by ABT-737 is entirely dependent on the presence of BAX/BAK.[30] Its in vitro killing activity also correlates to the expression of the relevant BCL2 family proteins such that reduction of MCL1, which is not targeted by ABT-737, sensitizes cell lines to death in response to the agent, and cell lines overexpressing BCL2 remain sensitive to death.[30,34] Furthermore, in mouse lymphoma models, its activity correlates with expression of relevant BCL2 family members.[35] ABT-737 and its orally available analogue ABT-263 demonstrate significant preclinical efficacy in a range of hematopoietic malignancies.[36–38]

ABT-737 was not suitable for clinical development as an oral agent. However, its orally bioavailable relative, ABT-263, also binds to and inhibits BCL2, $BCLx_L$, and BCLw (K_i <1 nM for all)[38] and entered clinical trials in 2006. ABT-263, also known as navitoclax, has provided the first insights into the potential benefits and pitfalls of this class of agent for the treatment of patients with lymphoid malignancies.

Navitoclax induced a 35% overall response rate (ORR) in patients with relapsed and refractory CLL in a Phase I/II trial.[39] The best outcome achieved in this study was partial remission (PR) when CLL burden was assessed in the blood, lymph nodes, and bone marrow. Responses were durable in many patients, and a progression-free survival (PFS) of 25 months was reported. Notably, responses were seen in patients with poor prognostic features, including del17p, bulky disease, and fludarabine-refractory cases. Final analyses of Phase II data in relapsed refractory CLL and in first-line CLL treatment are awaited.

In a Phase I trial of navitoclax involving patients with a variety of non-Hodgkin lymphomas (NHLs), an ORR of 22% was observed, composed entirely of PRs, and a PFS

of 16 months was estimated.[40] These clinical results represent a significant advance on clinical activity observed with obatoclax and AT-101. Given the apparently much weaker binding affinities of these two drugs compared with navitoclax, this finding suggests that BH3 mimetic agents require tight binding affinities in the low nanomolar range (or better) to have meaningful clinical activity.

Dose escalation in the Phase I CLL clinical trials of navitoclax (ABT-263) was limited by acute, dose-dependent thrombocytopenia,[39] and all patients experienced a predictable decrease in their platelet counts from baseline within several days on the recommended Phase II dose. This adverse effect on platelets is the result of "on-target" $BCLx_L$ inhibition. In mice, $BCLx_L$ is critical to maintaining the viability of peripheral circulating platelets,[15,41,42] and genetic experiments have excluded BCL2 as playing any role in maintaining the viability of circulating platelets.[15] This dose-limiting toxicity of thrombocytopenia meant that the maximum tolerated exposure to navitoclax (ABT-263) was capped, compromising assessment of efficacy rates at higher doses. Grade 3 and 4 neutropenia was also noted in up to 29% of patients[40] in the navitoclax (ABT-263) trials. Neutrophils do not undergo apoptosis in vitro when treated with ABT-737 or navitoclax (ABT-263),[43] and the mechanism for this toxicity is still under investigation. Lymphopenia was a common finding in the NHL cohort, affecting up to 14% of patients.[40] In mouse models, ABT-737 causes a predictable depletion of B and T lymphocyte subsets.[44]

Preclinical data had suggested that significant MCL1 expression would most likely prevent responses to navitoclax.[30] However, in the Phase I CLL study, MCL1 expression did not preclude clinical responses, although higher MCL1 expression was associated with lesser reduction of CLL.[39] Mechanistic data now indicate that in B lymphocytes, direct inhibition of BCL2 by navitoclax (and ABT-737) displaces BIM from BCL2, making it available to inhibit MCL1.[17,44] Thus, MCL1 expression per se is not a marker of resistance to navitoclax.

SELECTIVE INHIBITION OF BCL2 WITH THE BH3 MIMETIC ABT-199

The need to develop a BCL2-specific BH3 mimetic was apparent from the navitoclax (ABT-263) experience. It was anticipated that such a drug would be platelet sparing and not limited by thrombocytopenia, thus allowing complete definition of BCL2 as a therapeutic target. ABT-199 (GDC-0199/RG7601), generated by re-engineering navitoclax (ABT-263), is the first such drug described.[45] It has high binding affinity for BCL2 (K_i <1 nM) without significant binding to $BCLx_L$ (K_i, ~50 nM) or BCLw (K_i >200 nM) (Figure 5, Table 1). In preclinical studies, this drug has demonstrated significant activity against CLL peripheral blood samples but not platelets, in vitro, and no platelet toxicity in mice or dogs treated with ABT-199.[45]

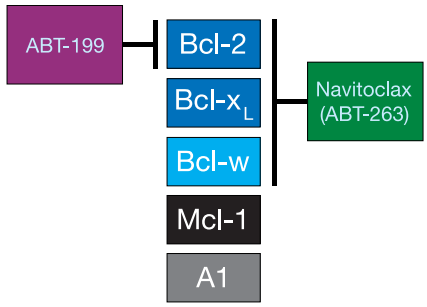

Figure 5. Selectivity of some BH3 mimetic compounds. Like the BH3-only protein BAD (as seen in Figure 3), navitoclax (ABT-263) binds tightly to BCL2, $BCLx_L$, and BCLw but spares MCL1 and BFL1/A1.[38] However, dosing of navitoclax (ABT-263) is limited by thrombocytopenia triggered by the targeting of BCLxL.[39,41,42] By engineering navitoclax (ABT-263), the selective BCL2 inhibitor ABT-199 was developed.[45] Because thrombocytopenia is not dose-limiting for ABT-199, it should allow the clinical impact of selectively inhibiting BCL2 to be fully evaluated.

Phase I clinical trials of ABT-199 as a single agent are currently underway in patients with CLL, NHL, and multiple myeloma.

ABT-199 has demonstrated the most promising clinical results of all the putative agents targeting BCL2 to date. Preliminary results from these Phase I studies demonstrate an 84% ORR in patients with relapsed or refractory CLL, including those with bulky disease, fludarabine-refractory disease, and del17p. In contrast to navitoclax, 23% of patients have achieved either a complete response (CR) or a CR with incomplete hematologic recovery.[46] These results hold for patients with ultra-high risk features (fludarabine refractoriness and del17p) with equivalent response rates (ORR, 82% and 89%, respectively) reported in abstract form. As yet, data for PFS have not reached maturity or been reported. The trial is ongoing, with accrual to safety expansion cohort at the recommended phase II dose of 400 mg to conclude in mid 2014.

The Phase I ABT-199 trial also includes an arm for patients with NHL. Preliminary data from this heterogeneous group of patients indicate an ORR of 53% comprising 44% PR and 8% CR.[47] An early signal of particular efficacy has been suggested for mantle cell lymphoma and Waldenström's macroglobulinemia, with ORRs of 82% and 100%, respectively, albeit in a small number of patients. Results for other subtypes of NHL have been less impressive, with ORRs of 27% and 38% in the follicular lymphoma and diffuse large B-cell lymphoma subgroups. Dose escalation is ongoing for patients with NHL.[47]

The higher CLL response rate seen with ABT-199 compared with navitoclax (ABT-263) (ORR, 84% vs 35%) most likely represents higher levels of inhibition of BCL2. ABT-199 has approximately two-fold higher specific bioactivity in vitro against CLL and has been

Table 1. Target Binding Profiles of BH3 Mimetic Compounds, and the Clinical Outcomes of Inhibiting Bcl-2 and Its Relatives With These Compounds

		Obatoclax	AT-101	Navitoclax (ABT-263)	ABT-199
Target binding (K_i)*					
BCL2		>500 nM	>200 nM	<1 nM	<1 nM
$BCLx_L$		>500 nM	>1000 nM	<1 nM	~50 nM
MCL-1		>500 nM[23]	>200 nM[33]	>500 nM[38]	>400 nM[45]
Clinical efficacy					
CLL	Overall response rate	4%[24]	0%[31]	35%[39]	84%[46]
	PR	4%	0%	35%	61%
	CR/CRi	0%	0%	0%	23%
	Median PFS	NR	NR	25 months	NR
NHL	Overall response rate	3%[25]	NA	22%[40]	53%[47]
	PR	3%		22%	44%
	CR	0%		0%	8%
	Median PFS	NR		16 months	NR
Hematopoietic toxicity (grades 3/4)					
CLL	Neutropenia	25%[24]	0%[31]	28%[39]	36%[46]
	Thrombocytopenia	3% (grade 4)	0%	18% (all grade 4)	9%
NHL	Neutropenia	NA	0%[32]	18%[40]	13%[47]
	Thrombocytopenia		0%	29%	11%
	Lymphopenia		NA	14%	NA
Dose-limiting toxicity		Neurologic disturbances (ataxia, somnolence, euphoria) and infusion-related reactions[24]	Abnormal liver function tests[31]	Thrombocytopenia[39]	Tumor lysis syndrome[48]

Abbreviations: CLL, chronic lymphocytic leukemia; CR, complete response; CRi, CR with incomplete hematologic recovery; NA, not available; NHL, non-Hodgkin lymphoma; NR, not reported; PFS, progression-free survival; PR, partial remission.

*The smaller value of K_i represents tighter binding to the target and hence more potent activity. Note that the values cited are indicative only.

escalated to higher doses than possible with navitoclax. Although grade 3 or 4 thrombocytopenia was reported in 9% of ABT-199–treated patients in the CLL arm of the Phase I trial, the majority of these subjects had pre-existing CLL-related immune thrombocytopenia, and no correlation between changes in platelet count and dose has been observed.[48] As for navitoclax, grade 3 and 4 neutropenia has been observed in heavily pretreated patients and is responsive to treatment with granulocyte colony-stimulating factor. Episodes of febrile neutropenia are rare, affecting just 5% of patients with CLL.[46] Mature data are required to determine if ABT-199 treatment is associated with a higher or lesser risk of infections than seen in other patients with advanced CLL.

The most significant dose-limiting toxicity for ABT-199 has been tumor lysis syndrome (TLS). In vitro, ABT-199 induces apoptosis of CLL very rapidly within 8 hours (unpublished data, Mary Ann Anderson, 2014). Remarkably, TLS has been observed in the same time frame in several patients at doses ranging between 50 and 1200 mg.[48] To date, between the Phase I study and a Phase Ib trial in combination with rituximab, the rate of significant TLS in CLL patients is 5%, including two deaths and one acute renal failure. However, the risk seems to be ameliorated by the implementation of protocols for the prophylaxis of TLS, especially in patients judged to be high risk by using objective criteria.[46]

TARGETING BCL2 AS PART OF COMBINATION THERAPY

Although some patients with CLL are achieving CRs with ABT-199 monotherapy, the potential impact of BH3 mimetic therapy has always been considered to be greater when used in combination with other anticancer agents. Although an intrinsic part of their biology, BCL2 overexpression is not considered the "driver" lesion in lymphoid cancers, and it thus seems improbable that even sustained inhibition of BCL2 will be curative. As discussed earlier, BCL2 overexpressing cells are "primed for death" and are variably susceptible to various stressors.[16,17] As with ABT-737 and navitoclax (ABT-263), ABT-199 exhibits potent synergy with various anti-cancer drugs in preclinical models of mantle cell lymphoma, myeloma, and diffuse large B-cell lymphoma.[45]

Clinical trials have begun combination treatment with anti-CD20 monoclonal antibodies and chemoimmunotherapy in patients with CLL (ClinicalTrials.gov identifiers NCT01682616, NCT01671904, NCT01889186, NCT01685892, and NCT02005471) and lymphoma (NCT01594229) or with bortezomib in patients with myeloma (NCT01794507). Combinatorial platelet toxicity is not predicted to be problematic as it was in trials adding navitoclax (ABT-263) to chemotherapy in patients with solid tumors.[49,50] However, neutropenia may be limiting when ABT-199 is combined with cytotoxic agents in previously treated patients, and variations in scheduling and dose may be required to find the most favorable balance of efficacy and toxicity.

PERSPECTIVES AND FUTURE DIRECTIONS

The clinical trial data with navitoclax (ABT-263) and, in particular, ABT-199, unequivocally validate BCL2 as a target for therapy in B-cell lymphoproliferative disorders. The exploration of the potential for BH3 mimetic compounds as inhibitors of BCL2 is an active area of clinical and laboratory research, and many key questions are yet to be resolved. For example, other than high-level BCL2 expression, are there biomarkers which distinguish lymphoid malignancies that will respond to ABT-199 as monotherapy? What is the long-term clinical significance of the CRs being observed in almost one quarter of patients with relapsed or refractory CLL? What are the consequences of BCL2 inhibition, and is therapy required to maintain PFS in patients with CLL? What are the molecular mechanisms causing primary and/or secondary treatment failures with ABT-199? In combination settings, the highest priority question must be to determine if ABT-199 can synergize with cytotoxic agents to overcome chemoresistance in currently incurable cancers (eg, "double-hit" lymphomas).

Hemato-oncologists now have a suite of powerful new agents that have the potential to change our current paradigms for treating several lymphoid malignancies. As with the introduction of previous novel therapeutic approaches (eg, combination cytotoxic chemotherapy, rituximab, and [more recently] tyrosine kinase inhibitors of B-cell–signaling pathways), time will be required to answer all the critical questions about how best to deploy these agents.

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