Selective Bcl-2 Inhibition to Treat Chronic Lymphocytic Leukemia and Non-Hodgkin Lymphoma

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Abstract: ABT-199, a second-generation BH3 mimetic, is an orally bioavailable, small molecule inhibitor that selectively targets B-cell lymphoma/leukemia 2 (Bcl-2). Bcl-2 is a key protein that inhibits the intrinsic mitochondrial pathway of apoptosis. First-generation BH3 mimetics such as navitoclax (ABT-263) had a broad range of inhibitory activity against Bcl-2 family members, including Bcl-2, Bcl-X_L, and Bcl-w. This drug demonstrated antitumor activity in patients with relapsed/refractory chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (NHL); however, on-target Bcl-X_L inhibition led to dose-dependent thrombocytopenia and posed a barrier to maximizing the activity of this agent. Through an elegant reengineering of navitoclax, ABT-199 was developed as a Bcl-2–selective small molecule inhibitor. In preclinical studies, ABT-199 was shown to have greater than 100-fold selectivity for Bcl-2 over Bcl-X_L. This selectivity has been consistent with the early results of the ongoing phase 1 clinical trial of ABT-199 in which the drug has demonstrated high rates of activity in relapsed/refractory CLL and NHL without dose-dependent thrombocytopenia. On-target tumor lysis syndrome (TLS) has been observed in a subset of patients treated with ABT-199, but changes in initial dosing and stepwise dose escalation have now been implemented to mitigate this risk. Ongoing correlative studies are being performed to help identify patients with the highest chance of response and the greatest risk for TLS.

Keywords
Apoptosis, chronic lymphocytic leukemia, non-Hodgkin lymphoma

Introduction

The antiapoptotic protein B-cell lymphoma/leukemia 2 (Bcl-2) has been inextricably linked to lymphoid malignancies from the time it was initially cloned from the breakpoints of the t(14;18) translocations associated with B-cell non-Hodgkin lymphomas (NHLs), in particular follicular lymphoma.[1] A high level of Bcl-2 expression has been recognized as a poor prognostic marker in both cutaneous[2] and nodal large B-cell lymphomas.[3] High Bcl-2 expression confers a survival advantage to cells that would otherwise be destined to undergo programmed cell death through apoptosis. Bcl-2 is the

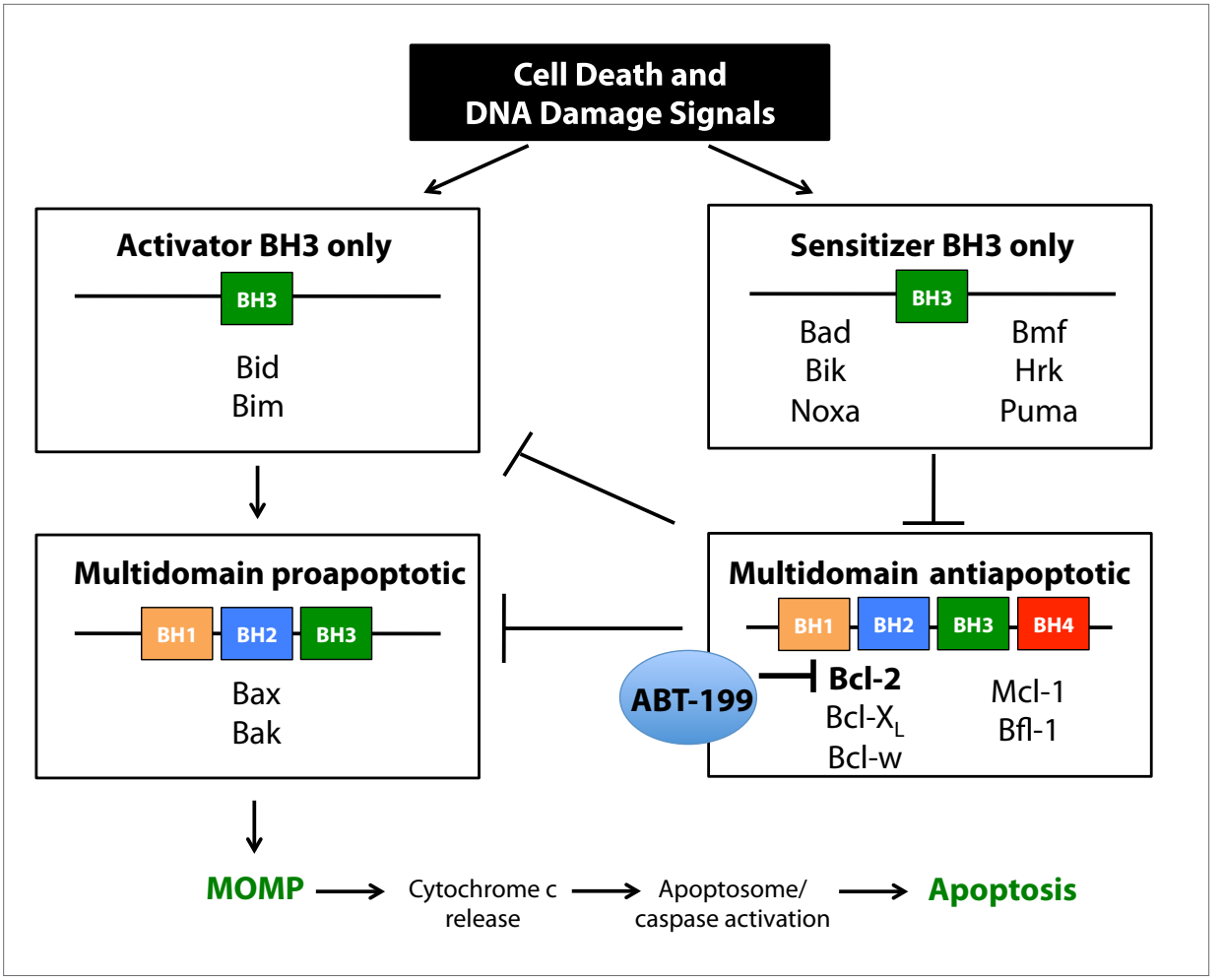

Figure. This illustration provides a schematic depiction of the Bcl-2 family of proteins. DNA damage from chemotherapy or other treatments, and cell death signals such as genomic instability, oncogene activation, or checkpoint violation, can activate the BH3-only class of Bcl-2 proteins. The activator BH3 subclass of these proteins can directly initiate the process of mitochondrial outer membrane permeabilization (MOMP) by promoting oligomerization of the multidomain proapoptotic class of proteins. This oligomerization ultimately leads to MOMP, which is characterized by a loss of mitochondrial membrane integrity and leads to release of cytochrome c, apoptosome activation, and subsequent apoptosis. This process is inhibited by the multidomain antiapoptotic proteins. A subclass of BH3-only proteins known as sensitizers can alternatively initiate apoptosis indirectly by displacing activators or effector multidomain proapoptotic proteins from multidomain antiapoptotic proteins, thereby allowing these proapoptotic proteins to initiate MOMP.

Figure adapted from Davids MS, Letai A. Targeting the B-cell lymphoma/leukemia 2 family in cancer. *J Clin Oncol.* 2012;30(25):3127-3135.

founding member of a family of apoptosis-regulating proteins whose interactions are the final regulatory step before the irreversible commitment to apoptosis. Because of this fundamental role in lymphoma pathophysiology, Bcl-2 is an attractive target for molecular therapy, and a potent small molecule Bcl-2–specific inhibitor, ABT-199, has recently been introduced into the clinic. Here, we will provide a brief introduction to the molecular interactions of Bcl-2 family proteins, review the development of ABT-199, and highlight the preclinical and early clinical data demonstrating the cytotoxic activity of ABT-199 in lymphoproliferative disorders.

The Bcl-2 Family

There are 3 general classes of Bcl-2 family proteins: proapoptotic effector proteins, proapoptotic BH3-only proteins, and antiapoptotic proteins. Proapoptotic effector proteins such as Bax and Bak directly participate in forming mitochondrial pores that initiate mitochondrial outer membrane permeabilization (MOMP), the final common pathway of the intrinsic pathway of mitochondrial apoptosis (see the figure). MOMP results in the release of cytochrome c from the mitochondrial intermembrane space. In concert with other proapoptotic factors, cytochrome c forms a multimeric

complex known as the apoptosome and begins the process of caspase activation and nuclear condensation, leading to irreversible cell death by apoptosis.[4-6]

The second class of Bcl-2 family proteins consists of proapoptotic proteins with homology only to Bcl-2 domain 3, hence the name BH3 (Bcl-2 homology 3)–only proteins. These include both activator and sensitizer BH3-only proteins. The activator proteins Bid and Bim can bind to Bax or Bak, thereby promoting a conformational change that can lead to Bax/Bak oligomerization and initiation of the irreversible commitment to apoptosis through MOMP.[7-9] Sensitizer proteins, such as Bad and Noxa, can displace activators such as Bid and Bim from antiapoptotic proteins, and thereby lead indirectly to Bax/Bak oligomerization and subsequent MOMP.[10]

The third class of Bcl-2 family proteins are the antiapoptotics: Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, and Bfl-1. They act by binding and thereby sequestering proapoptotic BH3-only proteins.[11,12] They may also directly bind to Bax and Bak and thereby prevent them from initiating pore formation and subsequent MOMP. Because of the high levels of antiapoptotic proteins (in particular Bcl-2) in malignant lymphocytes, and the fundamental role that these antiapoptotic proteins play in dampening the effect of the intrinsic pathway of apoptosis, they are a particularly attractive therapeutic target in lymphoid malignancies.

First-Generation BH3 Mimetic Drugs

Preclinical Data

The physiologic proapoptotic activity of the BH3-only proteins demonstrates an attractive mechanism for therapeutic activation of apoptosis. One strategy to develop a drug targeting this pathway was to create a molecule that would mimic the proapoptotic effects of the BH3-only proteins, a so-called "BH3 mimetic." Once the structures of Bak and Bad bound to Bcl-2 and Bcl-X_L were solved using nuclear magnetic resonance technology, a team at Abbott Laboratories (now AbbVie) embarked on the development of a small molecule inhibitor that would bind Bcl-2 with high affinity and specificity.[13] On a structural level, the BH3-binding domain consists of 2 hydrophobic alpha helices bordered by 6 or 7 amphipathic helices. This defined pocket could be used as a target to interfere with protein-protein interactions. The interacting surfaces of these protein-protein interactions previously had been considered too large to be disrupted with small molecule inhibitors. Using a structure-activity relationship analysis, several compounds that could bind to Bcl-2 and Bcl-XL were developed: first ABT-737,[14] and then the orally bioavailable navitoclax (ABT-263).[15] Both ABT-737 and navitoclax bind to Bcl-2, Bcl-X_L, and Bcl-w, but navitoclax is orally available, which allowed it to move into clinical development.

Clinical Development

Navitoclax showed promising activity in a phase 1 trial in patients with lymphoid malignancies. Chronic lymphocytic leukemia (CLL) patients had the greatest sensitivity to the drug, with all 7 CLL patients with baseline lymphocytosis achieving at least a 50% reduction in their lymphocyte count, while 50% (8 of 16 patients) had their tumors shrink. Surprisingly, patients with follicular lymphoma, which nearly always has strong Bcl-2 protein expression, had only modest sensitivity to the drug.[16] It is possible that sufficient drug levels were not achieved with navitoclax owing to dose-limiting toxicity (see paragraph below). A second phase 1 trial of navitoclax enrolled 29 patients with CLL. Lymphocytosis was reduced by more than 50% in 19 of 21 patients where this was assessed, spleen size was reduced in 10 of 13 patients, and nodal disease was reduced in 21 of 29 patients.[17] By the 1996 National Cancer Institute Working Group criteria, a modest overall response rate of 31% (9/29 patients) was achieved.

In the two phase 1 studies of navitoclax, thrombocytopenia was observed in 53% (29/55)[16] and 28% (8/29)[17] of patients, and was clearly dose-dependent. Thrombocytopenia was thought to be an on-target effect of the drug through its binding to Bcl-X_L, which is the antiapoptotic protein most important for platelet survival.[18] Additional studies investigating the effects of navitoclax and ABT-737 on platelets demonstrate that they induce platelet apoptosis via a mechanism distinct from platelet activation, and that they selectively kill older platelets, leaving younger platelets unaffected.[19] However, even surviving platelets had significant defects in activation.[20] This observation underscores the importance of Bcl-2 family members in promoting general hematopoietic homeostasis, and was a primary motivation to develop a more selective inhibitor of Bcl-2 that might spare platelets and thereby allow for greater peak drug levels with a larger therapeutic window to potentially allow for improved therapeutic efficacy.

Second-Generation BH3 Mimetic Drugs

Preclinical Data

Given that the on-target toxicity of thrombocytopenia observed with navitoclax was primarily caused by its inhibition of Bcl-X_L, a concerted effort was made to redesign the molecule to be more selective for Bcl-2. An elegant reverse engineering process yielded ABT-199, which is highly selective for Bcl-2, binding to it more avidly than to Bcl-X_L by more than 3 orders of magnitude.[21] In vitro studies showed that even at concentrations that were approximately 25-fold lower than navitoclax concentrations, ABT-199 was able to cause rapid induction of apoptosis in a Bcl-2–dependent acute lymphoblastic leukemia cell line within 4 hours of drug

exposure. Increased ABT-199 activity strongly correlated with higher levels of Bcl-2 expression in vitro.

During preclinical development, ABT-199 was found to significantly control tumor growth in 3 different mouse xenograft models of NHL, inhibiting xenograft growth and demonstrating synergy when combined with rituximab and bendamustine in all 3 models.[21] The molecular mechanism of ABT-199 was confirmed by experiments demonstrating that the drug is efficient at disrupting Bcl-2:Bim complexes and also that it is less likely to cause Bak-deficient Jurkat T cells to undergo apoptosis,[22] consistent with the observed apoptosis being mediated by an on-target effect. The selectivity of ABT-199 for Bcl-2 over other antiapoptotic proteins was independently confirmed in a murine pre–B-cell line engineered to overexpress Bcl-2 or a different member of the Bcl-2 family, Bcl-2L1.[22] In these experiments, cells overexpressing Bcl-2 showed approximately equivalent sensitivity to induction of apoptosis by navitoclax or ABT-199. In cells overexpressing Bcl-2L1, however, navitoclax induced apoptosis at concentrations more than 30-fold lower than those at which ABT-199 did.

CLL cells are known to rely on Bcl-2 for survival,[23] and therefore a Bcl-2–specific drug would theoretically be highly active against these cells. Ex vivo investigations of CLL cells have provided evidence that this is the case.[22] For example, ABT-199 induced apoptosis in CLL cells at concentrations that were 10-fold lower than those at which navitoclax did. Additionally, the cytotoxic effects of navitoclax, ABT-737, and ABT-199 on platelets were compared, and a 200-fold higher concentration of ABT-199 was required for induction of platelet apoptosis compared with navitoclax. Importantly, at levels well below those at which navitoclax was able to induce apoptosis of CLL cells, it induced a proportionally greater degree of platelet apoptosis. In contrast, ABT-199 efficiently induced CLL cell apoptosis in a dose-dependent fashion at concentrations that did not significantly increase platelet apoptosis.

Clinical Development

Published human data involving ABT-199 in patients are limited to the first 3 CLL patients treated in the phase 1 first-in-humans clinical trial for patients with relapsed/refractory CLL.[21] The 2 patients with baseline lymphocytosis had a greater than 95% reduction in lymphocyte count within the first 24 hours after receiving a single dose of ABT-199. All 3 patients in this cohort showed laboratory evidence of tumor lysis syndrome (TLS), which was easily managed and did not result in negative clinical sequelae. Thrombocytopenia was observed in 1 of these patients, but this was thought to be the result of concomitant disseminated intravascular coagulation associated with the TLS, as it improved with resolution of the TLS, despite ongoing dosing with ABT-199.

An interim analysis of the CLL arm of the ABT-199 phase 1 study was presented at the 2013 American Society of Hematology annual meeting.[24] All 38 CLL patients with evaluable lymphocytosis at enrollment had their lymphocyte count reduced by more than 50%. Of patients with evaluable nodal disease, 88% (50/57) had a greater than 50% reduction in nodal volume, and 89% (33/37) of patients who had bone marrow involvement had CLL infiltrate reduced by more than 50%. The overall response rate thus far with ABT-199 in relapsed/refractory CLL has been 84% (47/56), with equivalent activity in patients with fludarabine-refractory and del(17p) disease.

The most frequent adverse events observed at this interim evaluation of the CLL study include grades 1 to 2 diarrhea (43%), grade 3 neutropenia (36%), grades 1 to 2 nausea (40%), and grades 1 to 2 fatigue (33%). Grade 3 thrombocytopenia has been observed in 9%; importantly, this thrombocytopenia does not appear to be dose-dependent, and has improved over time even in patients continuing to receive ABT-199, suggesting that it is unlikely to be related to the drug. Interestingly, neutropenia has been observed in 37% of CLL patients. Given that neutrophils also depend on Bcl-2 for survival, this is likely an on-target effect of ABT-199, but thus far it does not appear to have led to higher rates of infection, as these patients can typically be supported with growth factor.

TLS has been observed in 9% of patients in this study, requiring an amendment to the study design that includes a lower initial dose of ABT-199 and more intensive monitoring during a weekly stepwise dose escalation. The revised study is now under way and will provide important safety data about ABT-199 in relapsed/refractory CLL patients. Hopefully this study will help identify which subgroups of CLL patients may be at highest risk for TLS from ABT-199.

Early results in relapsed/refractory NHL also appear to be promising, with an interim analysis revealing a 53% (19/36) overall best response rate.[25] Though the numbers are small, the activity of ABT-199 appears particularly strong in mantle cell lymphoma (MCL); 9 of the first 11 MCL patients treated so far (82%) have achieved a response, including 1 patient who achieved a complete response. Given that the hallmark of follicular lymphoma is a *BCL2* gene translocation with the immunoglobulin heavy chain t(14;18) leading to high levels of Bcl-2, the initial 27% response rate for follicular lymphoma patients seen with ABT-199 treatment has been lower than anticipated. However, several of the nonresponders were treated in earlier cohorts with lower doses of ABT-199, so time will tell whether a higher response rate is achieved in follicular lymphoma at higher doses. The adverse event profile in the NHL patients in this study has been similar to that in CLL patients, with grades 1 to 2 nausea (37%),

diarrhea (29%), upper respiratory tract infection (29%), fatigue (24%), thrombocytopenia (21%), and cough (21%) being the most frequently observed toxicities of any grade. The most frequently observed grade 3 toxicities were anemia (13%), neutropenia (13%), and thrombocytopenia (11%). The dose-limiting toxicities thus far have been neutropenia and febrile neutropenia in 1 patient each. Of note, significant issues with clinical TLS have not been observed in NHL patients.

Future Directions

Building on the exciting early clinical data with ABT-199, additional clinical trials are ongoing or planned for CLL and NHL patients. These include an ongoing phase 1b study of ABT-199 in combination with rituximab in relapsed/refractory CLL, a phase 2 study of ABT-199 in relapsed/refractory del(17p) CLL, and phase 2 studies of ABT-199 plus bendamustine and rituximab and of ABT-199 plus the monoclonal anti-CD20 antibody obinutuzumab in relapsed/refractory CLL.

Because BH3 mimetics have shown promising clinical activity with generally manageable toxicity, one might predict that the use of ABT-199 in conjunction with other targeted therapies would have the potential to increase antitumor activity without significantly increasing toxicity. In a preclinical model, a combination of the δ-isoform phosphoinositide 3-kinase inhibitor idelalisib (formerly CAL-101/GS-1101) with ABT-737 was found to overcome stroma-mediated treatment resistance in a coculture experiment with primary CLL cells,[23] suggesting that antagonism of the B-cell receptor signaling pathway might sensitize CLL cells that otherwise would be resistant to BH3 mimetic–mediated apoptosis. Ibrutinib (Imbruvica, Pharmacyclics/Janssen Biotech), a potent inhibitor of Bruton's tyrosine kinase, was recently approved by the US Food and Drug Administration for treating relapsed/refractory MCL and CLL. In MCL cell lines, ibrutinib decreased levels of several antiapoptotic proteins, including Bcl-X_L and Mcl-1,[26] suggesting that it might complement a Bcl-2–selective antagonist such as ABT-199 if used in combination. To determine whether these preclinical findings will translate to clinical efficacy, combination studies of ABT-199 with B-cell antigen receptor antagonists such as idelalisib and ibrutinib are urgently needed.

It will also be important to explore whether the activity of ABT-199 observed in indolent lymphoid malignancies can also be seen in aggressive lymphomas. Bcl-2 expression in diffuse large B-cell lymphoma (DLBCL) has been associated with poorer outcomes with cyclophosphamide, hydroxydoxorubicin, vincristine, and prednisone (CHOP) chemotherapy, but this was overcome with the addition of rituximab to CHOP (R-CHOP). With additional subclassification of DLBCL cases based on gene expression profiling into activated B-cell and germinal center B-cell (GCB) subtypes, recent data now suggest that *BCL2* expression and/or translocation in the GCB subtype is associated with poorer outcomes even with R-CHOP.[27,28] Given this observation, the combination of ABT-199 with R-CHOP chemotherapy should be explored, particularly in patients with GCB DLBCL.

Interrogation of the Bcl-2 pathway within CLL cells has the potential to yield predictive biomarkers for the efficacy of ABT-199. It has been shown in primary CLL cells that Bim was associated with Bcl-2 and that ABT-737–mediated displacement of Bim from Bcl-2 could promote apoptosis.[29] Interestingly, the sensitivity of CLL cells to ABT-737 was correlated with Mcl-1 but not Bcl-2 protein levels. The initial clinical experience with CLL patients treated with navitoclax showed that the maximum reduction in lymphocytosis was inversely correlated with levels of Mcl-1 protein and that high Bim:Mcl-1 ratios were associated with achieving clinical response.[17] Functional biomarker assays such as BH3 profiling[30,31] are also under investigation, and may eventually provide a clinically useful means of predicting ABT-199 efficacy and potentially identifying patients at higher risk for toxicities such as TLS.

Conclusion

Although most chemotherapeutic agents typically kill tumor cells through induction of MOMP via the intrinsic pathway of apoptosis, cells may adapt and develop resistance to chemotherapy. For example, CLL cells that develop del(17p), leading to decreased tumor protein p53 levels, or cells that develop *TP53* mutations may not be able to adequately transmit death signals downstream to mitochondria. ABT-199 is the culmination of a more than decade-long effort to produce a targeted therapy that bypasses these often defunct upstream pathways and directly induces MOMP to kill tumor cells. The promising preclinical data for ABT-199 in hematologic malignancies are just now beginning to be confirmed in patients with relapsed/refractory CLL and NHL. The direct molecular effect of this targeted therapy has begun to validate the years of research aimed at understanding the molecular biology of MOMP. The selective engineering of ABT-199 to mitigate the on-target toxicities of navitoclax, such as thrombocytopenia, validates the utility of rational drug design in this era of targeted therapies. Ultimately, the promising early clinical data on ABT-199 suggest that it may eventually become an important treatment option for patients with lymphoid malignancies. Given the fundamental role of Bcl-2 in cancer pathogenesis, this approach may benefit patients with a wide variety of other malignancies.

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