BH3-Only Mimetics

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Contents

Abstract

BH3 mimetics are a class of drugs designed to tip the balance of pro- and anti-apoptotic factors within a cell to favor apoptosis. They bind within a specific groove of anti-apoptotic Bcl-2 family survival factors in a similar manner to native proteins within the cell that regulate these factors. This primes the cell for apoptosis. Several small molecule BH3 mimetics are currently undergoing evaluation in clinical trials.

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J.L. Marshall (ed.), *Cancer Therapeutic Targets*,
DOI 10.1007/978-1-4419-0717-2_65

Keywords
ABT-737 • Anti-apoptotic Bcl-2 family • Bad • Bcl-2 • ABT-737 and ABT-263 • Assessment • B-cell lymphoma and mantle cell lymphoma • BH3-only mimetics • BH3-only proteins • Clinical trials • CLL and ALL • Gossypol • Hydrocarbon stapling • Mcl-1 overexpression • Navitoclax • Obatoclax • Preclinical studies • Role in cancer • BH3-only proteins • Activators • Chemotherapy • Overall survival in glioblastoma multiforme • Predictors of survival • Sensitizers • Therapeutics • Bid • Bim • Pro-apoptotic Bcl-2 proteins • Puma and Noxa

Target: Pro-survival Proteins via the Administration of BH3-Only Mimetics

The Bcl-2 family of proteins are intracellular factors important in regulating cellular apoptosis. All members of the family contain at least one BH (for "Bcl-2 homology") domain. Anti-apoptotic Bcl-2 family members (such as Bcl-2, Bcl-xL, Mcl-1, Bcl-w, and A1) consist of multiple BH domains (BH1–BH4) and a conserved C-terminal membrane domain that allows anchoring at the outer mitochondrial membrane or the endoplasmic reticulum. These anti-apoptotic globular proteins contain a hydrophobic pocket where pro-apoptotic factors bind with high affinity, inhibiting their ability to initiate apoptosis. The pro-apoptotic Bcl-2 proteins Bax and Bak have a similar structure to the anti-apoptotic Bcl-2 proteins (Elkholi et al. 2011), but initiate apoptosis following activation by appropriate intracellular signals. When activated, they homo-oligomerize to form pores in the outer mitochondrial membrane, releasing mitochondrial factors into the cytosol and initiating activation of the caspase enzymes that orchestrate cell destruction. Cells lacking both Bax and Bak are almost entirely resistant to apoptosis. The balance of anti-apoptotic and free pro-apoptotic Bcl-2 family proteins regulates entrance into the death cycle (Happo et al. 2012).

A third subclass of the Bcl-2 family, the BH3-only proteins (Bad, Bid, Bim, Bmf, Hark, Noxa, Puma), responds to cellular stressors and extracellular death signals by altering the balance between the pro- and anti-apoptotic factors, directing the cell toward apoptosis (Lomonosova and Chinnadurai 2008). All BH3-only proteins contain just a single BH domain, called BH3. The BH3 domain alone is both necessary and sufficient for the apoptotic function of these proteins (Chen et al. 2005). BH3 consists of 9–16 amino acids and forms an amphipathic α-helix capable of interacting with Bcl-2 family members of both the anti- and pro-apoptotic subclasses. Some BH3-only proteins called activators (Bid, Bim) can interact directly with pro-apoptotic Bcl-2 family members to promote apoptosis. Others, called sensitizers, bind to anti-apoptotic Bcl-2 family members at the same hydrophobic groove as the pro-apoptotic factors, reducing the number of binding sites available to sequester activated pro-apoptotic factors, favoring progress down the death pathway (Khosravi-Far and White 2008). Some BH3-only proteins are capable of binding and thereby inhibiting the anti-apoptotic function of all anti-apoptotic

proteins in the Bcl-2 family (Bid, Bim, and Puma), while others are selective for particular factors (Lomonosova and Chinnadurai 2008).

BH3-only proteins respond to a wide variety of stressors and are closely regulated at both the transcriptional level and by posttranslational modifications. Under unstressed conditions, many BH3-only proteins are barely detectable, but are then rapidly produced in response to physiologic and pathologic stressors. For example, multiple BH3-only proteins are rapidly upregulated in response to chemotherapy administration and are important factors in mediating cancer cell death under these conditions (Lomonosova and Chinnadurai 2008). Growth factor withdrawal causes upregulation of Bim by the Forkhead transcription factor Foxo3a. Puma and Noxa are transcriptionally regulated by p53 and respond to genotoxic stress. In the posttranslational setting, intracellular sequestration away from the mitochondria, phosphorylation, and protein cleavage are also used to regulate activity of these proteins. Bid is activated through the caspase cascade by extracellular death signals. Bad is phosphorylated in response to cytokine and growth factor signaling, resulting in its sequestration to binding proteins in the cytosol. Bim is sequestered to the microtubule-associated dynein motor complex and released with loss of cell adhesion (Elkholi et al. 2011). Each BH3-only protein is uniquely able to transduce the apoptotic signal in response to specific cellular stressors.

Biology of the Target

Bcl-2 was initially identified as a gene frequently translocated in follicular lymphoma. Relocation of *bcl-2* from its usual position on chromosome 18 to the q31:32 region of chromosome 14, called t(14,18):(q32;q21), places transcriptional regulation of this anti-apoptotic factor under the control of the immunoglobulin heavy chain promoter resulting in profound overexpression. Cells with these augmented levels of Bcl-2 have no change in their proliferation rate, but do not die when exposed to growth factor restriction (Kelly and Strasser 2011). When tested in vivo, translocation of the *bcl-2* gene to the heavy chain promoter region in mice caused a profound B-cell lymphocytosis. Adding this genetic abnormality to mice already overexpressing the *myc* oncogene accelerated tumor formation; all of these mice die from their tumors by 7 weeks of age, compared to just 40% with the *myc* translocation alone. Up to this point, all oncogenes previously discovered functioned by increasing cell growth. Bcl-2 was the first gene found to promote oncogenesis by inhibiting cell death. High levels of Bcl-2 are also found in diffuse large B-cell lymphoma and mantle cell lymphoma as well as lung, brain, and breast cancers. These cancers do not contain the t(14,18) translocation and overexpression is typically attributed to hypomethylation of the promoter. In chronic lymphocytic leukemia, mutational loss of microRNAs that control the level of Bcl-2 results in overexpression of the anti-apoptotic factor (DeVita et al. 2011). Overexpression of other anti-apoptotic Bcl-2 family members has also been found in cancer specimens. Bcl-xL overexpression is common in multiple myeloma. Mcl-1 overexpression is

seen in acute myelogenous leukemia, multiple myeloma, and cholangiocarcinoma (Kelly and Strasser 2011).

Given their role as inhibitors of anti-apoptotic Bcl-2 proteins (like Bcl-2, itself), BH3-only proteins were anticipated to be strong tumor suppressors; however, no consistent evidence has emerged to support this hypothesis. Mouse knockouts of each BH3-only family member have been created and show no developmental phenotype. Later in life, Bad knockout mice do form B-cell lymphoma, and Bid knockout mice develop myeloid hyperplasia which ultimately transforms to leukemia; however, the late onset of these tumors suggests tumorigenesis requires additional mutations accumulated through the aging process (Lomonosova and Chinnadurai 2008). The minimal effect of individual BH3-only protein knockout is attributed to the high physiologic redundancy between BH3-only family members.

Target Assessment

During diagnostic work-up, evaluation of tumor Bcl-2 expression level by immunohistochemistry is part of the standard of care for patients with a suspected B-cell malignancy. Assessment of tumor cells for the presence or absence of the t(14:18) translocation of the *bcl-2* gene by RT-PCR, FISH, and/or cytogenetic studies is also recommended as part of the standard of care work-up for many B-cell malignancies including follicular, marginal zone, mantle cell, and diffuse large B-cell lymphomas (DeVita et al. 2011).

There are no commercial clinically validated assays available to assess levels of BH3-only proteins. Testing for BH3-only proteins is not currently recommended for any cancer type.

Role of the Target in Cancer

Rank: “unknown” to 10 unknown to-1-2-3-4-5-6-7-8-9-10: 7

High-Level Overview

Diagnostic, Prognostic, and Predictive

Bcl-2 expression level is not a consistent predictor of prognosis in cancer (Davids and Letai 2012). CLL and ALL have the highest levels of Bcl-2 of any cancers, but are exquisitely chemosensitive. Conversely, overexpression of Bcl-2 has been seen in the majority of SCLC (75–95%) (DeVita et al. 2011) and is associated with decreased sensitivity to chemotherapy.

There is no evidence that levels of BH3-only proteins are prognostic or predictive of outcome in the large majority of cancers, although some isolated associations have been reported. Levels of some BH3-only factors may be independent predictors

of survival and of response to 5-FU-based chemotherapy in colon cancer (Sinicrope et al. 2008a, b). The expression level of Bim is predictive for response to prednisone in childhood ALL (Jiang et al. 2011). Breast cancer cells expressing Bad are more sensitive to taxanes than those lacking Bad expression (Craik et al. 2010). Interestingly, levels of BH3-only proteins taken in composite have been predicted to correlate with overall survival in glioblastoma multiforme, although expression level of any individual protein is non-correlative (Cartron et al. 2012). Studies in many other cancer types have shown no such association. This is consistent with the hypothesis that there is considerable redundancy of function among BH3-only proteins.

Therapeutics

BH3-only mimetics are small molecules or peptide agents designed to bind within the hydrophobic groove of anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-xL, and Mcl-1), mimicking the interaction of native BH3-only proteins. Occupation of these binding sites by a mimetic results in increased levels of free pro-apoptotic Bax and Bak, theoretically lowering the cellular threshold for apoptosis rendering the cell more susceptible to killing by cytotoxic agents. Cells with high levels of Bcl-2-like proteins (as is seen in many cancers and particularly in CLL and SCLC) are frequently "primed" with a large population of bound and sequestered pro-apoptotic Bax and Bak ready to be released upon administration of a BH3-only mimetic, which can result in spontaneous induction of apoptosis with just the single agent (Davids and Letai 2012). Currently, there are no FDA-approved BH3-only mimetics, but many are under active development.

Attempts have been made to create a peptidic BH3-only therapeutic, but multiple hurdles were encountered in this process. Studies have shown that the entire BH3 domain with intact α-helical structure is required for activity. Unfortunately, these peptides are poorly soluble in aqueous solution, fold poorly if solubilized, and cannot pass the cell membrane without the attachment of internalization tags (Khosravi-Far and White 2008). Modification of BH3 peptides by "hydrocarbon stapling" resulted in stabilization of the α-helix with more favorable solubility and permeability properties and increased half-life in vivo. In vivo apoptotic activity was also demonstrated with some of these "stapled" molecules (Walensky et al. 2004); however, none of these compounds have been tested in clinical trials.

Gossypol is a natural product derived from cotton seed pigments. The (-) enantiomer was developed into a therapeutic called AT-101, which binds Bcl-2, Bcl-xL, and Mcl-1 with moderate affinity. Administration of this compound in multiple cell lines has shown to cause release of mitochondrial contents into the cytosol, triggering apoptosis. In a phase I study in prostate cancer patients, dose-limiting toxicity was grade 3 small bowel obstruction (Liu et al. 2009). Sufficient activity was seen that the compound was advanced into phase II trials in combination with docetaxel, but results of this study are not yet available. A phase I trial also showed activity in breast cancer, but a phase I/II study for small-cell lung cancer in combination with

topotecan was negative (Heist et al. 2010). Results of early stage clinical trials of gossypol both alone and in combination with chemotherapeutic or targeted agents for additional cancers including CLL, esophageal cancer, small-cell lung cancer, squamous head and neck cancers, and adrenocortical cancer are ongoing (Hartman and Czyz 2012).

Obatoclax (GX015-070) is a small-molecule inhibitor of Bcl-2, Bcl-xL, and Mcl-1 at low micromolar concentrations. The prodigiosin parent molecule was identified by high-throughput screen of a natural products library and then chemically optimized to produce obatoclax. Obatoclax has been shown to kill lung cancer cells at low-micromolar IC50, but produces strong synergistic response with numerous chemotherapeutic agents as well as gefitinib, lapatinib, and bortezomib in vitro. The compound is considered to have clinically relevant Bcl-2-family-independent activity because (1) binding affinity for anti-apoptotic molecules is quite low, (2) double knockout of Bak and Bax does not inhibit cell killing by the compound, and (3) it has been demonstrated to cause cell cycle arrest (Lessene et al. 2008). In a phase I clinical trial of heavily pretreated patients with CLL, dose-limiting toxicity was transient adverse CNS toxicity during infusion (O'Brien et al. 2009). A phase II trial of the single agent in patients with relapsed or refractory Hodgkin lymphoma was stopped early due to lack of response (Oki et al. 2012). The compound has also been tested in combination for small-cell lung cancer. A phase I trial in combination with carboplatin and etoposide in chemotherapy-naive patients established safety for this regimen and produced an 88% response rate (Chiappori et al. 2012), but no increased efficacy was seen in phase II testing (Davids and Letai 2012). Similar lack of efficacy was seen in combination with topotecan in relapsed patients (Paik et al. 2011). Combination trials are ongoing in CLL, non-Hodgkin lymphoma, mantle cell lymphoma, and follicular lymphoma (Hartman and Czyz 2012).

ABT-737 and its oral equivalent ABT-263 (navitoclax) were discovered by directed screen for small-molecule-binding partners of the Bcl-xL hydrophobic groove. Both bind to Bcl-2 and Bcl-xL with IC50s in the nanomolar range, but have little affinity for Mcl-1. Consequently, cells with high levels of Mcl-1 tend to be resistant to these compounds in vitro. All preclinical studies support a mechanism of action consistent with perturbation of the pro- and anti-apoptotic balance of Bcl-2 family proteins. In preclinical studies and in clinical trials, ABT-737 and ABT-263 cause dose-dependent thrombocytopenia within hours of administration through direct killing of circulating platelets. Prolonged administration results in a compensatory upregulation of platelet production that can partially abrogate this toxicity. Consequently, administration is begun at smaller doses and then ramped up to allow time for the compensatory response; however, this adverse effect remains a barrier to clinical use particularly in patients with platelet production already suppressed by their malignancy or by prior treatment regimens (Davids and Letai 2012). Navitoclax has shown significant activity as a single agent in both phase I and phase II studies of CLL (Roberts et al. 2012). In combination with rituximab/fludarabine/cyclophosphamide or rituximab/bendamustine, an overall response rate of 81% was seen in patients with relapsed or refractory disease, with some patients achieving complete remission (Davids and Letai 2012).

Success has been more limited in solid tumors. A partial response and several patients with stable disease were reported in single agent phase I trial in SCLC, but limited activity was again seen in phase II trial (Rudin et al. 2012). Results of combination trials in solid tumors have yet to be reported.

Preclinical Summary

Active investigation continues to identify a molecular pattern that could predict which cancers are best "primed" to die by perturbations in Bcl-2 family proteins. It is hypothesized that treatment with chemotherapy or targeted agents could synergize with BH3 mimetics to produce a dramatic effect, but this has not yet been seen in vivo. Further research is needed to identify which antitumor agents are best suited for this role (Cragg et al. 2009).

ABT-263 (navitoclax) shows promise in the early stage clinical trials conducted so far; however, tumors expressing high levels of Mcl-1 show resistance in preclinical work. Modeled after the native Bad protein, ABT-263 does not bind Mcl-1. Research is ongoing to develop a molecule capable of interacting with all anti-apoptotic Bcl-2 family members at the high-affinity ABT-263 partners with Bcl-2 and Bcl-xL (Lessene et al. 2008).

Clinical Summary

Multiple BH3-only mimetics are currently undergoing testing in clinical trials and have shown limited activity as single agents. Results of combination trials for the most specific agent, ABT-263, are not yet available; however, the use of this compound in the clinic is limited by on-target thrombocytopenia. Recently, ABT-263 has been reengineered to decrease affinity for Bcl-xL (implicated in the adverse effect on circulating platelets) and increase affinity for Bcl-2. The new compound, ABT-199, shows decreased toxicity to platelets with no loss of activity against CLL cells in vitro. In first-in-human studies in CLL patients, a single dose of ABT-199 reduced tumor burden by 95% within 24 h of administration without causing significant thrombocytopenia (Souers et al. 2013). There are currently multiple open phase I trials testing ABT-199 in combination with therapy for CLL and non-Hodgkin lymphoma (http://www.clinicaltrials.gov).

Anticipated High-Impact Results

Results of clinical trials with ABT-199.

Results of clinical trials with ABT-263 in combination with other agents.

Cross-References

- ▶ Anti-apoptotic Bcl-2
- ▶ Caspase
- ▶ P53, Immunology

References

Cartron PF, Loussouarn D, Campone M, Martin SA, Vallette FM. Prognostic impact of the expression/phosphorylation of the BH3-only proteins of the BCL-2 family in glioblastoma multiforme. Cell Death Dis. 2012;3:e421.

Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM, Huang DC. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell. 2005;17:393–403.

Chiappori AA, Schreeder MT, Moezi MM, Stephenson JJ, Blakely J, Salgia R, Chu QS, Ross HJ, Subramaniam DS, Schnyder J, et al. A phase I trial of pan-Bcl-2 antagonist obatoclax administered as a 3-h or a 24-h infusion in combination with carboplatin and etoposide in patients with extensive-stage small cell lung cancer. Br J Cancer. 2012;106:839–45.

Cragg MS, Harris C, Strasser A, Scott CL. Unleashing the power of inhibitors of oncogenic kinases through BH3 mimetics. Nat Rev Cancer. 2009;9:321–6.

Craik AC, Veldhoen RA, Czernick M, Buckland TW, Kyselytzia K, Ghosh S, Lai R, Damaraju S, Underhill DA, Mackey JR, et al. The BH3-only protein Bad confers breast cancer taxane sensitivity through a nonapoptotic mechanism. Oncogene. 2010;29:5381–91.

Davids MS, Letai A. Targeting the B-cell lymphoma/leukemia 2 family in cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2012;30:3127–35.

DeVita VT, Lawrence TS, Rosenberg SA. Cancer: principles & practice of oncology: primer of the molecular biology of cancer. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2011.

Elkholi R, Floros KV, Chipuk JE. The role of BH3-only proteins in tumor cell development, signaling, and treatment. Genes Cancer. 2011;2:523–37.

Happo L, Strasser A, Cory S. BH3-only proteins in apoptosis at a glance. J Cell Sci. 2012;125:1081–7.

Hartman ML, Czyz M. Pro-apoptotic activity of BH3-only proteins and BH3 mimetics: from theory to potential cancer therapy. Anti Cancer Agents Med Chem. 2012;12:966–81.

Heist RS, Fain J, Chinnasami B, Khan W, Molina JR, Sequist LV, Temel JS, Fidias P, Brainerd V, Leopold L, et al. Phase I/II study of AT-101 with topotecan in relapsed and refractory small cell lung cancer. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer. 2010;5:1637–43.

Jiang N, Koh GS, Lim JY, Kham SK, Ariffin H, Chew FT, Yeoh AE. BIM is a prognostic biomarker for early prednisolone response in pediatric acute lymphoblastic leukemia. Exp Hematol. 2011;39:321–9. 329 e321-323.

Kelly PN, Strasser A. The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. Cell Death Differ. 2011;18:1414–24.

Khosravi-Far R, White E. Programmed cell death in cancer progression and therapy. Berlin: Springer; 2008.

Lessene G, Czabotar PE, Colman PM. BCL-2 family antagonists for cancer therapy. Nat Rev Drug Discov. 2008;7:989–1000.

Liu G, Kelly WK, Wilding G, Leopold L, Brill K, Somer B. An open-label, multicenter, phase I/II study of single-agent AT-101 in men with castrate-resistant prostate cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2009;15:3172–6.

Lomonosova E, Chinnadurai G. BH3-only proteins in apoptosis and beyond: an overview. Oncogene. 2008;27 Suppl 1:S2–19.

O'Brien SM, Claxton DF, Crump M, Faderl S, Kipps T, Keating MJ, Viallet J, Cheson BD. Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. Blood. 2009;113:299–305.

Oki Y, Copeland A, Hagemeister F, Fayad LE, Fanale M, Romaguera J, Younes A. Experience with obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist in patients with relapsed or refractory classical Hodgkin lymphoma. Blood. 2012;119:2171–2.

Paik PK, Rudin CM, Pietanza MC, Brown A, Rizvi NA, Takebe N, Travis W, James L, Ginsberg MS, Juergens R, et al. A phase II study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in relapsed small cell lung cancer. Lung Cancer. 2011;74:481–5.

Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, Carney DA, He SZ, Huang DC, Xiong H, 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 Off J Am Soc Clin Oncol. 2012;30:488–96.

Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, Chu Q, Giaccone G, Khaira D, Ramalingam SS, et al. Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2012;18:3163–9.

Sinicrope FA, Rego RL, Foster NR, Thibodeau SN, Alberts SR, Windschitl HE, Sargent DJ. Proapoptotic Bad and Bid protein expression predict survival in stages II and III colon cancers. Clin Cancer Res Off J Am Assoc Cancer Res. 2008a;14:4128–33.

Sinicrope FA, Rego RL, Okumura K, Foster NR, O'Connell MJ, Sargent DJ, Windschitl HE. Prognostic impact of bim, puma, and noxa expression in human colon carcinomas. Clin Cancer Res Off J Am Assoc Cancer Res. 2008b;14:5810–8.

Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202–8.

Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL, Korsmeyer SJ. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Science. 2004;305:1466–70.