The BCL2 Family: Key Mediators of the Apoptotic Response to Targeted Anticancer Therapeutics

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ABSTRACT
The ability of cancer cells to suppress apoptosis is critical for carcinogenesis. The BCL2 family proteins comprise the sentinel network that regulates the mitochondrial or intrinsic apoptotic response. Recent advances in our understanding of apoptotic signaling pathways have enabled methods to identify cancers that are “primed” to undergo apoptosis, and have revealed potential biomarkers that may predict which cancers will undergo apoptosis in response to specific therapies. Complementary efforts have focused on developing novel drugs that directly target antiapoptotic BCL2 family proteins. In this review, we summarize the current knowledge of the role of BCL2 family members in cancer development and response to therapy, focusing on targeted therapeutics, recent progress in the development of apoptotic biomarkers, and therapeutic strategies designed to overcome deficiencies in apoptosis.

Significance: Apoptosis, long known to be important for response to conventional cytotoxic chemotherapy, has more recently been shown to be essential for the efficacy of targeted therapies. Approaches that increase the likelihood of a cancer to undergo apoptosis following therapy may help improve targeted treatment strategies. Cancer Discov; 5(5); 1–13. ©2015 AACR.

INTRODUCTION
In 2002, Sydney Brenner, Robert Horvitz, and John Sulston were awarded the Nobel Prize in Physiology or Medicine largely for their contributions to the understanding of the highly regulated form of cell death known as apoptosis. On the basis of their work and that of many others, it is now well appreciated that apoptosis is a highly conserved mechanism critical for normal development and tissue homeostasis, with roughly 50 to 70 million cells undergoing apoptosis daily in an adult human (1). As there are significant pathologic consequences of unrestrained apoptosis, it is perhaps not surprising that apoptosis is governed by a complex network of molecular sentinels—the BCL2 family of proteins. Diverse inputs, such as DNA damage, energy stress, loss of growth factor signaling, and hypoxia, can trigger apoptosis by activation of these proteins (Fig. 1). In cancer, suppression of apoptotic signaling contributes significantly to carcinogenesis and tumor progression (2). Over the past two decades, many studies have elucidated the mechanisms by which this occurs in cancers, and these insights have laid the groundwork for therapies that directly target the apoptotic machinery.

THE BCL2 PROTEIN FAMILY
Thirty years ago, several groups reported a novel translocation between chromosomes 14 and 18, t(14;18), resulting in fusion of the immunoglobin heavy chain and BCL2 loci in acute B-cell leukemia and follicular lymphoma cells, leading to overexpression of BCL2 (3–7). It was subsequently shown that BCL2 enhanced the survival of these cells by inhibiting apoptosis (8–11). Additional genes with varying degrees of homology to BCL2 have since been identified that code for both antiapoptotic and proapoptotic proteins (12). The antiapoptotic BCL2 family proteins, which include BCL2, BCLXL, BCLW, MCL1, and BFL1/A1, share structural homology in the BCL2 homology (BH) 1, 2, 3, and 4 domains. These antiapoptotic proteins directly interact with the proapoptotic BH3-only proteins BIM, PUMA, BAD, BID, BIK, BMF, HRK, and NOXA, which share homology solely in the BH3 domain. Apoptotic stimuli lead to upregulation of BH3-only proteins...
and/or downregulation of antiapoptotic BCL2 family proteins. This change in the balance of pro- versus antiapoptotic BCL2 family proteins leads to activation of the multidomain (BH1, 2, 3) effector proteins BAK and BAX, which assemble into multimeric pores in the mitochondrial membrane and facilitate mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release into the cytosol (13).

Recent studies have clarified how the BCL2 family proteins interact to prevent or induce apoptosis (Fig. 1; ref. 14). “Activator” BH3-only proteins (BID, BIM, and PUMA) directly interact to prevent or induce apoptosis, while “effector” BAX and/or BAK proteins, inducing conformational changes that lead to the assembly of BAX/BAK multimeric pores in the mitochondrial membrane (15–21). Recent data suggest that activators may possess functional differences, with BIM preferentially activating BAX, and BID preferentially activating BAK (22). Antiapoptotic BCL2 family members (BCL2, BCLXL, MCL1, BCLW, and BFL1/A1) inhibit apoptosis by sequestering the activators from engaging BAX and BAK (23–25). “Sensitizer” BH3 proteins (e.g., BAD and NOXA) induce apoptosis by binding to antiapoptotic proteins, thereby displacing activators that are then free to activate BAX and BAK (25, 26). In addition, antiapoptotic BCL2 family proteins may bind activated BAX and BAK in some settings, thus promoting cell survival by both directly inhibiting BAX and BAK (27–29) as well as sequestering BH3-only proteins.

The complex network of interactions between pro- and antiapoptotic BCL2 family proteins tightly regulates the mitochondrial apoptotic response, allowing for a swift response to specific stimuli, while preventing unwanted cell death during normal cellular functioning. The binding affinities of the various pro- and antiapoptotic BCL2 family protein interactions have been characterized in solution using BH3 peptides and truncated proteins (23); however, this may not reflect the nature of the interactions between proteins that occur at the mitochondrial membrane (25, 30).

Recent work has focused on visualizing interactions between BCL2 family members in intact living cells, and has revealed complex spatiotemporal dynamics that govern activation of BAX and BAK (29, 31). In addition, there are marked differences in expression profiles of the BCL2 family proteins in different tissue and cell types (32). This complexity poses distinct challenges in elucidating the exact roles of individual BCL2 family proteins in regulating apoptosis in different cancer types, but also suggests that there could be a high degree of specificity for therapeutic modalities that directly target these proteins.

### BCL2 Family Proteins and Cancer

Overexpression of antiapoptotic BCL2 family proteins is observed in many cancers, and can result from chromosomal translocations, gene amplification, increased gene transcription, and/or altered posttranslational processing. As mentioned above, increased expression of BCL2 resulting from the t(14;18) translocation occurs in follicular lymphoma (3–5) and diffuse large B-cell lymphoma (33). Although this translocation is rarely seen in solid tumors, BCL2 protein overexpression is observed in some breast and prostate cancers (34–36), and other mechanisms of BCL2 overexpression have been identified, such as transcriptional activation by NF-κB signaling (37) or promoter hypomethylation (38). MCL1 and BCL2L1 (BCLXL) are frequently amplified or overexpressed in numerous tumor types (39–42), and increased MCL1 transcription can result from amplification of the transcription factor DEK (43) or constitutive activation of STAT3 (44). Posttranslational mechanisms that negatively regulate protein degradation pathways may also contribute to elevated expression of antiapoptotic BCL2 family proteins. For instance, MCL1 protein overexpression can result from enhanced protein stability due to genetic inactivation of the ubiquitin ligase complex protein FBW7 (39, 45, 46).
Overexpression of antiapoptotic BCL2 family proteins facilitates tumorigenesis and tumor progression (for more comprehensive reviews, see refs. 47, 48). Transgenic mice overexpressing BCL2 or MCL1 develop B-cell lymphomas (11, 49), but the long latency period and low tumor incidence (in the case of BCL2) suggests a permissive, rather than causative, role. Supporting this notion, numerous studies using transgenic mouse models have demonstrated that BCL2, BCLXL, and MCL1 can accelerate the development of MYC-driven lymphoma and leukemia (9, 50–54). Similarly, BCL2 has also been shown to cooperate with MYC and accelerate tumorigenesis in a mouse breast cancer model (50, 51). Once a tumor is established, antiapoptotic BCL2 family proteins also facilitate tumor cell maintenance and survival. For example, loss of BCL2 in a transgenic mouse leukemia model driven by BCL2 and e-MYC led to leukemic cell death and prolonged survival (55). MCL1 has been demonstrated to play a particularly critical role in the survival of multiple myeloma cells, and ablation of MCL1 expression alone stimulates apoptosis and leads to decreased cell survival (56, 57). As discussed in detail below, this provides a rationale for therapeutic targeting of specific antiapoptotic BCL2 family proteins in cancer.

Conversely, decreased expression of proapoptotic BH3-only proteins facilitates tumor formation and progression (58). Suppression of BH3-only protein expression permits the survival of malignant clones, and similar to the role of antiapoptotic BCL2 proteins in tumorigenesis, animal models reveal a largely permissive effect of loss of BH3-only protein expression. BIM- (59), BID- (60), PUMA- (61, 62), and NOXA (61)-deficient mice exhibit apoptotic defects but do not spontaneously develop cancers. BAD-deficient mice develop diffuse large B-cell lymphomas late in life, which can be accelerated by sublethal doses of radiation, supporting a role for BAD in facilitating the survival of tumorigenic lymphocyte clones (63). Similarly, genetic disruption of one Bcl2l11 (Bim) allele, resulting in haploinsufficiency, accelerates the formation of B-cell leukemias in Ep-Myc transgenic mice (64). Bcl2l11 loss has also been shown to cooperate with cyclin D1 overexpression in the development of mantle cell lymphoma in mice (65), mimicking human mantle cell lymphomas that exhibit cyclin D1 overexpression [due to a t(11;14) translocation] and, in some cases, homozygous deletions of Bcl2l11 (66).

Apoptotic stimuli, such as DNA damage, activate the tumor-suppressor p53, leading to apoptosis via upregulation of proapoptotic genes, including PUMA, NOXA, BID, and BAX (61, 67–70). TP53 is the most frequently altered gene across all cancers, and loss of TP53 accelerates and potentiates tumorigenesis in multiple murine cancer models (71). PUMA (p53 upregulated mediator of apoptosis) is the primary mediator of p53-induced apoptosis in response to DNA damage (67, 68), and the observation that TP53 mutations typically occur as late events in tumorigenesis (72) raises the possibility that loss of p53-induced expression of BH3-only proteins, such as PUMA, may contribute to disease progression. In one study, decreased PUMA expression was observed in melanoma compared with dysplastic nevi, and metastatic compared with primary lesions (73). Although alterations in TP53 were not examined in this study, another study reported that BRAF-mutant melanomas have impaired expression of p53 target genes compared with nevi (74), suggesting a link between loss of p53 signaling, downregulation of PUMA, and melanoma disease progression.

Under homeostatic conditions, the expression of proapoptotic BH3-only proteins is regulated by growth-promoting signaling pathways. Hyperactivation of these same pathways by oncogenic kinases can lead to diminished expression or function of BH3-only proteins by suppressing transcription or by posttranslational modifications that decrease BH3-only protein stability or lead to sequestration away from the mitochondria. Phosphorylation of BIM by ERK leads to RSK1/2-sensitive, βTrCP-mediated proteasomal degradation (75, 76), suggesting that hyperactivation of MAP kinase signaling may allow cancer cells to suppress BIM protein levels and evade apoptosis. Indeed, we speculate that this may be one of the key downstream effectors of activation of ERK signaling in cancers (77, 78). Similarly, BAD can be phosphorylated by both AKT and MAPK, thereby promoting binding to 14–3–3 proteins and sequestration (79–82). In addition to regulation by p53, PUMA expression can be modulated by growth factor stimulation via PI3K and FOXO3A (83). Thus, as discussed further below, suppression of BH3-only protein activity by activation of the MEK–ERK and PI3K–AKT signaling pathways may play a central role in the survival of cancers driven by constitutively activated oncogenic kinases such as EGFR (84–88), BRAF (89), KRAS (90), and BCR–ABL (91).

BCL2 FAMILY PROTEINS AND RESPONSE TO TARGETED THERAPIES

Although cancers typically harbor numerous genetic alterations, certain genetic events may lead to activation of oncogenic signaling pathways that are required for cancer cell survival—so-called “oncogene addiction.” The discovery that the ABL kinase inhibitor imatinib could inhibit the survival of chronic myelogenous leukemia (CML) cells harboring the BCR–ABL translocation ushered in the era of targeted therapies (92, 93). In 2004, non–small cell lung cancers (NSCLC) harboring activating mutations in EGFR were demonstrated to have exquisite sensitivity to the EGFR inhibitors gefitinib and erlotinib (94–96), and EGFR inhibitors have now supplanted chemotherapy as first-line therapy for EGFR-mutant NSCLC (97–100). Subsequently, dramatic clinical responses of BRAF-mutant melanoma (101) and EML4–ALK NSCLC (102–104) to BRAF and ALK inhibitors, respectively, have been observed. With recent advances in genomics, additional oncogenic driver mutations in different cancer types have been identified, and a myriad of novel therapies targeting many different signaling pathways are currently being evaluated in clinical trials.

Over the years, it has become clear that the induction of apoptosis is a critical component of effective targeted therapies. The majority of targeted therapies currently approved or in clinical trials are inhibitors of kinase signaling cascades, and thus lead to perturbation of BCL2 family proteins to affect apoptosis. Because many oncogenic drivers activate common downstream signaling pathways, such as MEK–ERK and PI3K–AKT–FOXO3A, therapies targeting different oncogenic kinases often lead to similar changes in BCL2 family proteins. Targeted therapies that lead to inhibition of...
MEK–ERK signaling almost invariably increase BIM protein levels, whereas those that cause downstream inhibition of mTORC1 typically induce PUMA expression. Importantly, multiple BCL2 family proteins may be affected simultaneously, which contributes to response to therapy (Fig. 2). For example, induction of both PUMA and BIM have been shown to be important in the response of mouse models of EGFR-mutant and HER2-positive breast cancer to EGFR and HER2 inhibitors, respectively (105). In KRAS-mutant NSCLC, combined MEK and PI3K inhibitors lead to upregulation of PUMA and BIM, both of which are necessary for the induction of an apoptotic response (90). In BRAF-mutant melanoma, BIM, PUMA, and BMF contribute to apoptosis induced by BRAF and/or MEK inhibitor treatment (89, 106, 107). Both BIM and BAD have been implicated in the apoptotic response of CML to imatinib (91). Conversely, downregulation of antiapoptotic BCL2 proteins may also play a role in response to targeted therapies, often in concert with upregulation of proapoptotic proteins. For instance, in EGFR-mutant NSCLC treated with EGFR inhibitors, the suppression of PI3K–mTORC1 signaling leads to a reduction in MCL1 expression that acts in concert with BIM induction to trigger an apoptotic response and induce tumor regression in vivo (108, 109).

**Figure 2.** Targeted therapies inhibit oncogenic kinase signaling cascades and modulate BCL2 family proteins to induce apoptosis. Examples of commonly occurring cancers driven by specific oncogenic driver mutations that result in constitutively activated downstream kinase signaling pathways and suppression of the mitochondrial apoptotic pathway. By inhibiting these pathways, targeted therapies lead to upregulation of proapoptotic BH3-only proteins and/or downregulation of prosurvival BCL2 family proteins, ultimately inducing apoptosis. [See references: EGFR (84, 87, 88, 105, 108); BRAF (89, 106, 181); KRAS (90); and CML (76, 91, 113).]
Although these studies have demonstrated that targeted therapies may affect multiple BCL2 family proteins, in a complex manner, BIM has repeatedly emerged as a critical mediator of targeted therapy–induced apoptosis in multiple cancer types, perhaps because many of the current kinase inhibitor–targeted therapy paradigms involve modulation of the MEK–ERK and PI3K–FOXO3 signaling axes. Indeed, BIM expression may serve as a potential biomarker useful for predicting response to targeted therapies (110). The first clear evidence that oncogenic signaling led to BIM suppression was provided by studies of BCR–ABL signaling in CML. BCR–ABL-induced ERK signaling leads to suppression of BIM protein levels via phosphorylation and subsequent proteasomal degradation, and treatment of BCR–ABL–positive cells with imatinib increases BIM protein levels and induces apoptosis (111, 112). Importantly, siRNA targeting of BIM protects these cells from imatinib-induced cell death. In addition, BIM is transcriptionally upregulated following inhibition of BCR–ABL by imatinib via activation of FOXO3A (113). Thus, multiple pathways regulated by BCR–ABL converge on BIM, making it a key effector of apoptosis induced by ABL kinase inhibitors.

Subsequently, other groups have reported that BIM is essential for induction of apoptosis in multiple cancer types in response to various targeted therapies. In EGFR–mutant NSCLC, EGFR inhibition results in downregulation of PI3K–AKT and MEK–ERK signaling (114), and loss of MEK–ERK signaling leads to accumulation of BIM. Depletion of BIM by RNAi abrogates the apoptotic response to EGFR inhibition (84, 85, 87, 88). The central role of BIM in promoting apoptosis in response to targeted therapies has also been demonstrated in other targeted therapy paradigms, including HER2–amplified breast cancers (115), ALK–positive NSCLCs (116), BRAF–mutant melanomas (106), BRAF–mutant colorectal cancers (117), and PIK3CA–mutant breast cancers (115). These studies provide strong experimental evidence that loss of apoptotic signaling—specifically, reduced BIM expression—significantly hinders the response to targeted therapies that either directly or indirectly inhibit MEK–ERK and/or PI3K–AKT signaling pathways.

**Assessment of BCL2 Family Proteins as Biomarkers of Response to Anticancer Therapies**

Given the central role of BCL2 family proteins in mediating the apoptotic response to anticancer therapies, there has been interest in determining whether they may have the potential to serve as biomarkers predicting treatment response. Deng and colleagues (23, 118) recently developed an experimental method termed “BH3 profiling” that quantifies the intrinsic propensity of a cell to undergo apoptosis, or apoptotic “priming.” Conceptually, priming can be understood as the proximity of a tumor cell to the apoptotic threshold, and is a function of the collective expression of pro–versus antiapoptotic BCL2 family proteins. BH3 profiling indirectly assesses this balance of BCL2 family proteins by perturbing cells with exogenous BH3 peptides that mimic the proapoptotic activity of promiscuous BH3–only proteins such as BIM, BMF, and PUMA (23, 118). In this assay, cells are challenged with low concentrations of BH3 peptides, and the degree of MOMP is measured using a fluorescent dye that is sensitive to mitochondrial membrane potential. In cells with a low degree of priming, the relative excess of antiapoptotic BCL2 family proteins will bind the exogenously added BH3 peptides without displacement of bound endogenous BH3 activator proteins, and no MOMP will be observed. In contrast, in cells with greater expression of endogenous activator BH3 proteins (or lower relative expression of antiapoptotic BCL2 family proteins), binding of BH3 peptides to antiapoptotic BCL2 family proteins will liberate the activators to bind BAX and BAK with subsequent MOMP. Thus, the experimentally observed MOMP can be interpreted to be a function of the relative balance of endogenous proapoptotic BH3 activator proteins sequestered by antiapoptotic BCL2 family proteins.

BH3 profiling has been successfully used to predict chemotherapeutic sensitivity of lymphoma cell lines (118), as well as the clinical response of a diverse set of cancers, including acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), multiple myeloma, and ovarian cancer (119, 120). Chemoresistant cells have significantly higher apoptotic priming than traditionally chemoresistant cancer subtypes or normal cells, suggesting a possible explanation for the therapeutic window for chemotherapeutic agents. In addition to conventional chemotherapy, BH3 profiling also appears to be effective for identifying highly primed cancers that are more likely to respond to BH3 mimetics (24, 121, 122), as these agents act by directly binding to antiapoptotic BCL2 proteins and liberating BH3 proteins (123, 124). Whether baseline global BH3 profiling or assessment of specific BCL2 family proteins will be useful in predicting the response of oncogene–addicted cancers to targeted therapies such as kinase inhibitors remains an open question (90). For example, BRAF–mutant melanoma and EGFR–mutant NSCLC are relatively chemoresistant, yet they are exquisitely sensitive to Braf and EGFR inhibitors, respectively, which induce apoptosis by altering expression of specific BCL2 family proteins. Performing BH3 profiling on these cancers following drug treatment, a technique recently described as Dynamic BH3 Profiling, may more effectively predict induction of apoptosis by targeted kinase inhibitor therapy (125).

It is notable that recent work has suggested that pretreatment BIM expression levels may indicate the likelihood of response to an array of targeted therapies. Indeed, BIM protein expression levels predict the apoptotic response of EGFR–mutant, BRAF–mutant, and HER2–amplified cell lines to the appropriate targeted therapies (115). Furthermore, we previously observed that pretreatment BIM mRNA expression levels in EGFR–mutant NSCLC specimens correlated with both the magnitude and the duration of response to EGFR inhibitor therapy, suggesting that low BIM expression may be a biomarker of poor response despite the presence of an activating EGFR mutation. This concept has been supported by analysis of BIM mRNA levels in patients enrolled in the EURTAC trial of erlotinib for EGFR–mutant NSCLC, which revealed that high BIM expression was associated with an overall response rate (ORR) of 87.5% and progression–free survival (PFS) of 12.9 months in the erlotinib treatment group, whereas those patients with low or moderate BIM expression had an ORR of 34.6% and PFS of 7.2 months.
Importantly, elevated BIM expression levels also correlated with improved overall survival (126). Interestingly, a germline polymorphism in intron 2 of \textit{BIM} that results in aberrant RNA splicing and decreased levels of \textit{BIM} transcripts containing the BH3 domain was associated with decreased responsiveness of \textit{EGFR}-mutant NSCLC to \textit{EGFR} inhibitor therapy (127–130). This same polymorphism has also been associated with decreased duration of remission induced by imatinib in CML (131), and a separate polymorphism in the \textit{BIM} BH3 domain has been identified that is associated with decreased \textit{BIM} mRNA expression and prolonged time to major molecular response after initiation of imatinib treatment (132). Altogether, these data suggest that BIM expression levels may have prognostic value in predicting response to kinase inhibitors in oncogene-addicted cancers.

**THERAPEUTIC TARGETING OF BCL2 FAMILY PROTEINS**

If a minimal apoptotic response to a given targeted therapy translates into a poor clinical response, it follows that drugs that specifically target apoptotic regulators may be useful to enhance the apoptotic response and improve clinical outcomes. As discussed above, the apoptotic response of a cell is governed by the relative balance of pro- and antiapoptotic BCL2 proteins. Therefore, direct inhibition of antiapoptotic BCL2 family members may be useful in cancers with marked overexpression of these proteins, or in combination with other therapies whose efficacy is limited by the expression of antiapoptotic BCL2 proteins. As a class, agents that inhibit antiapoptotic BCL2 family proteins act by binding within the BH3-binding groove of antiapoptotic BCL2 proteins and disrupting the interaction with BH3 proteins and are thus termed “BH3 mimetics.” Currently, there are inhibitors of BCL2 family proteins under development, including pan-BCL2 inhibitors, as well as selective inhibitors of BCL2/ BCLXL, BCL2 only, or MCL1. However, achieving a high degree of selectivity for induction of apoptosis via inhibition of BCL2 family proteins has proven to be challenging, with many putative BH3 mimetics leading to cell death in a BAX/ BAK–independent manner (133).

The most clinically advanced BCL2 family inhibitors target either BCL2 and BCLXL (BCL2/BCLXL inhibitors) or BCL2 only. ABT-737 and its clinical analogue ABT-263 (navitoclax) are small-molecule BAD BH3 mimetics that bind the hydrophobic BH3-binding groove of BCL2, BCLXL, and BCLW and prevent binding of proapoptotic family members such as BIM, BID, and BAD (123, 134). Initial studies suggested single-agent efficacy in cancer models characterized by BCL2 overexpression, such as B-cell malignancies and small cell lung cancer (SCLC). Recent clinical trials of navitoclax have demonstrated activity in CLL (135); however, the efficacy of single-agent BCL2/BCLXL inhibitors in SCLC has been underwhelming (136). Use of navitoclax is currently limited by its major dose-limiting toxicity of thrombocytopenia, an on-target consequence of BCLXL inhibition in platelets (137). In contrast, ABT-199 (venetoclax/GDC-0199), which selectively inhibits BCL2 but not BCLXL and thus does not cause thrombocytopenia, may be useful for malignancies in which BCL2 plays a more central role than BCLXL, such as in CLL and AML (122). Indeed, a phase I study of ABT-199 for relapsed/refractory CLL showed an overall objective response rate of 79%, with equivalent response rates in del(17p) and chemorefractory patients (124, 138).

Given the importance of BCL2 family proteins in regulating the response to kinase pathway inhibition, there has been interest in combining BCL2/BCLXL inhibitors with kinase inhibitors. ABT-737/navitoclax has been shown to enhance the efficacy of \textit{EGFR} inhibitors against \textit{EGFR}-mutant NSCLC cells (84, 87) and MEK or BRAF inhibitors for \textit{BRAF}-mutant melanoma (89, 139, 140). Whether the additional combination benefit of targeting BCL2/BCLXL outweighs the potential increase in toxicity for \textit{EGFR}- or \textit{BRAF}-mutant cancers, which generally respond well to tyrosine kinase inhibitor (TKI) alone, remains to be determined. The triple combination of dabrafenib (BRAF), trametinib (MEK), and navitoclax is currently being tested in a phase I/II trial for advanced \textit{BRAF}-mutant melanoma, which will provide a direct assessment of the benefit of adding navitoclax to the current standard of care BRAF/MEK inhibitor combination (clinicaltrials.gov NCT01989585).

BCL2/BCLXL inhibitors may also be useful for lowering the apoptotic threshold in cancers for which a kinase inhibitor alone is insufficient to induce an apoptotic response. We and others have explored the combination of MEK and BCL2/BCLXL inhibitors for \textit{KRAS}-mutant cancers, for which single-agent MEK inhibition is largely ineffective (141–143). In these cancers, inhibition of MEK leads to stabilization and accumulation of BIM; however, this only leads to apoptosis when BCLXL is simultaneously neutralized (Fig. 3). Given that inhibition of BCLXL appears to be more important than inhibition of BCL2 for the anticancer effect, it remains to be seen whether the unavoidable thrombocytopenia due to BCLXL inhibition will limit the clinical efficacy of this combination, which is currently under clinical development (Clinicaltrials.gov; NCT02079740). Should toxicity prevent the use of full doses of navitoclax, intermittent dosing strategies that take full advantage of inducing pronounced apoptosis could be explored.

Beyond BCLXL and BCL2, the MCL1 gene is frequently amplified (39) or aberrantly regulated in cancer, resulting in high expression levels in a wide range of both solid and hematologic malignancies (32), including lung cancer (41), breast cancer (144), prostate cancer (145), pancreas cancer (146), and leukemia (45, 147, 148). In particular, high MCL1 expression levels are important for the survival of multiple myeloma cells (56, 57, 149). In addition, elevated expression of MCL1 confers resistance to antitubulin chemotherapy (46) and BCL2/BCLXL inhibitors (150–152). Thus, there has been keen interest in developing drugs that selectively bind and inhibit MCL1 that might be useful as single agents or in combination with chemotherapy or other targeted therapies.

To date, no selective MCL1 inhibitors have entered clinical trials. The pan-BCL2 inhibitor obatoclax, which inhibits MCL1 as well as BCL2, BCLXL, and BCLW (153), has been tested in the clinic as a single agent and in combination with chemotherapy for a number of cancers, including hematologic malignancies, NSCLC, and SCLC (154–157). Overall, the clinical activity of obatoclax has been disappointing, and unexpected central nervous system toxicity, including...
disorientation and ataxia, has been observed, possibly due to off-target drug activity independent of induction of apoptosis via inhibition of BCL2 proteins (158). In addition to obatoclax, apogossypol derivatives BI-97C1 (sabutoclax) and BI112D1 that inhibit BH3 peptide binding to BCL2, BCLXL, and MCL1 have been reported by Wei and colleagues (159). Sabutoclax induces apoptosis in MCL1-dependent preclinical cancer models in a BAK/BAX–dependent manner (159), as well as mitochondrial fragmentation in an MCL1-dependent, BAX/BAK–independent manner (160), but it has yet to be evaluated in clinical trials.

Additional putative small-molecule inhibitors of MCL1 have been described; however, the clinical promise of many of these compounds is diminished by poor selectivity and/or potency (133, 161). Several groups have combined fragment-based screening and structure-based design—alogous to the development of ABT-737—to generate MCL1 inhibitors with improved potency and selectivity (162, 163). Most recently, AbbVie has reported the development of a series of small-molecule MCL1 inhibitors identified via high-throughput screening and subsequently refined via iterative structure-guided design using drug:MCL1 cocrystal structures (164). The resulting compounds exhibit subnanomolar binding affinities and high selectivity, and are capable of disrupting MCL1:BIM complexes in intact cells and inducing apoptosis in MCL1-dependent cancer cell models (165). These advances raise the exciting possibility that potent and selective MCL1 inhibitors may soon be available for clinical examination.

In the absence of direct inhibitors of MCL1, pharmacologic strategies that indirectly suppress MCL1 activity by diminishing MCL1 protein expression have been developed. In contrast to the other antiapoptotic BCL2 family proteins, the MCL1 protein has a short half-life (<4 hours), so alterations in transcription, translation, and degradation can rapidly affect cellular MCL1 protein levels. Wei and colleagues (166) used a chemical genomic screen to identify several compounds, including anthracycline chemotherapeutics (e.g., doxorubicin, daunorubicin, and epirubicin), that led to transcriptional repression of MCL1 and subsequent apoptosis. Importantly, restoration of physiologic MCL1 protein levels was capable of rescuing cells from the apoptotic effects of these MCL1 transcriptional repressor compounds, suggesting that MCL1 suppression may contribute to the clinical activity of anthracyclines. It has also been shown that MCL1 protein expression can be suppressed by inhibition of mTOR-mediated translation (167), though this effect appears specific to the ATP-competitive TORC inhibitors rather than allosteric TORC1 inhibitors such as rapamycin (108, 168–171). Interestingly, in EGFR-mutant NSCLC, EGFR inhibitors lead to inhibition of PI3K–mTOR signaling and downregulation of MCL1, which contributes significantly to the apoptotic response (Fig. 2; ref. 108). Exploiting this regulation of MCL1 protein expression by mTOR, we recently investigated combining mTOR inhibitors (targeting MCL1) with ABT-263 (targeting BCL2/BCLXL) and found that this combination was highly effective in preclinical models of KRAS- and BRAF-mutant colorectal cancers as well as SCLCs (170, 172). However, it remains to be determined whether this combination will be tolerable in the clinic.

**THERAPEUTIC STRATEGIES FOR ENHANCING BIM ACTIVITY**

As discussed above, oncogene-addicted cancers with decreased BIM expression may have a poor response to targeted therapies. Although loss of BIM expression may result from genetic mechanisms in some cases, in other cases, BIM expression may be suppressed by epigenetic mechanisms...
such as histone modifications or promoter hypermethylation (173). Thus, drugs that target epigenetic regulators might be useful for increasing BIM expression levels and overcoming apoptotic resistance in these cancers (Fig. 3).

Aberrant promoter hypermethylation occurs frequently in cancer and may result in transcriptional repression of tumor-suppressor genes (174). The \textit{BCL2L11} promoter contains an extensive CpG-rich region, and hypermethylation of this region is associated with low BIM expression. Notably, \textit{BCL2L11} promoter hypermethylation has been correlated with poor prognosis in CML (175) and Burkitt lymphoma (176), which are typically characterized by excellent clinical responses to imatinib and multiagent chemotherapy, respectively. Demethylating agents (decitabine and azacytidine, currently approved for the treatment of myelodysplastic syndrome) may be useful for reversing BIM suppression due to promoter hypermethylation, possibly by disrupting transcriptional corepressor complexes (177). The addition of decitabine to imatinib has been shown to restore BIM expression and imatinib-induced apoptosis in CML cells with \textit{BCL2L11} promoter hypermethylation (175). Importantly, this study demonstrates the potential of using agents to restore BIM expression in order to sensitize low BIM-expressing cancers to targeted therapies.

Histone modifications, such as acetylation, may also lead to transcriptional repression of BIM. Histone deacetylase (HDAC) inhibitors, such as vorinostat, have been shown to restore BIM expression in models of anaplastic large cell lymphoma, CLL, and pediatric ALL (177–179). The combination of an EGFR inhibitor with vorinostat resulted in increased expression of BH3 domain–containing BIM in \textit{EGFR}-mutant NSCLC. Therapies that directly target the apoptotic response by inhibiting antiapoptotic BCL2 family proteins (e.g., navitoclax, ABT-199) have shown clinical promise for cancers that depend on overexpression of BCL2, such as CLL, and may be useful in combination with kinase inhibitors for solid tumors. The rational use of these agents has the potential for improving currently available therapies as well as yielding novel therapeutic approaches for a wide range of cancers.

**Disclosure of Potential Conflicts of Interest**

A.N. Hata is a consultant/advisory board member for Amgen. J.A. Engelman reports receiving commercial research grants from Novartis, Sanofi Aventis, AstraZeneca, Amgen, Jounce, and GlaxoSmithKline; has ownership interest (including patents) in Ventana; is a consultant/advisory board member for Aisling, Amgen, FStar, G1 Therapeutics, Pathway Therapeutics, Genentech, GlaxoSmithKline, Janssen, Merck, Novartis, Red Sky, Roche/Ventana, Third Rock, Sanofi Aventis, Guidepoint Global, Quintiles, Madalon Consulting, Piramal, Clovis, AstraZeneca, Aveo/Biosdx, Cell Signaling Technology, Chucret, Cytoxms, Morgan Stanley, and Endo; and has provided expert testimony for Merrimack and Pfizer. No potential conflicts of interest were disclosed by the other author.

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