REVIEW

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

Aaron N. Hata[1,2], Jeffrey A. Engelman[1,2], and Anthony C. Faber[3]

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); 475–87. ©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

[1]Massachusetts General Hospital Cancer Center, Charlestown, Massachusetts. [2]Department of Medicine, Harvard Medical School, Boston, Massachusetts. [3]Virginia Commonwealth University Philips Institute for Oral Health Research, School of Dentistry and Massey Cancer Center, Richmond, Virginia.

Corresponding Authors: Jeffrey A. Engelman, Massachusetts General Hospital Cancer Center, Building 149, 13th Street, Boston, MA 02129. Phone: 617-724-7298; Fax: 617-724-9648; E-mail: jengelman@partners.org; and Anthony C. Faber, Philips Institute for Oral Health Research, Perkinson Building, Room 4134, 1101 East Leigh Street, Richmond, VA 23298. Phone: 804-828-0841; Fax: 804-828-0150; E-mail: acfaber@vcu.edu

doi: 10.1158/2159-8290.CD-15-0011

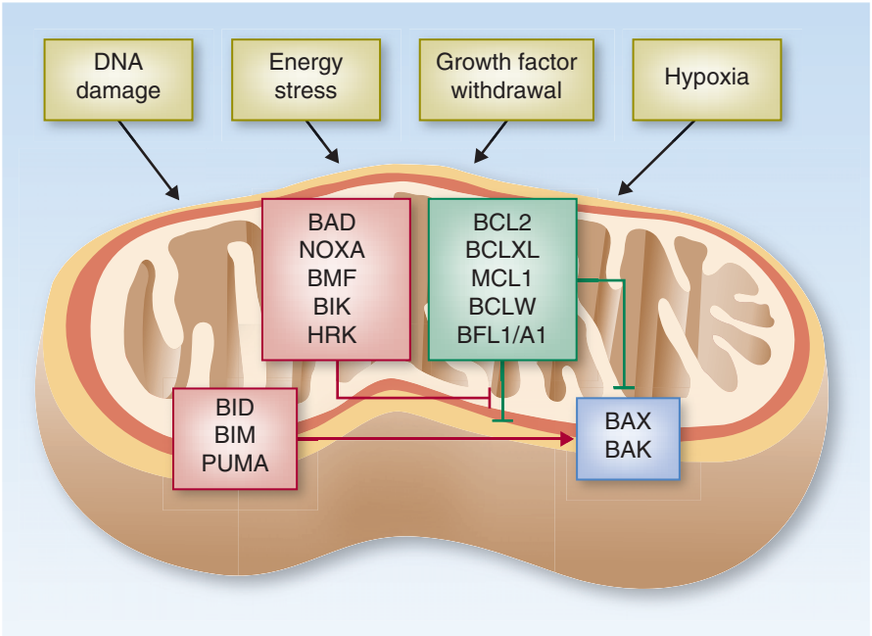

Figure 1. The intrinsic apoptotic pathway is regulated by BCL2 family proteins at the level of the mitochondria. Multiple cellular stressors modulate the expression levels of pro- and antiapoptotic BCL2 family proteins (red and green, respectively), leading to the activation of BAX and/or BAK and mitochondrial depolarization.

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 with "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). More 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 c-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 *Eμ-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

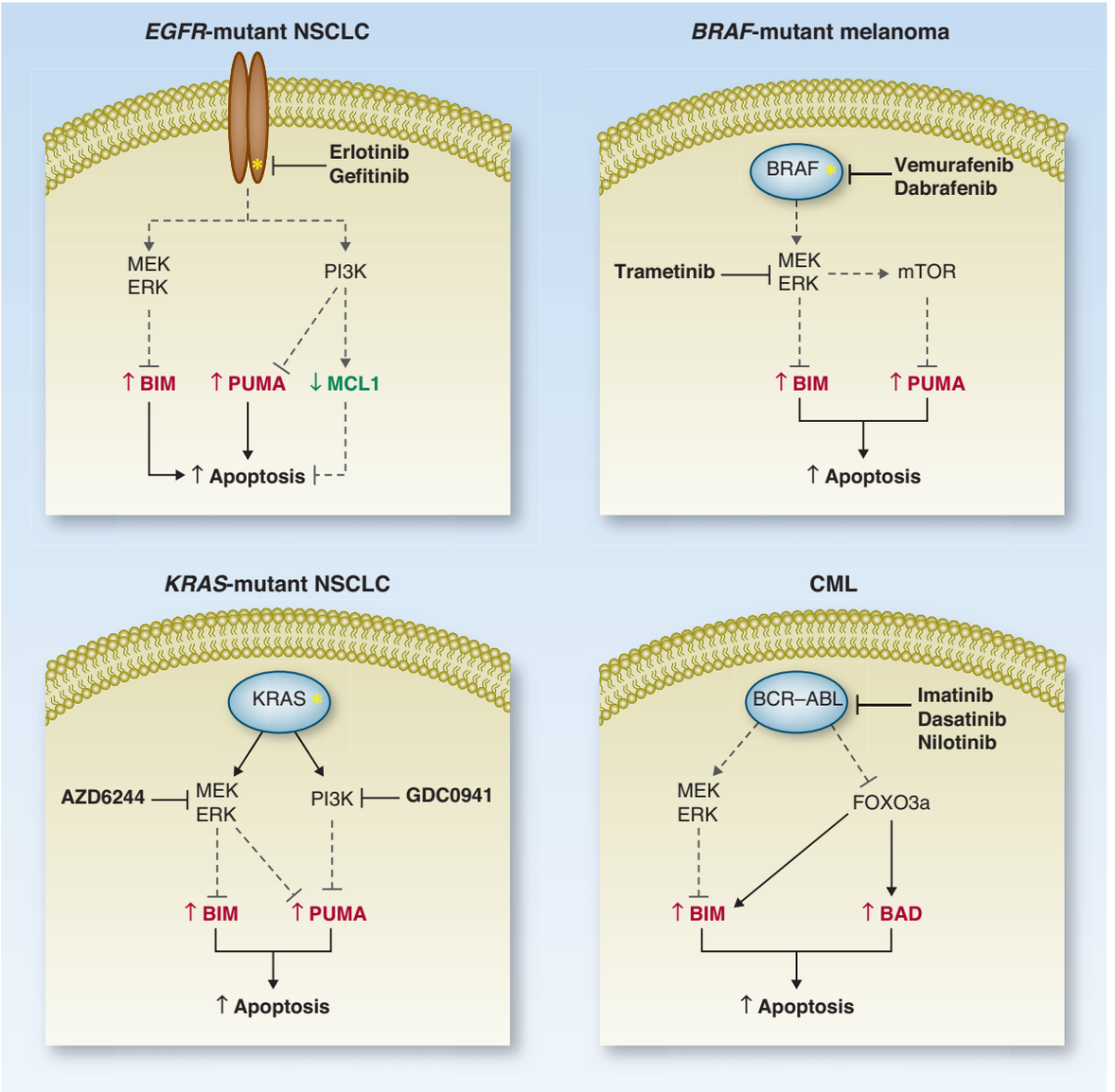

Figure 2. Targeted therapies inhibit oncogenic kinase signaling cascades and modulate BCL2 family proteins to induce apoptosis. Examples of commonly occurring cancers driven by specific oncogenic driver mutations that result in constitutively activated downstream kinase signaling pathways and suppression 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).]

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).

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–FOXO3A 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). Chemosensitive cancer 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 *BIM* that results in aberrant RNA splicing and decreased levels of *BIM* transcripts containing the BH3 domain was associated with decreased responsiveness of *EGFR*-mutant NSCLC to 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 *BIM* BH3 domain has been identified that is associated with decreased *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 EGFR inhibitors against *EGFR*-mutant NSCLC cells (84, 87) and MEK or BRAF inhibitors for *BRAF*-mutant melanoma (89, 139, 140). Whether the additional combination benefit of targeting BCL2/BCLXL outweighs the potential increase in toxicity for *EGFR*- or *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 *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 *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

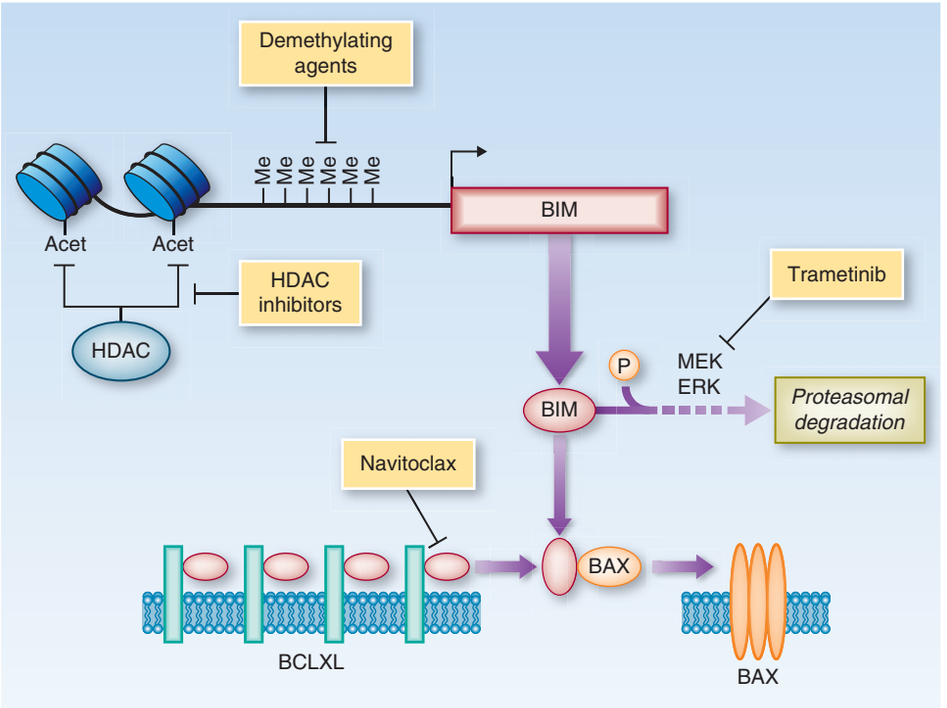

Figure 3. Strategies for enhancing the proapoptotic activity of BIM. Demethylating agents or HDAC inhibitors may overcome epigenetic repression of *BIM* transcription, whereas MEK inhibitors (e.g., trametinib) decrease BIM degradation—all leading to increased cellular BIM protein levels and increased activation of BAX. BCL2/BCLXL inhibitors (e.g., navitoclax) block the ability of antiapoptotic BCL2 family members such as BCL2 and BCLXL to neutralize BIM. Acet, acetyl group; Me, methyl group.

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—analogous 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 subnanonmolar 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 *BCL2L11* promoter contains an extensive CpG-rich region, and hypermethylation of this region is associated with low BIM expression. Notably, *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 azacytadine, currently approved for the treatment of myelodyspastic 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 *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 *EGFR*-mutant lung cancers that harbor the intronic deletion polymorphism discussed above (180). In this context, HDAC inhibition increased expression of the wild-type BIM protein and resensitized to EGFR inhibitor treatment *in vivo*. Thus, similar to demethylating agents, HDAC inhibitors may be useful in combination with targeted therapies for cancers with low BIM expression. Indeed, a clinical trial investigating the combination of erlotinib and the HDAC inhibitor romidepsin for advanced NSCLC has recently completed enrollment (Clinicaltrials.gov; NCT01302808). However, this trial was not restricted to lung cancers with *EGFR* mutations and, therefore, may not address the concept of restoring BIM levels to increase sensitivity to targeted therapies designed for genetically defined subsets of cancer.

CONCLUSION

It is now well established that the BCL2 family of proteins plays an important role in tumorigenesis and tumor maintenance, as well as in the response of cancers to both classic chemotherapies and targeted therapies. However, our understanding of the precise mechanisms of apoptotic signaling networks in specific cancer paradigms continues to evolve. Novel methods of interrogating the BCL2 family proteins to quantify the proximity of a cancer to the apoptotic threshold may be useful for predicting the response of specific cancers to chemotherapy and BH3 mimetics. Moreover, BIM levels alone may predict the apoptotic response to TKI treatment for certain oncogene-addicted cancers, such as *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/Biodesix, Cell Signaling Technology, Chugai, Cytomx, Morgan Stanley, and Endo; and has provided expert testimony for Merrimack and Pfizer. No potential conflicts of interest were disclosed by the other author.

Grant Support

J.A. Engelman is supported by NIH-NCI R01CA164273, NIH-NCI 2RO1CA140594, NIH-NCI 2P50CA127003, and NIH-NCI R01CA137008.

Received January 9, 2015; revised March 26, 2015; accepted March 27, 2015; published OnlineFirst April 20, 2015.

REFERENCES

1. Reed JC. Dysregulation of apoptosis in cancer. J Clin Oncol 1999;17:2941–53.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
3. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 1984;226:1097–9.
4. Cleary ML, Sklar J. Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci U S A 1985;82:7439–43.
5. Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, et al. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around JH on chromosome 14 and near a transcriptional unit on 18. Cell 1985;41:899–906.
6. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science 1985;228:1440–3.
7. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell 1986;47:19–28.
8. Henderson S, Rowe M, Gregory C, Croom-Carter D, Wang F, Longnecker R, et al. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 1991;65:1107–15.
9. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988;335:440–2.
10. McDonnell TJ, Deane N, Platt FM, Nunez G, Jaeger U, McKearn JP, et al. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 1989;57:79–88.

11. McDonnell TJ, Korsmeyer SJ. Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18). Nature 1991;349:254–6.
12. Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. The BCL-2 family reunion. Mol Cell 2010;37:299–310.
13. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell 2004;116:205–19.
14. Villunger A, Labi V, Bouillet P, Adams J, Strasser A. Can the analysis of BH3-only protein knockout mice clarify the issue of 'direct versus indirect' activation of Bax and Bak? Cell Death Differ 2011;18:1545–6.
15. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 2002;111:331–42.
16. Czabotar PE, Westphal D, Dewson G, Ma S, Hockings C, Fairlie WD, et al. Bax crystal structures reveal how BH3 domains activate Bax and nucleate its oligomerization to induce apoptosis. Cell 2013;152:519–31.
17. Gavathiotis E, Suzuki M, Davis ML, Pitter K, Bird GH, Katz SG, et al. BAX activation is initiated at a novel interaction site. Nature 2008;455:1076–81.
18. Gavathiotis E, Reyna DE, Davis ML, Bird GH, Walensky LD. BH3-triggered structural reorganization drives the activation of proapoptotic BAX. Mol Cell 2010;40:481–92.
19. Ren D, Tu HC, Kim H, Wang GX, Bean GR, Takeuchi O, et al. BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. Science 2010;330:1390–3.
20. Kim H, Tu HC, Ren D, Takeuchi O, Jeffers JR, Zambetti GP, et al. Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. Mol Cell 2009;36:487–99.
21. Leshchiner ES, Braun CR, Bird GH, Walensky LD. Direct activation of full-length proapoptotic BAK. Proc Natl Acad Sci U S A 2013;110:E986–95.
22. Sarosiek KA, Chi X, Bachman JA, Sims JJ, Montero J, Patel L, et al. BID preferentially activates BAK while BIM preferentially activates BAX, affecting chemotherapy response. Mol Cell 2013;51:751–65.
23. Certo M, Del Gaizo Moore V, Nishino M, Wei G, Korsmeyer S, Armstrong SA, et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell 2006;9:351–65.
24. Del Gaizo Moore V, Brown JR, Certo M, Love TM, Novina CD, Letai A. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. J Clin Invest 2007;117:112–21.
25. Lovell JF, Billen LP, Bindner S, Shamas-Din A, Fradin C, Leber B, et al. Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax. Cell 2008;135:1074–84.
26. Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, et al. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell 2005;17:525–35.
27. Willis SN, Chen L, Dewson G, Wei A, Naik E, Fletcher JI, et al. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev 2005;19:1294–305.
28. Merino D, Giam M, Hughes PD, Siggs OM, Heger K, O'Reilly LA, et al. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. J Cell Biol 2009;186:355–62.
29. Edlich F, Banerjee S, Suzuki M, Cleland MM, Arnoult D, Wang C, et al. Bcl-x(L) retrotranslocates Bax from the mitochondria into the cytosol. Cell 2011;145:104–16.
30. Leber B, Lin J, Andrews DW. Still embedded together binding to membranes regulates Bcl-2 protein interactions. Oncogene 2010;29:5221–30.
31. Aranovich A, Liu Q, Collins T, Geng F, Dixit S, Leber B, et al. Differences in the mechanisms of proapoptotic BH3 proteins binding to Bcl-XL and Bcl-2 quantified in live MCF-7 cells. Mol Cell 2012;45:754–63.
32. Placzek WJ, Wei J, Kitada S, Zhai D, Reed JC, Pellecchia M. A survey of the anti-apoptotic Bcl-2 subfamily expression in cancer types provides a platform to predict the efficacy of Bcl-2 antagonists in cancer therapy. Cell Death Dis 2010;1:e40.
33. Nunez G, Seto M, Seremetis S, Ferrero D, Grignani F, Korsmeyer SJ, et al. Growth- and tumor-promoting effects of deregulated BCL2 in human B-lymphoblastoid cells. Proc Natl Acad Sci U S A 1989;86:4589–93.
34. Callagy GM, Pharoah PD, Pinder SE, Hsu FD, Nielsen TO, Ragaz J, et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Clin Cancer Res 2006;12:2468–75.
35. Bhargava V, Kell DL, van de Rijn M, Warnke RA. Bcl-2 immunoreactivity in breast carcinoma correlates with hormone receptor positivity. Am J Pathol 1994;145:535–40.
36. Catz SD, Johnson JL. BCL-2 in prostate cancer: a minireview. Apoptosis 2003;8:29–37.
37. Catz SD, Johnson JL. Transcriptional regulation of bcl-2 by nuclear factor kappa B and its significance in prostate cancer. Oncogene 2001;20:7342–51.
38. Hanada M, Delia D, Aiello A, Stadtmauer E, Reed JC. bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. Blood 1993;82:1820–8.
39. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
40. Cho-Vega JH, Rassidakis GZ, Admirand JH, Oyarzo M, Ramalingam P, Paraguya A, et al. MCL-1 expression in B-cell non-Hodgkin's lymphomas. Hum Pathol 2004;35:1095–100.
41. Song L, Coppola D, Livingston S, Cress D, Haura EB. Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non–small cell lung cancer cells. Cancer Biol Ther 2005;4:267–76.
42. Zhang H, Guttikonda S, Roberts L, Uziel T, Semizarov D, Elmore SW, et al. Mcl-1 is critical for survival in a subgroup of non–small-cell lung cancer cell lines. Oncogene 2011;30:1963–8.
43. Khodadoust MS, Verhaegen M, Kappes F, Riveiro-Falkenbach E, Cigudosa JC, Kim DS, et al. Melanoma proliferation and chemoresistance controlled by the DEK oncogene. Cancer Res 2009;69:6405–13.
44. Epling-Burnette PK, Liu JH, Catlett-Falcone R, Turkson J, Oshiro M, Kothapalli R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. J Clin Invest 2001;107:351–62.
45. Inuzuka H, Shaik S, Onoyama I, Gao D, Tseng A, Maser RS, et al. SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature 2011;471:104–9.
46. Wertz IE, Kusam S, Lam C, Okamoto T, Sandoval W, Anderson DJ, et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature 2011;471:110–4.
47. 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.
48. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol 2014;15:49–63.
49. Zhou P, Levy NB, Xie H, Qian L, Lee CY, Gascoyne RD, et al. MCL1 transgenic mice exhibit a high incidence of B-cell lymphoma manifested as a spectrum of histologic subtypes. Blood 2001;97:3902–9.
50. Strasser A, Harris AW, Bath ML, Cory S. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. Nature 1990;348:331–3.
51. Jager R, Herzer U, Schenkel J, Weiher H. Overexpression of Bcl-2 inhibits alveolar cell apoptosis during involution and accelerates c-myc-induced tumorigenesis of the mammary gland in transgenic mice. Oncogene 1997;15:1787–95.
52. Linden M, Kirchhof N, Carlson C, Van Ness B. Targeted overexpression of Bcl-XL in B-lymphoid cells results in lymphoproliferative disease and plasma cell malignancies. Blood 2004;103:2779–86.
53. Swanson PJ, Kuslak SL, Fang W, Tze L, Gaffney P, Selby S, et al. Fatal acute lymphoblastic leukemia in mice transgenic for B cell-restricted bcl-xL and c-myc. J Immunol 2004;172:6684–91.

54. Xiang Z, Luo H, Payton JE, Cain J, Ley TJ, Opferman JT, et al. Mcl1 haploinsufficiency protects mice from Myc-induced acute myeloid leukemia. J Clin Invest 2010;120:2109–18.
55. Letai A, Sorcinelli MD, Beard C, Korsmeyer SJ. Antiapoptotic BCL-2 is required for maintenance of a model leukemia. Cancer Cell 2004;6:241–9.
56. Zhang B, Gojo I, Fenton RG. Myeloid cell factor-1 is a critical survival factor for multiple myeloma. Blood 2002;99:1885–93.
57. Derenne S, Monia B, Dean NM, Taylor JK, Rapp MJ, Harousseau JL, et al. Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x(L) is an essential survival protein of human myeloma cells. Blood 2002;100:194–9.
58. 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.
59. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science 1999;286:1735–8.
60. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, et al. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. Nature 1999;400:886–91.
61. Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. Science 2003;302:1036–8.
62. Jeffers JR, Parganas E, Lee Y, Yang C, Wang J, Brennan J, et al. Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. Cancer Cell 2003;4:321–8.
63. Ranger AM, Zha J, Harada H, Datta SR, Danial NN, Gilmore AP, et al. Bad-deficient mice develop diffuse large B cell lymphoma. Proc Natl Acad Sci U S A 2003;100:9324–9.
64. Egle A, Harris AW, Bouillet P, Cory S. Bim is a suppressor of Myc-induced mouse B cell leukemia. Proc Natl Acad Sci U S A 2004;101:6164–9.
65. Katz SG, Labelle JL, Meng H, Valeriano RP, Fisher JK, Sun H, et al. Mantle Cell Lymphoma in Cyclin D1 Transgenic Mice with Bim-deficient B-cells. Blood 2013;123:884–93.
66. Tagawa H, Karnan S, Suzuki R, Matsuo K, Zhang X, Ota A, et al. Genome-wide array-based CGH for mantle cell lymphoma: identification of homozygous deletions of the proapoptotic gene BIM. Oncogene 2005;24:1348–58.
67. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001;7:683–94.
68. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. Mol Cell 2001;7:673–82.
69. Yu J, Wang Z, Kinzler KW, Vogelstein B, Zhang L. PUMA mediates the apoptotic response to p53 in colorectal cancer cells. Proc Natl Acad Sci U S A 2003;100:1931–6.
70. Sax JK, Fei P, Murphy ME, Bernhard E, Korsmeyer SJ, El-Deiry WS. BID regulation by p53 contributes to chemosensitivity. Nat Cell Biol 2002;4:842–9.
71. Donehower LA, Lozano G. 20 years studying p53 functions in genetically engineered mice. Nat Rev Cancer 2009;9:831–41.
72. Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 tumor suppressor gene: important milestones at the various steps of tumorigenesis. Genes Cancer 2011;2:466–74.
73. Karst AM, Dai DL, Martinka M, Li G. PUMA expression is significantly reduced in human cutaneous melanomas. Oncogene 2005;24:1111–6.
74. Yu H, McDaid R, Lee J, Possik P, Li L, Kumar SM, et al. The role of BRAF mutation and p53 inactivation during transformation of a subpopulation of primary human melanocytes. Am J Pathol 2009;174:2367–77.
75. Dehan E, Bassermann F, Guardavaccaro D, Vasiliver-Shamis G, Cohen M, Lowes KN, et al. betaTrCP- and Rsk1/2-mediated degradation of BimEL inhibits apoptosis. Molecular cell 2009;33:109–16.
76. Luciano F, Jacquel A, Colosetti P, Herrant M, Cagnol S, Pages G, et al. Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function. Oncogene 2003;22:6785–93.
77. Wiggins CM, Band H, Cook SJ. c-Cbl is not required for ERK1/2-dependent degradation of BimEL. Cell Signal 2007;19:2605–11.
78. Ewings KE, Hadfield-Moorhouse K, Wiggins CM, Wickenden JA, Balmanno K, Gilley R, et al. ERK1/2-dependent phosphorylation of BimEL promotes its rapid dissociation from Mcl-1 and Bcl-xL. EMBO J 2007;26:2856–67.
79. del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 1997;278:687–9.
80. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997;91:231–41.
81. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14–3-3 not BCL-X(L). Cell 1996;87:619–28.
82. She QB, Solit DB, Ye Q, O'Reilly KE, Lobo J, Rosen N. The BAD protein integrates survival signaling by EGFR/MAPK and PI3K/Akt kinase pathways in PTEN-deficient tumor cells. Cancer Cell 2005;8:287–97.
83. You H, Pellegrini M, Tsuchihara K, Yamamoto K, Hacker G, Erlacher M, et al. FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal. J Exp Med 2006;203:1657–63.
84. Cragg MS, Kuroda J, Puthalakath H, Huang DC, Strasser A. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med 2007;4:1681–89; discussion 90.
85. Deng J, Shimamura T, Perera S, Carlson NE, Cai D, Shapiro GI, et al. Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. Cancer Res 2007;67:11867–75.
86. Costa DB, Nguyen KS, Cho BC, Sequist LV, Jackman DM, Riely GJ, et al. Effects of erlotinib in EGFR mutated non–small cell lung cancers with resistance to gefitinib. Clin Cancer Res 2008;14:7060–7.
87. Gong Y, Somwar R, Politi K, Balak M, Chmielecki J, Jiang X, et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. PLoS Med 2007;4:1655–68.
88. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 2007;4:1669–79; discussion 80.
89. Cragg MS, Jansen ES, Cook M, Harris C, Strasser A, Scott CL. Treatment of B-RAF mutant human tumor cells with a MEK inhibitor requires Bim and is enhanced by a BH3 mimetic. J Clin Invest 2008;118:3651–9.
90. Hata AN, Yeo A, Faber AC, Lifshits E, Chen Z, Cheng KA, et al. Failure to induce apoptosis via BCL-2 family proteins underlies lack of efficacy of combined MEK and PI3K inhibitors for KRAS-mutant lung cancers. Cancer Res 2014;74:3146–56.
91. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl +leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci U S A 2006;103:14907–12.
92. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nature medicine 1996;2:561–6.
93. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001;344:1038–42.
94. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.

95. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004;305:1163–7.
96. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
97. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.
98. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non–small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121–8.
99. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non–small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239–46.
100. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non–small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8.
101. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507–16.
102. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. N Engl J Med 2010;363:1693–703.
103. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013;368:2385–94.
104. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med 2014;370:1189–97.
105. Bean GR, Ganesan YT, Dong Y, Takeda S, Liu H, Chan PM, et al. PUMA and BIM are required for oncogene inactivation-induced apoptosis. Science signaling 2013;6:ra20.
106. Wang YF, Jiang CC, Kiejda KA, Gillespie S, Zhang XD, Hersey P. Apoptosis induction in human melanoma cells by inhibition of MEK is caspase-independent and mediated by the Bcl-2 family members PUMA, Bim, and Mcl-1. Clin Cancer Res 2007;13:4934–42.
107. VanBrocklin MW, Verhaegen M, Soengas MS, Holmen SL. Mitogen-activated protein kinase inhibition induces translocation of Bmf to promote apoptosis in melanoma. Cancer Res 2009;69:1985–94.
108. Faber AC, Li D, Song Y, Liang MC, Yeap BY, Bronson RT, et al. Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. Proc Natl Acad Sci U S A 2009;106:19503–8.
109. Faber AC, Wong KK, Engelman JA. Differences underlying EGFR and HER2 oncogene addiction. Cell Cycle 2010;9:851–2.
110. Faber AC, Ebi H, Costa C, Engelman JA. Apoptosis in targeted therapy responses: the role of BIM. Adv Pharmacol 2012;65:519–42.
111. Aichberger KJ, Mayerhofer M, Krauth MT, Vales A, Kondo R, Derdak S, et al. Low-level expression of proapoptotic Bcl-2-interacting mediator in leukemic cells in patients with chronic myeloid leukemia: role of BCR/ABL, characterization of underlying signaling pathways, and reexpression by novel pharmacologic compounds. Cancer Res 2005;65:9436–44.
112. Kuribara R, Honda H, Matsui H, Shinjyo T, Inukai T, Sugita K, et al. Roles of Bim in apoptosis of normal and Bcr-Abl-expressing hematopoietic progenitors. Mol Cell Biol 2004;24:6172–83.
113. Essafi A, Fernandez de Mattos S, Hassen YA, Soeiro I, Mufti GJ, Thomas NS, et al. Direct transcriptional regulation of Bim by FoxO3a mediates STI571-induced apoptosis in Bcr-Abl-expressing cells. Oncogene 2005;24:2317–29.
114. Mukohara T, Engelman JA, Hanna NH, Yeap BY, Kobayashi S, Lindeman N, et al. Differential effects of gefitinib and cetuximab on non–small-cell lung cancers bearing epidermal growth factor receptor mutations. J Natl Cancer Inst 2005;97:1185–94.
115. Faber AC, Corcoran RB, Ebi H, Sequist LV, Waltman BA, Chung E, et al. BIM expression in treatment naïve cancers predicts responsiveness to kinase inhibitors. Cancer Discov 2011;1:352–65.
116. Takezawa K, Okamoto I, Nishio K, Janne PA, Nakagawa K. Role of ERK-BIM and STAT3-survivin signaling pathways in ALK inhibitor-induced apoptosis in EML4-ALK-positive lung cancer. Clin Cancer Res 2011;17:2140–8.
117. Wickenden JA, Jin H, Johnson M, Gillings AS, Newson C, Austin M, et al. Colorectal cancer cells with the BRAF(V600E) mutation are addicted to the ERK1/2 pathway for growth factor-independent survival and repression of BIM. Oncogene 2008;27:7150–61.
118. Deng J, Carlson N, Takeyama K, Dal Cin P, Shipp M, Letai A. BH3 profiling identifies three distinct classes of apoptotic blocks to predict response to ABT-737 and conventional chemotherapeutic agents. Cancer Cell 2007;12:171–85.
119. Ni Chonghaile T, Sarosiek KA, Vo TT, Ryan JA, Tammareddi A, Moore Vdel G, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. Science 2011;334:1129–33.
120. Vo TT, Ryan J, Carrasco R, Neuberg D, Rossi DJ, Stone RM, et al. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. Cell 2012;151:344–55.
121. Del Gaizo Moore V, Schlis KD, Sallan SE, Armstrong SA, Letai A. BCL-2 dependence and ABT-737 sensitivity in acute lymphoblastic leukemia. Blood 2008;111:2300–9.
122. Pan R, Hogdal LJ, Benito JM, Bucci D, Han L, Borthakur G, et al. Selective BCL-2 Inhibition by ABT-199 causes on target cell death in acute myeloid leukemia. Cancer Discov 2013;4:362–75.
123. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677–81.
124. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013;19:202–8.
125. Montero J, Sarosiek KA, DeAngelo JD, Maertens O, Ryan J, Ercan D, et al. Drug-induced death signaling strategy rapidly predicts cancer response to chemotherapy. Cell 2015;160:977–89.
126. Costa C, Molina MA, Drozdowskyj A, Gimenez-Capitan A, Bertran-Alamillo J, Karachaliou N, et al. The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. Clin Cancer Res 2014;20:2001–10.
127. Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 2012;18:521–8.
128. Isobe K, Hata Y, Tochigi N, Kaburaki K, Kobayashi H, Makino T, et al. Clinical significance of BIM deletion polymorphism in non–small-cell lung cancer with epidermal growth factor receptor mutation. J Thorac Oncol 2014;9:483–7.
129. Zhao M, Zhang Y, Cai W, Li J, Zhou F, Cheng N, et al. The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non–small cell lung cancer. Cancer 2014;120:2299–307.
130. Lee JH, Lin YL, Hsu WH, Chen HY, Chang YC, Yu CJ, et al. Bcl-2-like protein 11 deletion polymorphism predicts survival in advanced non–small-cell lung cancer. J Thorac Oncol 2014;9:1385–92.
131. Katagiri S, Umezu T, Ohyashiki JH, Ohyashiki K. The BCL2L11 (BIM) deletion polymorphism is a possible criterion for discontinuation of imatinib in chronic myeloid leukaemia patients. Br J Haematol 2013;160:269–71.
132. Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F. A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PLoS One 2013;8:e78582.
133. Varadarajan S, Vogler M, Butterworth M, Dinsdale D, Walensky LD, Cohen GM. Evaluation and critical assessment of putative MCL-1 inhibitors. Cell Death Differ 2013;20:1475–84.

134. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 2008;68:3421–8.
135. Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, 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 2012;30:488–96.
136. Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, 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 2012;18:3163–9.
137. Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, et al. Programmed anuclear cell death delimits platelet life span. Cell 2007;128:1173–86.
138. Seymour JF, Davids MS, Pagel JM, Kahl BS, Wierda WG, Puvvada S, et al. ABT-199 (GDC-0199) in relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL): high complete- response rate and durable disease control. J Clin Oncol 2014;32:5s.
139. Serasinghe MN, Missert DJ, Asciolla JJ, Podgrabinska S, Wieder SY, Izadmehr S, et al. Anti-apoptotic BCL-2 proteins govern cellular outcome following B-RAF inhibition and can be targeted to reduce resistance. Oncogene 2015;34:857–67.
140. Frederick DT, Salas Fragomeni RA, Schalck A, Ferreiro-Neira I, Hoff T, Cooper ZA, et al. Clinical profiling of BCL-2 family members in the setting of BRAF inhibition offers a rationale for targeting *de novo* resistance using BH3 mimetics. PLoS ONE 2014;9:e101286.
141. Corcoran RB, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM, et al. Synthetic Lethal Interaction of Combined BCL-XL and MEK Inhibition Promotes Tumor Regressions in KRAS Mutant Cancer Models. Cancer Cell 2013;23:121–8.
142. Sale MJ, Cook SJ. The BH3 mimetic ABT-263 synergizes with the MEK1/2 inhibitor selumetinib/AZD6244 to promote BIM-dependent tumour cell death and inhibit acquired resistance. Biochem J 2013;450:285–94.
143. Tan N, Wong M, Nannini MA, Hong R, Lee LB, Price S, et al. Bcl-2/Bcl-xL inhibition increases the efficacy of MEK inhibition alone and in combination with PI3 kinase inhibition in lung and pancreatic tumor models. Mol Cancer Ther 2013;12:853–64.
144. Ding Q, He X, Xia W, Hsu JM, Chen CT, Li LY, et al. Myeloid cell leukemia-1 inversely correlates with glycogen synthase kinase-3beta activity and associates with poor prognosis in human breast cancer. Cancer Res 2007;67:4564–71.
145. Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, Song K, et al. Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol 1996;148:1567–76.
146. Miyamoto Y, Hosotani R, Wada M, Lee JU, Koshiba T, Fujimoto K, et al. Immunohistochemical analysis of Bcl-2, Bax, Bcl-X, and Mcl-1 expression in pancreatic cancers. Oncology 1999;56:73–82.
147. Kaufmann SH, Karp JE, Svingen PA, Krajewski S, Burke PJ, Gore SD, et al. Elevated expression of the apoptotic regulator Mcl-1 at the time of leukemic relapse. Blood 1998;91:991–1000.
148. Yoshimoto G, Miyamoto T, Jabbarzadeh-Tabrizi S, Iino T, Rocnik JL, Kikushige Y, et al. FLT3-ITD up-regulates MCL-1 to promote survival of stem cells in acute myeloid leukemia via FLT3-ITD-specific STAT5 activation. Blood 2009;114:5034–43.
149. Wuilleme-Toumi S, Robillard N, Gomez P, Moreau P, Le Gouill S, Avet-Loiseau H, et al. Mcl-1 is overexpressed in multiple myeloma and associated with relapse and shorter survival. Leukemia 2005;19:1248–52.
150. Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006;10:375–88.
151. van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 2006;10:389–99.
152. Lin X, Morgan-Lappe S, Huang X, Li L, Zakula DM, Vernetti LA, et al. 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737. Oncogene 2007;26:3972–9.
153. Nguyen M, Marcellus RC, Roulston A, Watson M, Serfass L, Murthy Madiraju SR, et al. Small molecule obatoclax (GX15–070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. Proc Natl Acad Sci U S A 2007;104:19512–7.
154. O'Brien SM, Claxton DF, Crump M, Faderl S, Kipps T, Keating MJ, et al. 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.
155. Chiappori A, Williams C, Northfelt DW, Adams JW, Malik S, Edelman MJ, et al. Obatoclax mesylate, a pan-bcl-2 inhibitor, in combination with docetaxel in a phase 1/2 trial in relapsed non–small-cell lung cancer. J Thorac Oncol 2014;9:121–5.
156. Paik PK, Rudin CM, Pietanza MC, Brown A, Rizvi NA, Takebe N, 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.
157. Parikh SA, Kantarjian H, Schimmer A, Walsh W, Asatiani E, El-Shami K, et al. Phase II study of obatoclax mesylate (GX15–070), a small-molecule BCL-2 family antagonist, for patients with myelofibrosis. Clin Lymphoma Myeloma Leuk 2010;10:285–9.
158. McCoy F, Hurwitz J, McTavish N, Paul I, Barnes C, O'Hagan B, et al. Obatoclax induces Atg7-dependent autophagy independent of beclin-1 and BAX/BAK. Cell Death Dis 2010;1:e108.
159. Wei J, Stebbins JL, Kitada S, Dash R, Placzek W, Rega MF, et al. BI-97C1, an optically pure Apogossypol derivative as pan-active inhibitor of antiapoptotic B-cell lymphoma/leukemia-2 (Bcl-2) family proteins. J Med Chem 2010;53:4166–76.
160. Varadarajan S, Butterworth M, Wei J, Pellecchia M, Dinsdale D, Cohen GM. Sabutoclax (BI97C1) and BI112D1, putative inhibitors of MCL-1, induce mitochondrial fragmentation either upstream of or independent of apoptosis. Neoplasia 2013;15:568–78.
161. Belmar J, Fesik SW. Small molecule Mcl-1 inhibitors for the treatment of cancer. Pharmacol Ther 2015;145:76–84.
162. Friberg A, Vigil D, Zhao B, Daniels RN, Burke JP, Garcia-Barrantes PM, et al. Discovery of potent myeloid cell leukemia 1 (Mcl-1) inhibitors using fragment-based methods and structure-based design. J Med Chem 2013;56:15–30.
163. Petros AM, Swann SL, Song D, Swinger K, Park C, Zhang H, et al. Fragment-based discovery of potent inhibitors of the anti-apoptotic MCL-1 protein. Bioorg Med Chem Lett 2014;24:1484–8.
164. Bruncko M, Wang L, Sheppard GS, Phillips DC, Tahir SK, Xue J, et al. Structure-guided design of a series of MCL-1 inhibitors with high affinity and selectivity. J Med Chem 2015;58:2180–94.
165. Leverson JD, Zhang H, Chen J, Tahir SK, Phillips DC, Xue J, et al. Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax). Cell Death Dis 2015;6:e1590.
166. Wei G, Twomey D, Lamb J, Schlis K, Agarwal J, Stam RW, et al. Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. Cancer Cell 2006;10:331–42.
167. Chen MH, Yang WL, Lin KT, Liu CH, Liu YW, Huang KW, et al. Gene expression-based chemical genomics identifies potential therapeutic drugs in hepatocellular carcinoma. PLoS ONE 2011;6:e27186.
168. Muller A, Zang C, Chumduri C, Dorken B, Daniel PT, Scholz CW. Concurrent inhibition of PI3K and mTORC1/mTORC2 overcomes resistance to rapamycin induced apoptosis by down-regulation of Mcl-1 in mantle cell lymphoma. Int J Cancer 2013;133:1813–24.
169. Hsieh AC, Costa M, Zollo O, Davis C, Feldman ME, Testa JR, et al. Genetic dissection of the oncogenic mTOR pathway reveals druggable addiction to translational control via 4EBP-eIF4E. Cancer Cell 2010;17:249–61.
170. Faber AC, Coffee EM, Costa C, Dastur A, Ebi H, Hata AN, et al. mTOR inhibition specifically sensitizes colorectal cancers with KRAS or BRAF mutations to BCL-2/BCL-XL inhibition by suppressing MCL-1. Cancer Discov 2014;4:42–52.

171. Preuss E, Hugle M, Reimann R, Schlecht M, Fulda S. Pan-mammalian target of rapamycin (mTOR) inhibitor AZD8055 primes rhabdomyosarcoma cells for ABT-737-induced apoptosis by down-regulating Mcl-1 protein. J Biol Chem 2013;288:35287–96.
172. Faber AC, Farago AF, Costa C, Dastur A, Gomez-Caraballo M, Robbins R, et al. Assessment of ABT-263 activity across a cancer cell line collection leads to a potent combination therapy for small-cell lung cancer. Proc Natl Acad Sci U S A 2015;112:E1288–96.
173. Harada H, Grant S. Targeting the regulatory machinery of BIM for cancer therapy. Crit Rev Eukaryot Gene Expr 2012;22:117–29.
174. Ohtani-Fujita N, Fujita T, Aoike A, Osifchin NE, Robbins PD, Sakai T. CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. Oncogene 1993;8:1063–7.
175. San Jose-Eneriz E, Agirre X, Jimenez-Velasco A, Cordeu L, Martin V, Arqueros V, et al. Epigenetic down-regulation of BIM expression is associated with reduced optimal responses to imatinib treatment in chronic myeloid leukaemia. Eur J Cancer 2009;45:1877–89.
176. Richter-Larrea JA, Robles EF, Fresquet V, Beltran E, Rullan AJ, Agirre X, et al. Reversion of epigenetically mediated BIM silencing overcomes chemoresistance in Burkitt lymphoma. Blood 2010;116:2531–42.
177. Piazza R, Magistroni V, Mogavero A, Andreoni F, Ambrogio C, Chiarle R, et al. Epigenetic silencing of the proapoptotic gene BIM in anaplastic large cell lymphoma through an MeCP2/SIN3a deacetylating complex. Neoplasia 2013;15:511–22.
178. Bachmann PS, Piazza RG, Janes ME, Wong NC, Davies C, Mogavero A, et al. Epigenetic silencing of BIM in glucocorticoid poor-responsive pediatric acute lymphoblastic leukemia, and its reversal by histone deacetylase inhibition. Blood 2010;116:3013–22.
179. Inoue S, Riley J, Gant TW, Dyer MJ, Cohen GM. Apoptosis induced by histone deacetylase inhibitors in leukemic cells is mediated by Bim and Noxa. Leukemia 2007;21:1773–82.
180. Nakagawa T, Takeuchi S, Yamada T, Ebi H, Sano T, Nanjo S, et al. EGFR-TKI resistance due to BIM polymorphism can be circumvented in combination with HDAC inhibition. Cancer Res 2013;73:2428–34.
181. Corcoran RB, Rothenberg SM, Hata AN, Faber AC, Piris A, Nazarian RM, et al. TORC1 suppression predicts responsiveness to RAF and MEK inhibition in BRAF-mutant melanoma. Sci Transl Med 2013;5:196ra98.