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Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy

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The BCL-2 protein family determines whether cells commit to apoptosis, an ancient cell suicide program that is essential for development, tissue homeostasis and immunity. Too little apoptosis can promote cancer and autoimmune diseases; too much apoptosis can augment ischemic conditions and drive neurodegeneration. We discuss the biochemical, structural and genetic studies that have clarified how the interplay between members of the BCL-2 family on mitochondria sets the apoptotic threshold. These mechanistic insights into the functions of the BCL-2 family are illuminating the physiological control of apoptosis, the pathological consequences of its dysregulation and the promising search for novel cancer therapies that target the BCL-2 family.

In multi-cellular organisms, altruistic cell suicide has many essential roles[1]. In the vertebrate epiblast, cell death culls the less fit cells[2]. Later, it sculpts the embryo by deleting superfluous cells, for example by removing interdigital cells during limb formation and by hollowing out ducts. Also, excess neurons are deleted to match target cell numbers, and excess male germ cells must be eliminated in early spermatogenesis to avoid overwhelming their supporting Sertoli cells **[G]**. Throughout life, cell death must balance cell proliferation, particularly in tissues with high turnover, such as the haematopoietic system and intestinal epithelium. In the immune system, cells that are useless or dangerous because their antigen receptors recognize self-tissues must be eliminated. Cell death also drives duct formation in the mammary gland and involution at weaning, as well as thymus atrophy with aging. Finally, the programmed suicide of infected cells limits pathogen spread[3].

The major mode of programmed cell death, apoptosis, was first recognised morphologically[4]. In cells undergoing apoptosis, the nuclei and cytoplasm, including the mitochondria, shrink, and the cell contents typically become encased in 'apoptotic bodies' surrounded by plasma membrane. In response to "eat me" signals **[G]** on their surface, these corpses are rapidly engulfed by nearby phagocytic cells and digested in their lysosomes. The clearance system is so effective that little cell death is apparent even in tissues with high levels of apoptosis, such as the thymus[1]. By contrast, necrosis (passive cell death), which involves swelling of the mitochondria and rupture of the plasma membrane, releases inflammatory cellular contents that might provoke presentation of self-antigens in an immunogenic form and culminate in autoimmune

tissue damage[5]. Apoptosis has been well studied in vertebrates, the flatworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Recently, other forms of programmed cell death, such as necroptosis **[G]**, have been recognized, but they have been expertly reviewed elsewhere[6, 7] and will not be addressed here.

Molecular insights into apoptosis first emerged during the 1980s and 1990s from a remarkable convergence of mammalian cancer cytogenetics and biochemistry with pioneering genetic studies on developmentally programmed cell death in *C. elegans*. The previously unknown gene *BCL2* was identified from the breakpoint region of a recurrent chromosomal translocation in human follicular lymphoma[8]. In seminal studies, enforced BCL-2 expression rendered haematopoietic cells refractory to death induced by cytokine deprivation[9] and promoted lymphocyte accumulation in mice[10-12], often culminating in autoimmune disease or cancer (see below). Notably, the function of CED-9, which prevents developmental cell death in *C. elegans*[13], could be replaced by human BCL-2[14, 15]. Similarly, the worm killer protein CED-3[16] resembled the mammalian aspartate-specific cysteine proteases, later to be known as caspases. Subsequent elegant biochemical studies showed that the mammalian proteolytic cascade induced by the BCL-2-regulated pathway starts on the scaffold protein APAF1[17], which is the homologue of worm CED-4[18], in response to cytochrome *c* released from mitochondria[17, 19]. These discoveries outlined a dedicated, evolutionarily conserved genetic programme to ensure the tidy (non-inflammatory) removal of unwanted cells.

This Review focuses on the commitment to apoptosis in vertebrates, as this pivotal step differs substantially in mechanism and complexity from that in invertebrates, and is highly germane to human pathology. We concentrate on the mitochondrial (also known as intrinsic, BCL-2-regulated or stress) pathway to cell death, in which the apoptotic threshold is set by interactions on the mitochondrial outer membrane between three functionally and structurally distinct subgroups of the BCL-2 protein family: the BH3-only proteins, which convey initiating apoptotic signals; the pro-survival cell guardians such as BCL-2 itself; and the pro-apoptotic effector proteins BAX and BAK (Figure 1). Thus, this family can be regarded as a tripartite apoptotic switch. When sufficient activation of BH3-only proteins occurs in response to various cytotoxic stresses to exceed the apoptotic threshold, BAX and BAK begin to form the oligomers that permeabilise the mitochondrial outer membrane. This releases apoptogenic factors into the cytosol, including particularly

cytochrome *c*[17, 19], which promotes the activation of apical caspase-9 on APAF1[20]; activated caspase-9 in turn processes and activates the effector caspases. We mention only briefly the independent but convergent death receptor-mediated (also known as extrinsic) pathway, which is triggered when certain members of the tumour necrosis factor receptor (TNFR) family are ligated on the plasma membrane and proceeds through the activation of caspase-8 to activation of the effector caspases (Figure 1), as that pathway has been reviewed recently elsewhere[21]. We do not address the structural aspects of caspase activation[22] or the downstream events that they induce[23].

We first describe the recent molecular insights, illuminated by structural studies, into how the BCL-2 family sets the apoptotic threshold and regulates commitment to apoptosis. We briefly consider how regulatory signals impinge on the family, and then address its physiological functions in cell death control, its roles in the development of pathology[24, 25] and the impact on cancer therapy. We briefly note the proposed involvement of the BCL-2 family in several non-apoptotic processes, discussed further in other reviews[26-29]. Finally, we describe how the insights on BCL-2 family structure and function are guiding the development of therapeutics that target BCL-2 family members to improve the treatment of cancer, and potentially also certain autoimmune and infectious diseases.

The tripartite BCL-2 apoptotic switch

Proteins of the BCL-2 family have blocks of sequence homology known as BCL-2 homology (BH) domains (Figure 2a). Taking into account both structural and sequence homology, it is now clear that the six human pro-survival family members (BCL-2 itself, BCL-X_L, BCL-W, MCL1, A1 (also known as BFL1) and BCL-B), as well as the pro-apoptotic effector proteins BAX, BAK and BOK, share four BH domains [30] and adopt similar globular structures: a helical bundle surrounding a central hydrophobic core helix (α5)[31] (Figure 2b, c). This fold generates a hydrophobic surface groove delineated by α-helices 2, 3, 4 and 5. This groove constitutes a crucial interface for interactions with the BH3 domain of pro-apoptotic members of the BCL-2 family, such as BIM (Figure 2d). These interactions occur primarily on intracellular membranes, particularly the mitochondrial outer membrane, to which many family members are directed by a C-terminal hydrophobic trans-membrane domain (Figure 2a).

Unlike the globular multi-BH domain family members, most BH3-only proteins (Figure 2a) are intrinsically disordered[32], although their BH3 region becomes an amphipathic helix upon interaction with protein partners (Figure 2d). BID, which links the death receptor-mediated apoptotic pathway to the mitochondrial apoptotic pathway to amplify caspase activation[33-35] (Figure 1), is the structural exception. Its fold resembles the multi-BH domain members, with key BH3 residues buried and thus inactive [36, 37]. Cleavage of BID, for example by caspase-8, generates the active truncated form (tBID), in which the BH3 domain is exposed. It is possible that under certain circumstances intact BID might function in a BAX- or BAK-like manner.

Interactions between BCL-2 family members. BH3-only proteins, which are induced transcriptionally or post-translationally (see below) by cytotoxic stress signals (reviewed in [38]), carry out their pro-apoptotic function by two mechanisms[39-41]: neutralisation of the pro-survival BCL-2 family proteins[42-44] and direct activation of the pro-apoptotic effectors BAX and BAK[45-48] (Box 1). Neutralisation of the pro-survival proteins has been well characterised, both structurally and functionally, and has informed the development of novel therapeutics that target these proteins. The BH3 amphipathic helix of BH3-only proteins binds the hydrophobic groove of pro-survival proteins predominantly by the insertion of four hydrophobic residues (h1–h4) along one face into hydrophobic pockets in the groove, and by a salt bridge between a conserved BH3 aspartyl residue and a conserved arginyl residue in the BH1 domain of the pro-survival proteins[49-51] (Figure 2d). However, mutational studies suggest that interactions across the entire interface contribute to binding[52, 53]. Owing to subtle differences in their BH3 domains, and in the grooves of the pro-survival proteins, some BH3-only proteins (such as BAD and NOXA) are selective for subsets of their pro-survival relatives, whereas others, particularly BIM, tBID and PUMA, probably neutralise all of the pro-survival proteins[39, 42, 45] (Figure 2e). Interestingly, structural studies have revealed that the BH3-binding groove of the pro-survival BCL-2 family members has considerable plasticity[54, 55], which probably contributes to their ability to associate with multiple distinct BH3 domains (Figure 2e, 2f).

Pro-survival proteins can also bind the BH3 domains of activated BAX and BAK (see below) and thereby restrain their pro-apoptotic activity[56-58]. All of the pro-survival proteins seem to bind BAX, but BAK seems to be restrained mainly by MCL1, A1 and BCL-X_L[43, 44, 59] (Figure 2f). The interfaces in complexes of pro-

survival proteins with BAX or BAK BH3 peptides closely resemble those in their complexes with the BH3 peptides of BH3-only proteins[49, 60, 61]. This suggests a simple competition model for apoptotic regulation, whereby increased BH3-only protein levels (resulting in increased binding of pro-survival proteins) prevent or overcome the restraint of activated BAX or BAK by pro-survival proteins[42-44]. An alternative, but not mutually exclusive, model (Box 1) suggests that the pro-survival proteins prevent apoptosis by neutralising the BH3-only proteins that can directly activate BAX and BAK[39, 45-47, 62]. Indeed, genetic and biochemical evidence supports the idea that both models operate within a tripartite network of interactions between the three sub-groups of the BCL-2 family[40, 41]. The dominant pathway may vary with biological context, such as non-transformed cells versus tumour cells, or according to the cell death stimulus.

Activation of BAX and BAK. In healthy cells, BAK resides on the mitochondria, with its trans-membrane domain (α9) spanning the outer membrane[63]. By contrast, BAX is primarily cytosolic in healthy cells, with its trans-membrane domain tucked within its canonical hydrophobic groove[64] (Figure 2c), but cytotoxic signals promote the accumulation of BAX on the mitochondria[64]. Recent evidence suggests that BAX regularly cycles to the outer mitochondrial membrane in healthy cells but is then translocated back, possibly by interactions with pro-survival proteins such as BCL-X_L[65, 66]. Hence, the mitochondrial enrichment of BAX during apoptosis may reflect either the activation of BAX in the cytosol, probably by a BH3-only protein (see below), or simply a shift in this equilibrium, such as less retro-translocation of BAX from the mitochondria.

During apoptosis, BAX and BAK change their structure from inert monomers with folds resembling the pro-survival proteins (Figure 2b,c) into homo-oligomers of unknown structure that can permeabilise the mitochondrial outer membrane. This transition can be driven by so-called activator BH3-only proteins **[G] (Box 1)**, such as BIM or tBID, and, experimentally, even by their BH3 peptides. For BAX, two distinct activation sites have been proposed: the canonical hydrophobic groove homologous to that of pro-survival proteins[67] (Figure 3a) and an alternative site on the opposite side of BAX[48] (Figure 3b).

Insights into the activation mechanism emerged recently from studies of a form of BAX truncated to remove its trans-membrane domain, BAX(ΔTM)[67]; this form mimics full-length BAX when its tail has inserted into the mitochondrial membrane. Notably, BAX(ΔTM) treated with a BID BH3 peptide (but not with BH3 peptides from so-called sensitiser BH3-only proteins **[G],** such as BAD (Box 1)), together with ionic detergent, yielded crystals with the BID peptide bound in the groove of BAX (Figure 3a). Mutagenesis studies based on this structure have defined the characteristics that distinguish 'activator' from 'sensitiser' BH3 domains and have enabled the design of novel sequences with activator function[67]. For example, activation by BID BH3 requires two isoleucines near its N terminus at 'h0', a few residues before the canonical BH3 region, h1–h4 (Figure 3a).

The BAX 'rear' activation site, which involves helices α1 and α6 (Figure 3b), was suggested by NMR studies using activator BH3 peptides modified with a hydrocarbon staple **[G]** and full-length BAX, which has its trans-membrane domain located within its canonical hydrophobic groove[48, 68]. The rear activation site might be a trigger for extruding the BAX trans-membrane domain from the groove and thereby accelerating the basal shuttling of BAX between the cytosol (inert BAX) and the outer mitochondrial membrane (activated BAX). The groove activation site may then drive subsequent transitions, as some biochemical evidence suggests[62, 68, 69]. However, the failure of mutations of the rear activation site to abolish apoptosis induction[70, 71] indicates that it might not be essential for BAX activation.

In the case of BAK, several groups have identified its canonical surface groove as the sole interaction site for activating BH3 domains [69, 72-74]. The complex of BAK with a bound stapled BID BH3 domain[74] closely resembles that of BAX with a bound BID BH3 peptide[67].

Conversion of BAX and BAK into killers. After cells receive a cytotoxic stimulus, early structural transitions in BAX and BAK, presumably driven by activator BH3-only proteins (Box 1), include the exposure of cryptic N-terminal residues[63, 75] and transient exposure of their BH3 domain before its insertion into the groove of a neighbouring BAX or BAK protein[74, 76-78]. The structure of BAX bound to an activating BH3 domain[67] unexpectedly revealed that this interaction produces a destabilising cavity inside BAX near the BAX BH3 domain, which might promote

extrusion of the BH3 domain, and that BAX rearranges into a 'core' domain consisting of α2–α5 (and possibly α1) and a 'latch' domain comprising α6–α8 (Figure 4). These observations are consistent with the transitory exposure of the BAX and BAK BH3 domains during commitment to apoptosis[76, 77] and the importance of BAX helices α2–α5 in oligomerisation[79]. BH3 peptide binding to BAK also produces N-terminal exposure and oligomerisation[73, 74, 78], but whether BAK undergoes an equivalent core–latch disengagement remains to be determined.

Cross-linking and biophysical studies have suggested several models of the interfaces within BAX and BAK oligomers[76, 77, 80-83]. In the best supported model[76], two activated BAK (or BAX) molecules each insert their BH3 domain into the canonical groove of the other molecule to form a novel 'symmetric' dimer[76, 77, 81, 82]. Crystallographic studies have shown the structure of the BAX core domain in such a BH3-in-groove dimer[67] (Figure 4, step 5). The presence of symmetric dimers within the BAX and BAK homo-oligomers argues against proposed head-to-tail ("daisy-chain") models of oligomerisation[84, 85]. Instead, the dimers must join by a second interface to build the oligomers[76, 80, 86]. Crosslinking studies have identified parallel α6 helices at or near a second interface[77, 80], but that interface and the structure of larger oligomers remain to be defined.

How BAX and BAK oligomers engage and perforate the outer mitochondrial membrane also remains unresolved. Cysteine-labelling studies led to the proposal that the BAX α5 and α6 helices insert as a hairpin into the mitochondrial outer membrane early in apoptosis[87], but this seems incompatible with the BH3-in-groove symmetric dimer structure (Figure 4), particularly because BAX with α5 pinned to α6 lost apoptotic function[67]. It seems pertinent that the BAX core BH3-in-groove symmetric dimer has a planar surface of α4 and α5 chains exposing multiple aromatic residues[67] (Figure 4). If this surface sits on the membrane in the BAX oligomers and inserts those side-chains between lipid head-groups in the outer membrane leaflet, the excess bulk in that leaflet might increase membrane tension and curvature. This could promote stable bilayer breaks[88], leading to proteolipidic pores (ones not bounded entirely by protein)[67]. Recent reports indicate that substantial oligomerization of BAK may precede pore formation[78] and that oligomer mass might determine pore size[89].

Thus, the cardinal interaction in the BCL-2 family is that of the BH3 domain of a pro-apoptotic family member, for example a BH3-only protein, with the surface

groove of a multi-domain relative. Whereas the high-affinity binding of a BH3-only protein to a pro-survival protein produces a stable complex, the lower-affinity, transient binding of certain BH3-only proteins to the groove of BAX or BAK instead leads to major structural transitions in them (Figure 4). Significantly, these conformational changes include the 'release' of their own BH3 domain, which can then engage the groove of another activated BAX or BAK molecule to form the symmetric dimers that constitute the central element of the oligomers that perforate the outer mitochondrial membrane. The understanding of the BH3–groove interaction, together with genetic insights into the physiological roles of BCL-2 family members that we address below, has galvanised the search for drugs that target BCL-2 family members (discussed last).

Signals regulating the BCL-2 family

Whereas the regulation of BCL-2 family proteins through interactions with other members of the family is becoming well defined (see above and Box 1), their control by signals from proteins outside the family remains poorly understood. For example, a great many unrelated proteins have been reported to bind to BCL-2 family members, but few such putative interactions have survived more detailed scrutiny. The reported interaction of a cytosolic form of the tumour suppressor p53 with several pro-survival BCL-2 family proteins and BAX still draws attention[90], but its physiological significance is questionable, because genetic data indicate that the ability of p53 to induce *PUMA* and *NOXA* transcription can account for all of its pro-apoptotic function[91]. The intriguing interaction on the ER between pro-survival BCL-2 family members and BECLIN1 (Box 2) seems to inhibit autophagy rather than drive apoptosis, because the BECLIN1 BH3 domain binds the pro-survival proteins only weakly. How NAF1 associates with BCL-2 and augments this interaction[92] (Box 2) remains to be defined.

BCL-2 family proteins are subject to many post-translational modifications, particularly phosphorylation and ubiquitylation, but the biological consequences are often controversial, as is the identity of the relevant kinases, phosphatases and ubiquitin ligases[93]. For example, the pro-survival BCL-2 family members are phosphorylated predominantly at several Ser or Thr residues within their flexible $\alpha 1 - \alpha 2$ loop, but these modifications (often of the same residues) have been variously

linked to decreased or increased pro-survival activity, increased or decreased protein stability, entry into mitosis (e.g. upon taxane treatment), ability to associate with other proteins and sub-cellular localisation[93, 94]. Some clarity is provided by recent evidence that phosphorylation of BCL-2 at Ser70 or several other loop residues alters the loop conformation, which greatly enhances binding to BAK and BIM and renders cells more refractory to chemotherapeutic agents[95]. Presumably different loop conformations affect the association of BCL-2 with other proteins.

MCL-1 is subject to exquisite regulation, in terms of its transcription, translation and protein turnover (half life < 30 min)[29, 96]. MCL-1 can be degraded by a ubiquitin-independent pathway, or targeted to the proteasome by several ubiquitin ligases (such as MULE, β-TrCP and FBW7) or stabilised by the deubiquitylase USP9X[29, 96-98]. The N-terminal region also affects MCL-1 stability, and excision of an N-terminal segment generates an isoform of MCL-1 that is found in the mitochondrial matrix and has been implicated in mitochondrial structure and function[29, 99] (Box 2).

The BH3-only proteins are regulated in diverse ways[93, 100]. The transcription of *PUMA* and *NOXA* is regulated by p53, but both can also be up-regulated in a p53-independent manner. The Forkhead box transcription factor FOXO3a is implicated in the induction of *BIM* and *PUMA* transcription, but recent *in vivo* studies question its physiological relevance for *BIM* transcription[101]. Post-translational modifications of the BH3-only proteins are common. Several proteases, most notably caspase-8, process BID into the active tBid (Figure 1). BAD is phosphorylated and sequestered by 14-3-3 proteins in the cytosol, but the biological effects of phosphorylation of most BH3-only proteins are controversial[93, 100]. For example, BIM_{EL}, the most abundant BIM isoform, is thought to be phosphorylated by ERK1 and ERK2 (at Ser65 in the mouse) and thereby targeted for proteasomal degradation, but removal of the ERK docking site by *in situ* modification of the *Bim* locus did not have any effect on cell populations in which BIM has crucial roles[102].

Collectively, these results suggest that a definitive understanding of how upstream signals impinge on the BCL-2 family may require physiological studies in normal cell populations after *in situ* gene modification in mice.

The BCL-2 family in normal physiology

Studies using transgenic and gene-targeted mice have clarified the functions of many BCL-2 family members in both normal physiology and diverse pathological settings. Collectively, they leave little doubt that these proteins control the survival of all mammalian cells. Some biochemical findings have led to proposals that family members also participate in non-apoptotic processes, including mitochondrial fission and fusion, autophagy and mitochondrial function (Box 2), as discussed further in other reviews[26-29], but physiological evidence for those links remains limited.

The pro-survival proteins. When over-expressed in transgenic mice, all pro-survival BCL-2 family members render many cell types resistant to diverse apoptotic stimuli[10, 12, 103]. Conversely, constitutive or conditional gene deletion has identified crucial roles for specific family members in distinct cell populations, presumably those in which the remaining expressed pro-survival BCL-2 family members cannot maintain the apoptotic threshold. BCL-2 ablation causes fatal polycystic kidney disease, owing to the death of renal epithelial progenitors, and it depletes mature lymphoid cells and melanocyte progenitors[104]. Remarkably, concomitant loss of the BH3-only protein BIM rescues all these defects[105]. BCL-X_L-deficient mice die near day 14 of embryogenesis owing to defective foetal erythropoiesis and neuronal attrition[106]. Moreover, loss of even one *BCL-X* allele impairs spermatogenesis[107] and reduces platelet numbers[108], whereas conditional loss of both alleles kills hepatocytes, producing liver fibrosis[109]. Concomitant BIM loss rescues the testicular and erythroid defects[110], but not the excess neuronal death, which may be driven by other BH3-only proteins. BCL-W-deficient mice have only a spermatogenic defect[111, 112], probably reflecting death of supporting Sertoli cells[113].

MCL1 ablation has the greatest physiological effect. Its constitutive loss precludes implantation, although whether this results from excess apoptosis remains unknown[114]. Conditional MCL1 deletion has revealed its crucial role in the survival of diverse cell types, including haematopoietic stem and progenitor cells[115, 116], as well as effector lymphocytes[117, 118]. Remarkably, conditional MCL1 deletion in cardiomyocytes rapidly provokes cardiomyopathy and heart failure[119, 120]. These defects can be attributed to cardiomyocyte apoptosis (and secondary necrosis), because concomitant deletion of the pro-apoptotic effectors BAX and BAK precluded them; however, certain mitochondrial defects remained[119], implicating MCL1 in an additional, non-cell death-related mitochondrial role (Box 2).

The functions of A1 (also known as BFL1 in humans) remain ill-defined, due to the difficulty of targeting the three functional and chromosomally closely linked *A1* genes in mice[121]. Nevertheless, loss of the *A1a* gene impaired granulocyte and mast cell survival *ex vivo*[121], and transgenic mice expressing a short hairpin RNA targeting all expressed *A1* genes also had a lymphocyte deficit[122]. Mouse knockout studies cannot clarify the roles of human BCL-B, because its mouse homologue (Boo/Diva) bears inactivating mutations that are not present in BCL-B[123].

The BH3-only proteins. Over-expressed BH3-only proteins, particularly those (BIM, BID and PUMA) that target all pro-survival BCL-2 family members (Figure 3a), can trigger apoptosis (for example [42]). Gene disruption has defined their essential individual roles and some overlapping functions (reviewed in detail elsewhere[24, 124]). In general, loss of the BH3-only proteins that bind all pro-survival proteins and/or directly activate BAX and BAK provokes major abnormalities, whereas ablation of individual more selective binders (such as BAD and BIK) has only limited impact. Thus, ablation of BIM, which is activated by cytokine withdrawal, deregulated calcium flux and certain other apoptotic stimuli, produces excess lymphoid and myeloid cells, showing its role in normal haematopoietic homeostasis[125], as well as in deletion of auto-reactive lymphocytes[126, 127] and shutdown of immune responses after clearance of an infection[128-131]. As BID is activated by the death receptor pathway (Figure 1), BID-deficient mice are spared the lethal hepatitis that is normally induced by activation of FAS[33, 34] or TNFR1[132].

PUMA and *NOXA* are direct transcriptional targets of the tumour suppressor protein p53[133, 134]. Accordingly, PUMA is crucial for the DNA damage-induced apoptosis of diverse cell types, particularly lymphocytes[135, 136]; NOXA assists in the lymphocyte attrition[135, 137] and has the greater role in the death of fibroblasts and keratinocytes induced by UV radiation[138]. Notably, PUMA and NOXA seem to account for all of the p53-induced pro-apoptotic activity, as their combined loss renders lymphocytes as resistant to apoptosis induced by γ-irradiation as does p53 loss[91]. PUMA and NOXA also drive the death of primordial follicle oocytes following DNA damage, a process mediated by the p53 relative p63[139]. PUMA also mediates the effects of several p53-independent apoptotic stimuli, including cytokine deprivation and treatment with glucocorticoids or phorbol ester[135, 136, 140].

Following BMF loss, B cells accumulate and are resistant to glucocorticoids and certain other pro-apoptotic stimuli[141]. Although BIK deficiency provokes no overt abnormalities[142], concomitant loss of BIM renders males infertile[143], because normal spermatogenesis requires apoptosis of excess primordial progenitors to adjust their numbers to their supporting Sertoli cells. BAD loss reportedly rendered fibroblasts slightly resistant to the pro-apoptotic effects of glucose or cytokine deprivation, but BAD-deficient mice seem largely normal[144], except for a small increase in the number of platelets[145, 146]. HRK seems to be expressed only in neurons, but HRK-deficient neurons have only minor apoptotic defects[147, 148].

Consistent with the ability of BIM and PUMA to neutralise multiple pro-survival relatives (Figure 3a) and/or activate BAX and BAK, mice lacking both BIM and PUMA have exacerbated apoptotic defects[149] and animals also lacking BID may have even more profound abnormalities[150].

The BAX-like proteins. Mice lacking BAK appear normal[151] but have excess platelets due to increased platelet lifespan[108]. *Bax*$^{-/-}$ mice also appear normal, but the males are sterile owing to survival of excess primordial germ cells, which impairs subsequent spermatogenesis (see above). BAX and BAK double-knockout mice, however, have profound abnormalities: most die perinatally (for still unknown reasons), and the few long-term survivors all develop lymphadenopathy and fatal systemic autoimmune disease[151, 152]. All *Bax*$^{-/-}$*Bak*$^{-/-}$ mice have webbed feet and many females have imperforate vagina, which are classic defects in developmentally programmed cell death[1, 6]. Diverse cell types from *Bax*$^{-/-}$*Bak*$^{-/-}$ mice are profoundly resistant to multiple apoptotic stimuli[151], including enforced expression of BH3-only proteins[153, 154]. Thus, the BH3-only proteins act upstream of BAX and BAK (Figure 1) and cannot cause cell death unless BAX or BAK is present.

Although BAX and BAK have essential and largely overlapping roles in apoptosis, several tissues that are thought to be shaped by apoptosis (such as luminal structures) seem normal in *Bax*$^{-/-}$*Bak*$^{-/-}$ mice. Does their close relative BOK contribute? Although mice lacking BOK[155] and even those lacking BOK and either BAX or BAK[156] are largely normal, the increased numbers of primordial follicle oocytes in aged BOK and BAX double-knockout females provides the first physiological indication that BOK has a pro-apoptotic function[156]. Analysis of BAX,

BAK and BOK triple-knockout mice will be required to reveal the overall contribution of the BCL-2-regulated apoptotic pathway to morphogenesis, but cell removal during development might also involve the death receptor pathway, non-apoptotic cell death mechanisms (such as necroptosis) or even non-death-related processes[6].

Collectively, the genetic manipulation of the BCL-2 family supports the concepts that all mammalian cells are poised to undergo cell death; that their survival depends on one or more pro-survival family member, BCL-X_L or MCL1 often being crucial; that BAX or BAK is an essential effector of apoptosis; that particular BH3-only proteins drive the responses to specific cytotoxic insults and developmental cues, BIM and PUMA often being essential; and that tissue homeostasis is determined primarily by the balance between the levels of BH3-only proteins and their pro-survival relatives.

Pathological roles of the BCL-2 family

Defects in the mitochondrial apoptotic pathway are strongly implicated in the development of several diseases, particularly cancer and autoimmunity. Over-expression of pro-survival BCL-2 causes a low incidence of lymphoma in transgenic mice[157, 158] and accelerates tumorigenesis driven by dysregulated expression of MYC and certain other oncogenes[9, 159]. Loss of certain pro-apoptotic BCL-2 family members, particularly BIM[160], PUMA[161, 162], BAD[163], BMF[163] and BAX[164], also accelerates tumorigenesis driven by MYC or certain other oncogenic lesions. Blocks to apoptosis presumably promote tumorigenesis by keeping cells alive long enough to acquire oncogenic mutations that drive their neoplastic progression, as well as by countering the apoptosis that oncogenes, such as *MYC*, drive. Accordingly, in humans, BCL-2 is over-expressed as a result of a t14;18 chromosomal translocation in ~90% of follicular centre B cell lymphomas[165]; *MCL1* or *BCLX* is amplified in diverse tumours[166]; both *BIM* alleles are deleted in 17% of mantle cell B lymphomas[167]; and *BIM* or *PUMA* expression is decreased in diverse malignancies owing to promoter hyper-methylation or other causes[161, 168].

One important checkpoint against autoimmune disease is the apoptosis of autoreactive T and B cells[169]. Accordingly, BCL-2 over-expression in B cells of

mice[11], loss of BIM[125] or of both BAX and BAK[152, 170], can provoke a fatal autoimmune kidney disease resembling human systemic lupus erythematosus.

Impact on therapy. Defects in the mitochondrial apoptotic pathway also contribute to the resistance of diverse tumours to cytotoxic drugs. For example, the expression of PUMA, NOXA and, surprisingly given that it is not a p53 target gene, BIM determines the response of lymphoma cells to DNA damage-inducing drugs[171]. BIM expression is also required for the killing of tumour cells by glucocorticoids[140, 172], paclitaxel[173], inhibitors of oncogenic kinases (such as imatinib for BCR-ABL[174] and Tarceva/Iressa for mutant epidermal growth factor receptor (EGFR)[175, 176]), or shutdown of the mutant B-RAF signalling pathway[177]. Accordingly, a human *BIM* splice site polymorphism causes resistance to imatinib in chronic myeloid leukaemia and to Tarceva/Iressa in lung cancers with EGFR mutations[178]. Thus, novel strategies to efficiently activate endogenous BH3-only proteins, or to introduce small molecules mimicking their function (see below), might improve treatment for diverse cancers.

BCL-2 proteins as therapeutic targets

Targeting the regulation or function of pro-survival BCL-2 family members has considerable appeal. Although cancer therapeutics can induce several types of cell death, activation of the BCL-2-regulated apoptotic pathway seems to be crucial for the therapeutic efficacy of most (perhaps all) conventional cytotoxic agents and inhibitors of oncogenic kinases. This process is, however, often blunted in tumours. For example, p53 mutation, which is prevalent in diverse tumour types, impairs the induction of PUMA and NOXA in response to DNA damage-inducing chemotherapeutics, and many lymphoid malignancies have increased levels of BCL-2 (see above). Somewhat paradoxically, however, most, if not all, tumours seem to be more 'primed to die' than their normal counterparts, probably due to increased levels of BH3-only proteins [25, 45, 179]. For example, lymphoid cells expressing high levels of pro-survival BCL-2 accumulate increased levels of pro-apoptotic BIM[179, 180], probably because the excess BCL-2 keeps alive stressed cells that induce BIM. Notably, the sensitivity of mitochondria from tumour cells to disruption by a BIM BH3 peptide correlates well with the success of conventional therapies[181, 182]. Thus,

many types of tumour cell should be vulnerable to novel therapies that directly turn on apoptosis.

Such considerations have galvanised the search for compounds ('BH3 mimetics') that induce apoptosis by mimicking the function of the BH3 domain. However, the long, shallow and mainly hydrophobic groove of the pro-survival BCL-2 proteins, to which the BH3 domain binds (Figure 2), is very challenging to target. Although numerous compounds reportedly bind various BCL-2 family members, most have only moderate affinity and nearly all seem to kill cells predominantly by off-target effects not requiring BAX or BAK[183, 184].

BH3 mimetic potential and targets. The potential of BH3 mimetics in cancer therapy has been amply demonstrated by ABT-737[185] and its orally available clinical derivative ABT-263 (now navitoclax)[186], which both bind avidly to BCL-2, BCL-X_L and BCL-W, but not significantly to MCL1 or A1. Accordingly, these agents have little activity against tumour cells with high levels of MCL1 or A1[183]. Surprisingly, their crucial intracellular target, at least in lymphoid cells, is not unoccupied BCL-2 but BIM–BCL-2 complexes[179, 180]. Furthermore, these BH3 mimetics disrupt BIM–BCL-2 complexes much more efficiently than BIM–BCL-X_L or BIM–BCL-W complexes, explaining why high BCL-2 levels promote sensitivity to ABT-737, whereas increased levels of BCL-X_L, similarly to increased MCL1 or A1, instead confer resistance[180]. To kill lymphoid cells, the BIM that is released by binding of ABT-737 to BCL-2 apparently must neutralise pro-survival MCL1 in these cells[180] and might also directly activate BAX or BAK[179]. The identification of BIM-BCL-2 complexes as the crucial target of navitoclax in lymphoid malignancies indicates that effective BH3 mimetics may require affinities for BCL-2 comparable to those of its natural ligands, such as BIM (low nM or even sub-nM), in order to efficiently displace these ligands and trigger apoptosis.

Early clinical trials of navitoclax as a single agent, focused on blood cell cancers or small cell lung cancer, have shown promise, particularly in chronic lymphocytic leukaemia[187]. Moreover, recent pre-clinical studies suggest that combining navitoclax with other anti-cancer drugs, either conventional agents[188, 189] or targeted therapies[190], can enhance efficacy, including in the treatment of diverse solid tumours. Many conventional agents probably synergise with navitoclax by

decreasing the level of MCL1, which has a high turnover at both the mRNA and protein levels[191]. Combination of navitoclax with agents that specifically target tumour cells, such as inhibitors of oncogenic kinases, should have particular promise[190].

The dose-limiting toxicity with navitoclax is a transient acute drop in the number of platelets[187], due to its inhibition of BCL-X_L, which controls platelet lifespan[108]. A BH3 mimetic that targets only BCL-2 should obviate this problem. Indeed, the recently developed ABT-199[192], which targets only BCL-2, appears from preclinical studies and experience with the first few treated patients with chronic lymphocytic leukaemia to be at least as potent as navitoclax but without decreasing platelet levels[192-194]. These results suggest that BH3 mimetics targeting single pro-survival proteins might be particularly useful. Thus, the recently described WEHI-539[195], which is highly specific for BCL-X_L, will help to define the roles of BCL-X_L in normal physiology as well as in cancer and possibly other diseases. Pertinently, in many solid tumours, BCL-X_L is thought to have a more important pro-survival role than BCL-2.

The development of MCL1 or A1 inhibitors has progressed more slowly, perhaps because their grooves are more rigid than those of BCL-X_L or BCL-2[51]; so far, only moderately potent MCL1 inhibitors have been reported (for example [196]). The importance of MCL1 in the development, sustained growth and therapeutic resistance of diverse cancers [166, 197], including acute myeloid leukaemia (AML)[191, 198] and melanoma[166, 197, 199], makes it an appealing target. Moreover, MCL1 and A1 induce resistance to the BH3 mimetics ABT-263, ABT-737, ABT-199 and WEHI-539[179, 183, 192, 195]. However, the crucial role of MCL1 in maintenance of non-transformed cells (see above) suggests that targeting it might produce severe side effects. Nevertheless, AML cells are more sensitive to loss of MCL1 than normal haematopoietic stem/progenitor cells[198], and mice lacking one *Mcl1* allele (mimicking 50% inhibition) seem to be normal[114]; thus, it may be possible to establish an adequate therapeutic window for MCL1-specific BH3 mimetics.

Structural data for compounds targeting BCL-2, BCL-X_L or MCL1 highlight commonalities in their binding mode (Figure 5). All are anchored in the canonical groove within the P2 hydrophobic pocket, and ABT-737, ABT-263, ABT-199 and WEHI-539 increase binding affinity by extensions into the P4 pocket. Intriguingly,

the selectivity of ABT-199 for BCL-2, and of WEHI-539 for BCL-$X_{L,}$ seems to reflect increased polar interactions with their targets (Figure 5b,c). The P2 pocket seems to be crucial for binding all these ligands because of its plasticity: to accommodate the BH3 mimetics, the P2 pocket morphs into unique deeper cavities that are not present in the complexes with endogenous ligands. Thus, the plasticity in the pro-survival grooves can be exploited to develop potent and specific ligands.

Alternative therapeutic approaches. The challenges of developing high-affinity small organic BH3 mimetics have prompted initial studies on modified BH3 peptides as potential drugs[200, 201]. A 'stapled' BIM BH3 peptide killed cultured leukaemia cells and abated the growth of a leukaemia xenograft *in vivo*[201]. Also, a stapled MCL1 BH3 peptide bound MCL1, preventing it from capturing BAK, and thereby sensitised cancer cells to apoptosis[202]. However, concerns have been raised regarding the affinity and cell permeability of some stapled peptides[70], and the therapeutic window for a BIM BH3 peptide, which should eliminate all pro-survival restraint (Figure 2e) and activate BAX, remains to be determined.

Because MCL1 is regulated in multiple ways and turns over rapidly (see above), targeting its regulation may prove easier than developing an effective BH3 mimetic[191]. Indeed, several conventional chemotherapeutics, including anti-tubulin drugs[98], lower the MCL1 level, and MCL1 is increased in certain tumours by up-regulation of the de-ubiquitylase USP9X, which could be targeted[97]. Drugs that up-regulate the MCL1 antagonist NOXA (Figure 2e) might also have promise.

Applications in autoimmune and infectious diseases. The major role of BCL-2 in the maintenance of lymphocytes suggests that ABT-737, ABT-263 and ABT-199 could be used to treat autoimmune diseases. Indeed, ABT-737 has significant efficacy in several animal models of autoimmune disease[203, 204].

To preclude elimination by host cell apoptosis, many viruses have evolved homologues of pro-survival BCL-2 proteins that often differ markedly in sequence from their host counterparts[3]. Hence, the development of BH3 mimetics specific for viral pro-survival proteins might decrease virus spread within the body. Certain parasites, such as schistosomes, also have distant BCL-2 pro-survival homologues that could be targeted[205].

BAX and BAK as therapeutic targets. The recent progress in clarifying the structural transitions that drive BAX and BAK activation (Figures 3 and 4) should facilitate the development of novel compounds that directly regulate their activity: either promoting their activation for cancer treatment, as reported for BAX[206], or inhibiting it for disorders involving pathologically increased apoptosis. Target sites might include their groove, the 'rear' site in BAX, the region near their BH3 domain to facilitate (or block) its release and the α5–α6 hairpin in BAX to promote or inhibit core–latch disengagement. Because increased cell death accompanies ischemic conditions, such as stroke, cardiac infarction and traumatic brain injury, and inhibition of apoptosis (for example, by BCL-2 over-expression) reduces the pathology[207], drugs inhibiting BAX and/or BAK activation could potentially moderate the devastating consequences. Chronic neurodegenerative disorders, such as Alzheimer's disease, also involve apoptosis, but whether the mitochondrial pathway has a major role is less clear[207].

Perspectives

Our understanding of how the three BCL-2 subfamilies regulate the apoptotic switch has advanced considerably in recent years, but major unresolved issues include the membrane topology of family members during apoptosis, how the membrane affects their association, the structure of BAX and BAK homo-oligomers and the nature and structure of the apoptotic pores they generate. We need better techniques for determining membrane protein structures and better imaging technologies to monitor associations in living cells, both between family members and with the membrane[208]. To provide more quantitative insights, we need to establish the abundance of family members within select cells and equilibrium constants for all their interactions.

Increased understanding of molecular mechanisms will guide further advances in targeting the BCL-2 family therapeutically. The potential for improved therapy of cancer appears high, particularly for lymphoid malignancies. Further advances in BH3 mimetic therapy are likely to emerge with agents targeting other pro-survival proteins (such as MCL1 and A1), new ways to assess drug–target engagement inside cells, identification of the most vulnerable tumour types for each BH3 mimetic and, of course, many tests of combination therapies[190]. The prospect of developing agents

that directly activate or inhibit BAX or BAK is also exciting; BAX/BAK inhibitors might open the way to better manage ischemic conditions and degenerative disorders. We predict that exploiting insights into apoptosis regulation for the betterment of health is only at its outset.

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Box 1: Models for control of the apoptotic switch

Three models addressing how interactions between members of the BCL-2 family control the apoptotic switch are depicted. In the direct activation model, certain BH3-only proteins, particularly tBID, BIM and perhaps PUMA, directly engage and activate the pro-apoptotic effectors BAX and BAK[39, 45-48, 62]. Detection of such complexes has been problematic, probably because the interactions are transient (see text), but the associations were inferred on the basis that BAX could permeabilise liposomes only together with certain BH3 peptides, such as those of BIM and BID[39, 45, 46, 62]. BH3-only proteins that lack this direct 'activator' function, such as BAD, are designated 'sensitisers' to indicate that they exclusively engage pro-survival BCL-2 family members, thereby releasing bound BH3-only activator proteins[47]. Precisely which BH3-only proteins are activators remains debatable.

Whereas the pro-survival proteins, such as BCL-2, primarily sequester BH3-only proteins in the direct activation model, in the indirect activation model[42-44], they must also sequester any BAX or BAK molecule that becomes activated and exposes its BH3 domain[57, 58]. In this model, BAX and BAK might be activated in multiple ways, such as by an unknown modification or spontaneously at a low rate. This model emphasises that BAX and BAK become free to permeabilise the mitochondrial outer membrane only if all the pro-survival proteins are neutralised by BH3-only proteins or their mimics[42-44].

The current consensus is that both the direct and indirect models apply in many circumstances. Thus, the unified model[41] requires that the pro-survival proteins sequester not only BH3-only proteins ('mode 1') but also activated BAX and BAK ('mode 2'). The effect of BIM with altered binding specificities on cell turnover *in vivo* is consistent with this model[40]. The similar 'embedded together' model[84], which is supported by an elegant analysis of the interactions between tBID, BAX and BCL-X_L on a synthetic membrane[209], emphasises that all the crucial interactions are influenced by the membrane in which the molecules are embedded [210].

Box 2 Proposed non-apoptotic roles for BCL-2 proteins

Mitochondrial fission and fusion. The mitochondrial network fragments early in apoptosis, potentially linking apoptosis to mitochondrial fission and fusion **[G]** (reviewed in[26]). However, the link remains controversial; for example, ablation of BAX and BAK only modestly decreases mitochondrial fusion and fission is unaffected[211]. One proposed link is DRP1, a soluble factor that is recruited to mitochondria to promote fission, but DRP1 seemingly is not required for mitochondrial membrane permeabilisation; nor is permeabilisation required for fragmentation[26]. Although no compelling molecular link has emerged, BAX and BAK may preferentially permeabilise sites where fission and/or fusion initiate[26].

Autophagy. During autophagy, an ancient process to mobilise metabolites and energy for cell survival under adverse conditions, cellular components are packaged within membranes and digested within lysosomes. BECLIN1, an essential inducer of autophagy, has a BH3-like domain, through which it was reported to engage pro-survival BCL-2 proteins, albeit apparently only on the endoplasmic reticulum, and these complexes seem to inhibit autophagy (reviewed in[28]). BCL-2 may be recruited to the ER by NAF1, which augments its sequestration of BECLIN1[92]. However, although NAF1 itself has no BH3 domain, the binding of BH3-only proteins to BCL-2 can release NAF1 and free BECLIN1 to induce autophagy. BECLIN1 can also be freed by phosphorylation of the BECLIN1 BH3 domain or of three sites in the BCL-2 flexible $\alpha1 - \alpha2$ loop. The loop phosphorylation may be crucial[94], because BCL-2 with those sites mutated *in situ* prevented autophagy induction in response to nutrient starvation[212].

Mitochondrial function. Although one MCL1 isoform resides as expected on the mitochondrial outer membrane, a truncated isoform resides inside the mitochondrial matrix. Notably, cells engineered to express only the apoptosis-regulating outer membrane isoform had abnormal mitochondrial morphology, less mitochondrial DNA and reduced respiration and ATP production[29, 99]. Conditional MCL1 deletion in cardiomyocytes provoked similar mitochondrial defects[119], but their basis remains unknown. BCL-X_L has also been implicated in entering mitochondria and affecting their function[213].

Figure Legends

Figure 1 **The mitochondrial and death receptor pathways to apoptosis.** Diverse cytotoxic stimuli, including oncogenic stress and chemotherapeutic agents, as well as developmental cues, engage the mitochondrial pathway, which is regulated by BCL-2 family members. These stimuli activate BH3-only family members (initiators), which inhibit the pro-survival BCL-2-like proteins (guardians), allowing activation of the pro-apoptotic effectors BAX and BAK, which then disrupt the mitochondrial outer membrane. The cytochrome *c* (Cyc *c*) released from the mitochondria promotes caspase-9 activation on the scaffold protein APAF1, whereas the released SMAC (also known as DIABLO)[214, 215] blocks the caspase inhibitor XIAP. The death receptor-mediated (or extrinsic) pathway of apoptosis is activated when certain death receptor ligands of the tumour necrosis factor (TNF) family (such as FAS ligand and TNF) engage their cognate death receptors (FAS and TNFR1, respectively) on the plasma membrane, leading to caspase 8 activation via FADD and TRADD. The two pathways converge at activation of the effector caspases (-3, -7 and -6). In addition, tBID, generated by caspase-8-mediated proteolysis of BID in the death receptor pathway, can engage the mitochondrial pathway to amplify the apoptotic response. This amplification mechanism is required for effective apoptosis in certain cells (denoted "type 2" cells), such as hepatocytes, but not in "type 1" cells, such as thymocytes[33, 34]. The level of XIAP distinguishes the two cell types, being higher in type 2 cells[35].

Figure 2 **The structure of BCL-2 family proteins and the selectivity in their interactions.** (a) BCL-2 family members are related by regions of sequence and structural homology. When structural as well as sequence homology is considered[30], the multi-domain members of the family (the pro-survival proteins and the effector proteins BAX, BAK and perhaps BOK) share four such regions (BH1-BH4), whereas the BH3-only proteins contain only the BH3 amphipathic helix, which mediates their interaction with the groove of multi-domain BCL-2 family members. Most family members also have a trans-membrane (TM) domain for anchoring to organelles, most

notably the mitochondria. The mouse homologue of human BCL-B, Boo/Diva, bears mutations that are thought to render it non-functional[123]. (b, c) The BCL-2 family structural fold, of seven amphipathic α-helices bundled around a central hydrophobic helix (α5), is shared by the pro-survival members of the family, illustrated here by BCL-X_L (b), and by the pre-activated forms of BAX and BAK, represented here by cytosolic BAX (c), which has its trans-membrane domain (α9) sequestered within its surface groove[64]. The surface groove of multi-domain BCL-2 family proteins mediates interactions with the BH3 domain of pro-apoptotic family members, shown here by the structure of a BIM BH3 peptide bound to MCL1[51] (d). All BH3 domains insert four hydrophobic residues (h1–h4) into hydrophobic pockets in the surface groove and extend an aspartate to a conserved arginine through a salt bridge. (e) Some BH3-only proteins can bind to and neutralise all pro-survival proteins (and *vice versa*), whereas others (such as BAD or NOXA) bind only a limited subset[39, 42, 45]. (f) BAK is inhibited predominantly by BCL-X_L, MCL1 and A1[44, 59], although BCL-2 can contribute in some contexts[95, 216], whereas BAX probably can be inhibited by all the pro-survival proteins[43].

Figure 3 **Direct activation of BAX by BH3-only proteins.** Two distinct sites on BAX have been proposed to allow certain BH3-only proteins to engage and activate it: (a) its canonical hydrophobic surface groove, based on the crystal structure of a BID BH3 peptide bound to BAX[67]; or (b) an alternative rear site, based on a molecular model calculated from NMR data of full-length BAX with a bound BIM stapled peptide[48]. In (a), the BID BH3 peptide contacts the BAX groove not only through the h1–h4 residues and the conserved aspartate residue that are used to engage pro-survival BCL-2 family members (compare with Figure 2d) but also, uniquely, through two isoleucine residues near its N terminus (denoted 'h0' on the structure), which are required for effective BAX activation by the BID BH3 domain[67]. Mutagenesis of BH3 peptides suggests that the other coloured residues shown in the BID BH3 sequence might also contribute to BID activator function[67]. In (b), the BIM SAHB (stabilised α helix of BCL-2 domains) lies across the α1 and α6 helices of BAX. Binding to this site is proposed[48, 62, 68] to shift the α1–α2 loop and allosterically displace the BAX trans-membrane domain (α9), which lies on the other side of cytosolic BAX.

Figure 4 **Model for** the a**ctivation and oligomerisation of BAX and BAK.** The drawings illustrate structural transitions in BAX (and BAK), proposed from cross-linking studies[76, 77, 80, 82], biophysical measurements[81, 83], BH3:protein association[67, 73, 74] and, where indicated, by the Bax 3D structures shown[67]. Step 1 illustrates the proposed shuttling of BAX to the mitochondrial outer membrane and its retro-translocation to the cytosol [65, 66]. The movement of BAX to the mitochondrial membrane may be triggered or enhanced by engagement of the rear BAX site by a BH3-only protein (not shown; see Figure 3b). The trans-membrane (TM) domain (grey) released from the BAX surface groove can insert across the mitochondrial outer membrane, generating a membrane-bound form of BAX (which resembles native BAK). In step 2, an activator BH3 domain binds to the BAX groove. In step 3, this binding promotes release of the 'latch' domain from the 'core' domain of BAX and destabilises the BAX BH3 domain, $\alpha 2$[67]; this step has not yet been documented for BAK. The initiating activator BH3 domain then disengages (step 4). In step 5, protrusion of the BAX BH3 domain allows two such molecules to form the BAX BH3-in-groove dimer[76, 77]. The structure of the BAX core ($\alpha 2$- $\alpha 5$) as a symmetrical dimer[67] reveals a hydrophobic layer of two $\alpha 4$ and two $\alpha 5$ chains with 12 protruding aromatic residues (side view). Although it is unclear how the larger oligomers form (step 6), the core $\alpha 4$-$\alpha 5$ surface might engage the outer leaflet of the outer mitochondrial membrane, thereby inducing tension and contributing to membrane permeabilisation[67].

Figure 5 **BH3 mimetics binding to the hydrophobic groove of their pro-survival BCL-2 family target proteins.** (a) ABT-737 bound to BCL-X_L. ABT-737 occupies hydrophobic pockets P2 and P4 and makes a charge interaction with BCL-X_L Glu96[217]. (b) A closely related analogue of ABT-199 in complex with BCL-2[192]. ABT-199 also occupies P2 and P4. The charge interaction between the aza-indole moiety and BCL-2 Asp103 confers selectivity for BCL-2 over BCL-X_L. In ABT-199, where X is nitrogen, contact of Arg 107 of BCL-2 with the nitrogen of ABT-199 enhances affinity[192]. (c) BCL-X_L-specific WEHI-539 [195] bound to BCL-X_L. WEHI-539 also occupies P2 and P4 of BCL-X_L and makes charge interactions with BCL-X_L Glu96 and the conserved Arg139. Two hydrogen bonds may confer selectivity for

BCL-X_L over BCL-2 and BCL-W: one between the benzothiazole nitrogen of WEHI-539 and the backbone NH of BCL-X_L Leu108; and one between the hydrazone NH of WEHI-539 and the backbone carbonyl of Ser106[195]. (d) A moderate affinity MCL1 inhibitor[196] bound to MCL1. It occupies only P2 and has a charge interaction with Arg263 of MCL1, which is equivalent to BCL-X_L Arg139. The proteins are represented as surface (grey, red, blue and yellow represent carbon, oxygen, nitrogen and sulphur atoms, respectively) with their ligands in stick representation (magenta, red, blue and yellow represent carbon, oxygen, nitrogen and sulphur atoms, respectively).

Glossary

Sertoli cells: cells that nourish developing sperm cells through the stages of spermatogenesis

"eat me" signals: surface markers on apoptotic cells, such as phosphatidylserine, that allow their recognition and phagocytosis by healthy cells

necroptosis: a programmed form of necrosis regulated by RIP kinases

activator BH3-only proteins: BH3-only proteins, such as BIM and tBID, that can directly bind and activate BAX or BAK

sensitiser BH3-only proteins: BH3-only proteins, such as BAD, that can activate BAX or BAK only indirectly by neutralising pro-survival BCL-2 family members, thereby preventing them from restraining BAX or BAK

staple: a hydrocarbon bridge introduced into a peptide, linking amino acids four or seven residues apart, to maintain the peptide in a helical conformation, which is thought to convey higher affinity for its target, as well as greater stability and perhaps cell penetration

mitochondrial fission and fusion: mitochondria divide by fission but also continually fuse into tubular networks; thus, their structure is dynamic.

REFERENCES

1. Golstein, P. Cell death in us and others. *Science* 281, 1283 (1998).

2. Claveria, C., Giovinazzo, G., Sierra, R. & Torres, M. Myc-driven endogenous cell competition in the early mammalian embryo. *Nature* 500, 39-44 (2013).

3. Vaux, D.L., Haecker, G. & Strasser, A. An evolutionary perspective on apoptosis. *Cell* 76, 777-779 (1994).

4. Kerr, J.F.R., Wyllie, A.H. & Currie, A.R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Brit. J. Cancer* 26, 239-257 (1972).

5. Nagata, S., Hanayama, R. & Kawane, K. Autoimmunity and the clearance of dead cells. *Cell* 140, 619-30 (2010).

6. Hotchkiss, R.S., Strasser, A., McDunn, J.E. & Swanson, P.E. Cell death. *New Engl. J. Med.* 361, 1570-83 (2009).

7. Yuan, J. & Kroemer, G. Alternative cell death mechanisms in development and beyond. *Gene. Dev.* 24, 2592-602 (2010).

8. Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E. & Croce, C.M. The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. *Science* 229, 1390-3 (1985).

9. Vaux, D.L., Cory, S. & Adams, J.M. *Bcl*-2 gene promotes haemopoietic cell survival and cooperates with c-*myc* to immortalize pre-B cells. *Nature* 335, 440-442 (1988). **The pro-survival function of BCL-2 was discovered through the finding that its over-expression prevented the death of haematopoietic cells deprived of cytokine. The demonstration that BCL-2 helps MYC to immortalize lymphocytes indicated that it has an oncogenic role, as first shown *in vivo* by REF. 159.**

10. McDonnell, T.J. et al. *bcl*-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell* 57, 79-88 (1989). **This study provided the first physiological evidence that BCL-2 can control tissue homeostasis by maintaining lymphocyte survival (see also REF. 11).**

11. Strasser, A. et al. Enforced *BCL2* expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *P. Natl. Acad. Sci. U. S. A.* 88, 8661-8665 (1991). **This study and later work in REF. 125 provided the first evidence that increased lymphocyte survival elicited by changes in regulation of the BCL-2 family could contribute to autoimmune disease.**

12. Strasser, A., Harris, A.W. & Cory, S. *Bcl-2* transgene inhibits T cell death and perturbs thymic self-censorship. *Cell* 67, 889-899 (1991).

13. Hengartner, M.O., Ellis, R.E. & Horvitz, H.R. *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* 356, 494-499 (1992).

14. Vaux, D.L., Weissman, I.L. & Kim, S.K. Prevention of programmed cell death in *Caenorhabditis elegans* by human *bcl*-2. *Science* 258, 1955-1957 (1992).

15. Hengartner, M.O. & Horvitz, H.R. *C.elegans* cell survival gene *ced*-9 encodes a functional homolog of the mammalian proto-oncogene *bcl*-2. *Cell* 76, 665-676 (1994).

16. Yuan, J., Shaham, S., Ledoux, S., Ellis, H.M. & Horvitz, H.R. The *C. elegans* cell death gene *ced*-3 encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* 75, 641-652 (1993).

17. Zou, H., Henzel, W.J., Liu, X., Lutschg, A. & Wang, X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of Caspase-3. *Cell* 90, 405-413 (1997).

18. Yuan, J. & Horvitz, H.R. The *Caenorhabditis elegans* cell death gene *ced-4* encodes a novel protein and is expressed during the period of extensive programmed cell death. *Development* 116, 309-320 (1992).

19. Kluck, R.M., Bossy-Wetzel, E., Green, D.R. & Newmeyer, D.D. The release of cytochrome *c* from mitochondria - a primary site for Bcl-2 regulation of apoptosis. *Science* 275, 1132-1136 (1997).

20. Li, P. et al. Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91, 479-489 (1997).

21. Strasser, A., Jost, P.J. & Nagata, S. The many roles of FAS receptor signaling in the immune system. *Immunity* 30, 180-92 (2009).

22. Riedl, S.J. & Salvesen, G.S. The apoptosome: signalling platform of cell death. *Nat. Rev. Mol. Cell Bio.* 8, 405-13 (2007).

23. Tait, S.W. & Green, D.R. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat. Rev. Mol. Cell Bio.* 11, 621-32 (2010).

24. Strasser, A., Cory, S. & Adams, J.M. Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases. *EMBO J.* 30, 3667-3683 (2011).

25. Letai, A.G. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. *Nat. Rev. Cancer* 8, 121-132 (2008).

26. Martinou, J.C. & Youle, R.J. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev. Cell* 21, 92-101 (2011).

27. Chipuk, J.E., Moldoveanu, T., Llambi, F., Parsons, M.J. & Green, D.R. The BCL-2 family reunion. *Mol. Cell* 37, 299-310 (2010).

28. Levine, B., Sinha, S. & Kroemer, G. Bcl-2 family members: dual regulators of apoptosis and autophagy. *Autophagy* 4, 600-6 (2008).

29. Perciavalle, R.M. & Opferman, J.T. Delving deeper: MCL-1's contributions to normal and cancer biology. *Trends Cell Biol* 23, 22-9 (2013).

30. Kvansakul, M. et al. Vaccinia virus anti-apoptotic F1L is a novel Bcl-2-like domain-swapped dimer that binds a highly selective subset of BH3-containing death ligands. *Cell Death Differ* 15, 1564-71 (2008).

31. Muchmore, S.W. et al. X-ray and NMR structure of human Bcl-x_L, an inhibitor of programmed cell death. *Nature* 381, 335-341 (1996).

32. Hinds, M.G. et al. Bim, Bad and Bmf: intrinsically unstructured BH3-only proteins that undergo a localized conformational change upon binding to prosurvival Bcl-2 targets. *Cell Death Differ.* 14, 128-136 (2007).

33. Yin, X.-M. et al. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* 400, 886-891 (1999).

34. Kaufmann, T. et al. The BH3-Only Protein Bid Is Dispensable for DNA Damage- and Replicative Stress-Induced Apoptosis or Cell-Cycle Arrest. *Cell* 129, 423-433 (2007).

35. Jost, P.J. et al. XIAP discriminates between type I and type II FAS-induced apoptosis. *Nature* 460, 1035-9 (2009).

36. Chou, J.J., Li, H., Salvesen, G.S., Yuan, J. & Wagner, G. Solution structure of BID, an intracellular amplifier of apoptotic signaling. *Cell* 96, 615-624 (1999).

37. McDonnell, J.M., Fushman, D., Milliman, C.L., Korsmeyer, S.J. & Cowburn, D. Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. *Cell* 96, 625-634 (1999).

38. Adams, J.M. & Cory, S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene* 26, 1324-1337 (2007).

39. Kuwana, T. et al. BH3 Domains of BH3-Only Proteins Differentially Regulate Bax-Mediated Mitochondrial Membrane Permeabilization Both Directly and Indirectly. *Mol. Cell* 17, 525-535 (2005).

40. Merino, D. et al. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. *J. Cell Biol.* 186, 355-62 (2009).

41. Llambi, F. et al. A Unified Model of Mammalian BCL-2 Protein Family Interactions at the Mitochondria. *Mol. Cell* 44, 517-31 (2011).

42. Chen, L. et al. Differential targeting of pro-survival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol. Cell* 17, 393-403 (2005). **Quantitative data revealed selectivity in the binding of BH3 domains to pro-survival BCL-2 family proteins and showed that efficient apoptosis requires engagement of most of the expressed pro-survival proteins (see also REF. 39).**

43. Willis, S.N. et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* 315, 856-859 (2007). **This study showed that effective induction of apoptosis requires that BH3-only proteins bind and neutralize all the pro-survival BCL-2 family members expressed in a cell.**

44. Willis, S.N. et al. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. *Gene. Dev.* 19, 1294-305 (2005). **This study showed that activation of BAK is restrained predominantly by MCL1 and BCL-X_L.**

45. Certo, M. et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell* 9, 351-365 (2006). **This study introduced the concept that cells with increased levels of BCL-2 are still sensitive to BCL-2 inhibition if they have been 'primed' by stress signals that have increased the level of a bound activator BH3-only protein such as BIM (see also REF. 179).**

46. Kim, H. et al. Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. *Nat Cell Biol* 8, 1348-1358 (2006).

47. Letai, A. et al. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* 2, 183-192 (2002). **This work, using BH3 peptides, introduced the**

hypothesis that BH3 domains are of two classes: those that can directly activate BAX and those that can only sensitize cells to apoptosis by engaging the pro-survival family members.

48. Gavathiotis, E. et al. BAX activation is initiated at a novel interaction site. *Nature* 455, 1076-81 (2008).

49. Sattler, M. et al. Structure of Bcl-x_L-Bak peptide complex: recognition between regulators of apoptosis. *Science* 275, 983-986 (1997). **Building on the structure of BCL-X_L (REF. 31), this study showed that a BAK BH3 peptide binds in the surface groove of BCL-X_L, revealing a principal mode of regulation of apoptosis by this family.**

50. Liu, X., Dai, S., Zhu, Y., Marrack, P. & Kappler, J.W. The structure of a Bcl-x_L/Bim fragment complex: Implications for Bim function. *Immunity* 19, 341-352 (2003).

51. Czabotar, P.E. et al. Structural insights into the degradation of Mcl-1 induced by BH3 domains. *P. Natl. Acad. Sci. U. S. A.* 104, 6217-22 (2007).

52. Lee, E.F. et al. A novel BH3 ligand that selectively targets Mcl-1 reveals that apoptosis can proceed without Mcl-1 degradation. *J. Cell Biol.* 180, 341-355 (2008).

53. Chen, T.S., Palacios, H. & Keating, A.E. Structure-based redesign of the binding specificity of anti-apoptotic Bcl-x(L). *J. Mol. Biol.* 425, 171-85 (2013).

54. Lee, E.F. et al. Conformational changes in Bcl-2 pro-survival proteins determine their capacity to bind ligands. *J Biol Chem* 284, 30508-17 (2009).

55. Smits, C., Czabotar, P.E., Hinds, M.G. & Day, C.L. Structural plasticity underpins promiscuous binding of the prosurvival protein A1. *Structure* 16, 818-29 (2008).

56. Oltvai, Z.N., Milliman, C.L. & Korsmeyer, S.J. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74, 609-619 (1993).

57. Wang, K., Gross, A., Waksman, G. & Korsmeyer, S.J. Mutagenesis of the BH3 domain of BAX identifies residues critical for dimerization and killing. *Mol. Cell. Biol.* 18, 6083-6089 (1998).

58. Fletcher, J.I. et al. Apoptosis is triggered when prosurvival Bcl-2 proteins cannot restrain Bax. *P. Natl. Acad. Sci. U. S. A.* 105, 18081-7 (2008).

59. Simmons, M.J. et al. Bfl-1/A1 functions, similar to Mcl-1, as a selective tBid and Bak antagonist. *Oncogene* 27, 1421-8 (2008).

60. Czabotar, P.E. et al. Mutations to Bax beyond the BH3 domain disrupts interactions with pro-survival proteins and promotes apoptosis. *J. Biol. Chem.* 286, 7123-31 (2011).

61. Ku, B., Liang, C., Jung, J.U. & Oh, B.H. Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX. *Cell Res.* (2010).

62. Kim, H. et al. Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. *Mol. Cell* 36, 487-99 (2009).

63. Griffiths, G.J. et al. Cell damage-induced conformational changes of the pro-apoptotic protein Bak in vivo precede the onset of apoptosis. *J. Cell Biol.* 144, 903-914 (1999).

64. Suzuki, M., Youle, R.J. & Tjandra, N. Structure of Bax: coregulation of dimer formation and intracellular localization. *Cell* 103, 645-654 (2000).

65. Edlich, F. et al. Bcl-x(L) Retrotranslocates Bax from the Mitochondria into the Cytosol. *Cell* 145, 104-16 (2011).

66. Schellenberg, B. et al. Bax exists in a dynamic equilibrium between the cytosol and mitochondria to control apoptotic priming. *Mol. Cell* 49, 959-71 (2013).

67. Czabotar, P.E. et al. Bax crystal structures reveal how BH3 domains activate Bax and nucleate its oligomerization to induce apoptosis. *Cell* 152, 519-31 (2013). **Structural analysis showed that BAX activation requires binding of certain BH3 domains into its surface groove. It identified the residues distinguishing such BAX activators, showed that BAX then unfolds into two segments and revealed that the core segment forms a BH3-in-groove symmetric dimer, thought to be the central element in the lethal BAX oligomers (see REF. 74 and 76).**

68. Gavathiotis, E., Reyna, D.E., Davis, M.L., Bird, G.H. & Walensky, L.D. BH3-triggered structural reorganization drives the activation of proapoptotic BAX. *Mol. Cell* 40, 481-92 (2010).

69. Leshchiner, E.S., Braun, C.R., Bird, G.H. & Walensky, L.D. Direct activation of full-length proapoptotic BAK. *P. Natl. Acad. Sci. U. S. A.* 110, E986-95 (2013).

70. Okamoto, T. et al. Stabilizing the pro-apoptotic BimBH3 helix (BimSAHB) does not necessarily enhance affinity or biological activity. *A.C.S. Chem. Biol.* 8, 297-302 (2013).

71. Peng, R. et al. Targeting Bax interaction sites reveals that only homo-oligomerization sites are essential for its activation. *Cell Death Differ.* 20, 744-54 (2013).

72. Moldoveanu, T. et al. The x-ray structure of a BAK homodimer reveals an inhibitory zinc binding site. *Molecular Cell* 24, 677-688 (2006).

73. Dai, H. et al. Transient binding of an activator BH3 domain to the Bak BH3-binding groove initiates Bak oligomerization. *J. Cell Biol.* 194, 39-48 (2011).

74. Moldoveanu, T. et al. BID-induced structural changes in BAK promote apoptosis. *Nat. Struct. Mol. Biol.* 20, 589-97 (2013). **A BID BH3 peptide is shown to bind into the groove of BAK and elicit rearrangements akin to those in BAX (REF. 67).**

75. Hsu, Y.T. & Youle, R.J. Nonionic detergents induce dimerization among members of the Bcl-2 family. *J. Biol. Chem.* 272, 13829-13834 (1997).

76. Dewson, G. et al. To trigger apoptosis Bak exposes its BH3 domain and homo-dimerizes via BH3:grooove interactions. *Mol. Cell* 30, 369-380 (2008). **Crosslinking studies showed that, to form oligomers, two activated BAK monomers first expose their BH3 domain and then bury it in the other monomer's surface groove, creating novel symmetric dimers, as later confirmed by structural studies on BAX (REF. 67).**

77. Dewson, G. et al. Bax dimerizes via a symmetric BH3:groove interface during apoptosis. *Cell Death Differ.* 19, 661-70 (2012).

78. Weber, K., Harper, N., Schwabe, J. & Cohen, G.M. BIM-Mediated Membrane Insertion of the BAK Pore Domain Is an Essential Requirement for Apoptosis. *Cell Rep* (2013).

79. George, N.M., Evans, J.J. & Luo, X. A three-helix homo-oligomerization domain containing BH3 and BH1 is responsible for the apoptotic activity of Bax. *Gene. Dev.* 21, 1937-1948 (2007).

80. Dewson, G. et al. Bak activation for apoptosis involves oligomerization of dimers via their alpha6 helices. *Mol. Cell* 36, 696-703 (2009).

81. Bleicken, S. et al. Molecular details of Bax activation, oligomerization, and membrane insertion. *J. Biol. Chem.* 285, 6636-47 (2010).

82. Zhang, Z. et al. Bax forms an oligomer via separate, yet interdependent, surfaces. *J. Biol. Chem.* 285, 17614-17627 (2010).

83. Oh, K.J. et al. Conformational changes in BAK, a pore-forming proapoptotic Bcl-2 family member, upon membrane insertion and direct evidence for the existence of BH3-BH3 contact interface in BAK homo-oligomers. *J. Biol. Chem.* 285, 28924-37 (2010).

84. Bogner, C., Leber, B. & Andrews, D.W. Apoptosis: embedded in membranes. *Curr. Opin. Cell. Biol.* 22, 845-51 (2010).

85. Pang, Y.P. et al. Bak Conformational Changes Induced by Ligand Binding: Insight into BH3 Domain Binding and Bak Homo-Oligomerization. *Sci .Rep.* 2, 257 (2012).

86. Westphal, D., Dewson, G., Czabotar, P.E. & Kluck, R.M. Molecular biology of Bax and Bak activation and action. *Biochim. Biophys. Acta* 1813, 521-31 (2011).

87. Annis, M.G. et al. Bax forms multispanning monomers that oligomerize to permeabilize membranes during apoptosis. *EMBO J.* 24, 2096-103 (2005).

88. Bleicken, S., Wagner, C. & Garcia-Saez, A.J. Mechanistic differences in the membrane activity of Bax and Bcl-xL correlate with their opposing roles in apoptosis. *Biophys. J.* 104, 421-31 (2013).

89. Bleicken, S., Landeta, O., Landajuela, A., Basanez, G. & Garcia-Saez, A.J. Proapoptotic Bax and Bak form stable protein-permeable pores of tunable size. *J Biol Chem* (2013).

90. Green, D.R. & Kroemer, G. Cytoplasmic functions of the tumour suppressor p53. *Nature* 458, 1127-30 (2009).

91. Michalak, E.M., Villunger, A., Adams, J.M. & Strasser, A. In several cell types the tumour suppressor p53 induces apoptosis largely via Puma but Noxa can contribute. *Cell Death Differ.* 15, 1019-1029 (2008).

92. Chang, N.C., Nguyen, M., Germain, M. & Shore, G.C. Antagonism of Beclin 1-dependent autophagy by BCL-2 at the endoplasmic reticulum requires NAF-1. *EMBO J* 29, 606-18 (2010).

93. Kutuk, O. & Letai, A. Regulation of Bcl-2 family proteins by posttranslational modifications. *Curr Mol Med* 8, 102-18 (2008).

94. Wei, Y., Pattingre, S., Sinha, S., Bassik, M. & Levine, B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell* 30, 678-88 (2008).

95. Dai, H. et al. Contribution of Bcl-2 Phosphorylation to Bak Binding and Drug Resistance. *Cancer Res* (2013).

96. Thomas, L.W., Lam, C. & Edwards, S.W. Mcl-1; the molecular regulation of protein function. *FEBS Lett* 584, 2981-9 (2010).

97. Schwickart, M. et al. Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* 463, 103-7 (2010).

98. Wertz, I.E. et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. *Nature* 471, 110-4 (2011).

99. Perciavalle, R.M. et al. Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. *Nat. Cell Biol.* 14, 575-83 (2012).

100. Elkholi, R., Floros, K.V. & Chipuk, J.E. The Role of BH3-Only Proteins in Tumor Cell Development, Signaling, and Treatment. *Genes Cancer* 2, 523-37 (2011).

101. Herold, M.J. et al. Foxo-mediated Bim transcription is dispensable for the apoptosis of hematopoietic cells that is mediated by this BH3-only protein. *EMBO Rep* (2013).

102. Clybouw, C. et al. Alternative splicing of Bim and Erk-mediated Bim(EL) phosphorylation are dispensable for hematopoietic homeostasis in vivo. *Cell Death Differ* 19, 1060-8 (2012).

103. Campbell, K.J. et al. Elevated Mcl-1 perturbs lymphopoiesis, promotes transformation of hematopoietic stem/progenitor cells, and enhances drug resistance. *Blood* 116, 3197-207 (2010).

104. Veis, D.J., Sorenson, C.M., Shutter, J.R. & Korsmeyer, S.J. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75, 229-240 (1993).

105. Bouillet, P., Cory, S., Zhang, L.-C., Strasser, A. & Adams, J.M. Degenerative disorders caused by Bcl-2 deficiency are prevented by loss of its BH3-only antagonist Bim. *Dev. Cell* 1, 645-653 (2001).

106. Motoyama, N. et al. Massive cell death of immature hematopoietic cells and neurons in Bcl-x deficient mice. *Science* 267, 1506-1510 (1995).

107. Rucker, E.B., 3rd et al. Bcl-x and Bax regulate mouse primordial germ cell survival and apoptosis during embryogenesis. *Mol. Endocrinol.* 14, 1038-1052 (2000).

108. Mason, K.D. et al. Programmed anuclear cell death delimits platelet life span. *Cell* 128, 1173-1186 (2007).

109. Takehara, T. et al. Hepatocyte-specific disruption of Bcl-xL leads to continuous hepatocyte apoptosis and liver fibrotic responses. *Gastroenterology* 127, 1189-1197 (2004).

110. Akhtar, R.S., Klocke, B.J., Strasser, A. & Roth, K.A. Loss of BH3-only protein Bim inhibits apoptosis of hemopoietic cells in the fetal liver and male germ cells but not neuronal cells in bcl-x-deficient mice. *J. Histochem. Cytochem.* 56, 921-7 (2008).

111. Print, C.G. et al. Apoptosis regulator Bcl-w is essential for spermatogenesis but appears otherwise redundant. *P. Natl. Acad. Sci. U. S. A.* 95, 12424-12431 (1998).

112. Ross, A.J. et al. Testicular degeneration in *Bclw*-deficient mice. *Nat. Genet.* 18, 251-256 (1998).

113. Ross, A.J. et al. BCLW mediates survival of postmitotic Sertoli cells by regulating BAX activity. *Dev. Biol.* 239, 295-308 (2001).

114. Rinkenberger, J.L., Horning, S., Klocke, B., Roth, K. & Korsmeyer, S.J. Mcl-1 deficiency results in peri-implantation embryonic lethality. *Gene. Dev.* 14, 23-27 (2000).

115. Opferman, J. et al. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. *Science* 307, 1101-1104 (2005).

116. Opferman, J.T. et al. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 426, 671-676 (2003).

117. Vikstrom, I. et al. Mcl-1 is essential for germinal center formation and B cell memory. *Science* 330, 1095-9 (2010).

118. Peperzak, V. et al. Mcl-1 is essential for the survival of plasma cells. *Nat. Immunol.* 14, 290-7 (2013).

119. Wang, X. et al. Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. *Gene. Dev.* 27, 1351-64 (2013).

120. Thomas, R.L. et al. Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure. *Gene. Dev.* 27, 1365-77 (2013).

121. Hamasaki, A. et al. Accelerated neutrophil apoptosis in mice lacking A1-a, a subtype of the *bcl-2*-related A1 gene. *J. Exp. Med.* 188, 1985-1992 (1998).

122. Ottina, E. et al. Targeting antiapoptotic A1/Bfl-1 by in vivo RNAi reveals multiple roles in leukocyte development in mice. *Blood* 119, 6032-42 (2012).

123. Rautureau, G.J., Day, C.L. & Hinds, M.G. The structure of Boo/Diva reveals a divergent Bcl-2 protein. *Proteins* 78, 2181-6 (2010).

124. Youle, R.J. & Strasser, A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* 9, 47-59 (2008).

125. Bouillet, P. et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 286, 1735-1738 (1999). **The BH3-only protein BIM was shown to be essential for specific cytotoxic responses and to maintain lymphoid homeostasis, thereby preventing autoimmunity (see REF. 126).**

126. Bouillet, P. et al. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 415, 922-926 (2002).

127. Enders, A. et al. Loss of the pro-apoptotic BH3-only Bcl-2 family member Bim inhibits BCR stimulation-induced apoptosis and deletion of autoreative B cells. *J. Exp. Med.* 198, 1119-1126 (2003).

128. Fischer, S.F. et al. Pro-apoptotic BH3-only protein Bim is essential for developmentally programmed death of germinal center-derived memory B cells and antibody forming cells. *Blood* 110, 3978-3984 (2007).

129. Hildeman, D.A. et al. Activated T cell death in vivo mediated by pro-apoptotic Bcl-2 family member, Bim. *Immunity* 16, 759-767 (2002).

130. Pellegrini, M., Belz, G., Bouillet, P. & Strasser, A. Shut down of an acute T cell immune response to viral infection is mediated by the pro-apoptotic Bcl-2 homology 3-only protein Bim. *P. Natl. Acad. Sci. U. S. A.* 100, 14175-14180 (2003).

131. Hughes, P.D. et al. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. *Immunity* 28, 197-205 (2008).

132. Kaufmann, T. et al. Fatal hepatitis mediated by tumor necrosis factor TNFalpha requires caspase-8 and involves the BH3-only proteins Bid and Bim. *Immunity* 30, 56-66 (2009).

133. Nakano, K. & Vousden, K.H. *PUMA*, a novel proapoptotic gene, is induced by p53. *Mol. Cell* 7, 683-694 (2001).

134. Yu, J., Zhang, L., Hwang, P.M., Kinzler, K.W. & Vogelstein, B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol. Cell* 7, 673-682 (2001).

135. Villunger, A. et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* 302, 1036-8 (2003). **The BH3-only proteins PUMA and to a lesser extent NOXA, which are induced by the tumour suppressor p53, were shown to be crucial**

mediators of the apoptosis elicited by genotoxic damage and certain drugs (see also REF. 136 and 137).

136. Jeffers, J.R. et al. Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. *Cancer Cell* 4, 321-328 (2003).

137. Shibue, T. et al. Integral role of Noxa in p53-mediated apoptotic response. *Gene. Dev.* 17, 2233-2238 (2003).

138. Naik, E., Michalak, E.M., Villunger, A., Adams, J.M. & Strasser, A. UV-radiation triggers apoptosis of fibroblasts and skin keratinocytes mainly via the BH3-only protein Noxa. *J. Cell Biol.* 176, 415-424 (2007).

139. Kerr, J.B. et al. DNA damage-induced primordial follicle oocyte apoptosis and loss of fertility require TAp63-mediated induction of Puma and Noxa. *Mol. Cell* 48, 343-52 (2012).

140. Erlacher, M. et al. BH3-only proteins Puma and Bim are rate-limiting for {gamma} -radiation and glucocorticoid-induced apoptosis of lymphoid cells in vivo. *Blood* 106, 4131-4138 (2005).

141. Labi, V. et al. Loss of the BH3-only protein Bmf impairs B cell homeostasis and accelerates gamma irradiation-induced thymic lymphoma development. *J. Exp. Med.* 205, 641-655 (2008).

142. Coultas, L. et al. Proapoptotic BH3-only Bcl-2 family member Bik/Blk/Nbk is expressed in hemopoietic and endothelial cells but is redundant for their programmed death. *Mol. Cell. Biol.* 24, 1570-1581 (2004).

143. Coultas, L. et al. Concomitant loss of proapoptotic BH3-only Bcl-2 antagonists Bik and Bim arrests spermatogenesis. *EMBO J.* 24, 3963-3973 (2005).

144. Ranger, A.M. et al. *Bad*-deficient mice develop diffuse large B cell lymphoma. *P. Natl. Acad. Sci. U. S. A.* 100, 9324-9329 (2003).

145. Kelly, P.N. et al. Individual and overlapping roles of BH3-only proteins Bim and Bad in apoptosis of lymphocytes and platelets and in suppression of thymic lymphoma development. *Cell Death Differ.* 17, 1655-64 (2010).

146. Baumgartner, F. et al. Minor cell-death defects but reduced tumor latency in mice lacking the BH3-only proteins Bad and Bmf. *Oncogene* 32, 621-30 (2013).

147. Imaizumi, K. et al. Critical role for DP5/Harakiri, a Bcl-2 homology domain 3-only Bcl-2 family member, in axotomy-induced neuronal cell death. *J. Neurosci.* 24, 3721-3725 (2004).

148. Coultas, L. et al. Hrk/DP5 contributes to the apoptosis of select neuronal populations but is dispensable for haematopoietic cell apoptosis. *J. Cell Sci.* 120, 2044-2052 (2007).

149. Erlacher, M. et al. Puma cooperates with Bim, the rate-limiting BH3-only protein in cell death during lymphocyte development, in apoptosis induction. *J. Exp. Med.* 203, 2939-2951 (2006).

150. Ren, D. et al. BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. *Science* 330, 1390-3 (2010).

151. Lindsten, T. et al. The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. *Mol. Cell* 6, 1389-1399. (2000). **Genetic evidence that the combined absence of BAX and BAK effectively ablated intrinsic apoptosis in mice showed that they are the crucial but mutually redundant effectors of this pathway (see also REFS 153,154).**

152. Mason, K.D. et al. Proapoptotic Bak and Bax guard against fatal systemic and organ-specific autoimmune disease. *P. Natl. Acad. Sci. U. S. A.* 110, 2599-604 (2013).

153. Zong, W.X., Lindsten, T., Ross, A.J., MacGregor, G.R. & Thompson, C.B. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. *Gene. Dev.* 15, 1481-1486 (2001).

154. Wei, M.C. et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* 292, 727-730 (2001). **Together with the physiological results showing that apoptosis requires BAX or BAK (REF. 151), this study and REF. 153 showed that BH3-only proteins must function upstream of and through BAX and BAK.**

155. Ke, F. et al. BCL-2 family member BOK is widely expressed but its loss has only minimal impact in mice. *Cell Death Differ.* 19, 915-25 (2012).

156. Ke, F. et al. Consequences of the combined loss of BOK and BAK or BOK and BAX. *Cell Death Dis.* 4, e650 (2013).

157. McDonnell, T.J. & Korsmeyer, S.J. Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). *Nature* 349, 254-256 (1991).

158. Strasser, A., Harris, A.W. & Cory, S. Eμ-*bcl*-2 transgene facilitates spontaneous transformation of early pre-B and immunoglobulin-secreting cells but not T cells. *Oncogene* 8, 1-9 (1993).

159. Strasser, A., Harris, A.W., Bath, M.L. & Cory, S. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between *myc* and *bcl*-2. *Nature* 348, 331-333 (1990). **Extending the *in vitro* analysis in REF. 9, the demonstration that BCL-2 accelerated MYC-driven tumour development provided the first direct evidence *in vivo* that BCL-2 over-expression is tumorigenic.**

160. Egle, A., Harris, A.W., Bouillet, P. & Cory, S. Bim is a suppressor of Myc-induced mouse B cell leukemia. *P. Natl. Acad. Sci. U. S. A.* 101, 6164-6169 (2004).

161. Garrison, S.P. et al. Selection against PUMA gene expression in Myc-driven B-cell lymphomagenesis. *Mol. Cell. Biol.* 28, 5391-402 (2008).

162. Michalak, E.M. et al. Puma and to a lesser extent Noxa are suppressors of Myc-induced lymphomagenesis. *Cell Death Differ.* 16, 684-96 (2009).

163. Frenzel, A. et al. Suppression of B-cell lymphomagenesis by the BH3-only proteins Bmf and Bad. *Blood* 115, 995-1005 (2010).

164. Eischen, C.M., Roussel, M.F., Korsmeyer, S.J. & Cleveland, J.L. Bax loss impairs Myc-induced apoptosis and circumvents the selection of p53 mutations during Myc-mediated lymphomagenesis. *Mol. Cell. Biol.* 21, 7653-7662 (2001).

165. Tsujimoto, Y., Finger, L.R., Yunis, J., Nowell, P.C. & Croce, C.M. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* 226, 1097-1099 (1984).

166. Beroukhim, R. et al. The landscape of somatic copy-number alteration across human cancers. *Nature* 463, 899-905 (2010).

167. Tagawa, H. et al. Genome-wide array-based CGH for mantle cell lymphoma: identification of homozygous deletions of the proapoptotic gene BIM. *Oncogene* 24, 1348-1358 (2005).

168. Zantl, N. et al. Frequent loss of expression of the pro-apoptotic protein Bim in renal cell carcinoma: evidence for contribution to apoptosis resistance. *Oncogene* 26, 7038-7048 (2007).

169. Goodnow, C.C. Multistep pathogenesis of autoimmune disease. *Cell* 130, 25-35 (2007).

170. Rathmell, J.C., Lindsten, T., Zong, W.-X., Cinalli, R.M. & Thompson, C.B. Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis. *Nature Immunology* 3, 932-939 (2002).

171. Happo, L. et al. Maximal killing of lymphoma cells by DNA-damage inducing therapy requires not only the p53 targets Puma and Noxa but also Bim. *Blood* 116, 5256-67 (2010).

172. Bachmann, P.S. et al. Epigenetic silencing of BIM in glucocorticoid poor-responsive pediatric acute lymphoblastic leukemia, and its reversal by histone deacetylase inhibition. *Blood* 116, 3013-22 (2010).

173. Tan, T.T. et al. Key roles of BIM-driven apoptosis in epithelial tumors and rational chemotherapy. *Cancer Cell* 7, 227-238 (2005).

174. Kuroda, J. 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. *P. Natl. Acad. Sci. U. S. A.* 103, 14907-14912 (2006).

175. Cragg, M.S., Kuroda, J., Puthalakath, H., Huang, D.C.S. & Strasser, A. Gefitinib-Induced Killing of NSCLC Cell Lines Expressing Mutant *EGFR* Requires Bim and Can Be Enhanced by BH3 Mimetics. *PLoS Med.* 4, 1681-89 (2007).

176. Costa, D.B. et al. BIM Mediates EGFR Tyrosine Kinase Inhibitor-Induced Apoptosis in Lung Cancers with Oncogenic EGFR Mutations. *PLoS Med.* 4, e315 (2007).

177. Cragg, M.S., Jansen, E.S., Cook, M., Strasser, A. & Scott, C.L. Treatment of B-RAF mutant human tumor cells with a MEK inhibitor requires Bim and is enhanced by a BH3 mimetic *J. Clin. Invest.* 118, 3651-3659 (2008).

178. Ng, K.P. et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat. Med.* 18, 521-528 (2012).

179. Del Gaizo Moore, V. et al. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. *J. Clin. Invest.* 117, 112-21 (2007).

180. Merino, D. et al. Bcl-2, Bcl-x(L), and Bcl-w are not equivalent targets of ABT-737 and navitoclax (ABT-263) in lymphoid and leukemic cells. *Blood* 119, 5807-16 (2012).

181. Chonghaile, T.N. et al. Pretreatment Mitochondrial Priming Correlates with Clinical Response to Cytotoxic Chemotherapy. *Science* 334, 1129-33 (2011).

182. Vo, T.T. et al. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. *Cell* 151, 344-55 (2012).

183. van Delft, M.F. 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* 10, 389-99 (2006).

184. Lessene, G., Czabotar, P.E. & Colman, P.M. BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug Discov.* 7, 989-1000 (2008).

185. Oltersdorf, T. et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435, 677-681 (2005). **The development of ABT-737 provided the first compelling evidence that BH3 mimetic therapy for cancer was likely to become a reality, and its orally bioavailable derivative ABT-263 (now navitoclax) (REF. 186) has shown promise in early clinical trials.**

186. Tse, C. et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* 68, 3421-3428 (2008).

187. Roberts, A.W. 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.* 30, 488-496 (2012).

188. Mason, K.D. et al. In vivo efficacy of the Bcl-2 antagonist ABT-737 against aggressive Myc-driven lymphomas. *P. Natl. Acad. Sci. U. S. A.* 105, 17961-6 (2008).

189. Tan, N. et al. Navitoclax enhances the efficacy of taxanes in non-small cell lung cancer models. *Clin. Cancer Res.* 17, 1394-404 (2011).

190. Cragg, M.S., Harris, C., Strasser, A. & Scott, C.L. Unleashing the power of inhibitors of oncogenic kinases through BH3 mimetics. *Nat. Rev. Cancer* 9, 321-6 (2009).

191. Gores, G.J. & Kaufmann, S.H. Selectively targeting Mcl-1 for the treatment of acute myelogenous leukemia and solid tumors. *Gene. Dev.* 26, 305-11 (2012).

192. Souers, A.J. et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat. Med.* 19, 202-8 (2013). **Pre-clinical studies and early results from the first clinical trial suggest that ABT-199, the first potent and highly selective BCL-2 antagonist, has considerable promise, particularly for lymphoid malignancies.**

193. Vandenberg, C.J. & Cory, S. ABT-199, a new Bcl-2-specific BH3 mimetic, has in vivo efficacy against aggressive Myc-driven mouse lymphomas without provoking thrombocytopenia. *Blood* 121, 2285-8 (2013).

194. Vaillant, F. et al. Targeting BCL-2 with the BH3 Mimetic ABT-199 in Estrogen Receptor-Positive Breast Cancer. *Cancer Cell* 24, 120-9 (2013).

195. Lessene, G. et al. Discovery, structure-guided design and validation of a novel, potent and selective inhibitor of the pro-survival BCL-2 family member BCL-XL. *Nature Chem Biol* 9, 390-397 (2013).

196. Friberg, A. et al. Discovery of potent myeloid cell leukemia 1 (mcl-1) inhibitors using fragment-based methods and structure-based design. *J. Med. Chem.* 56, 15-30 (2013).

197. Placzek, W.J. et al. 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.* 1, e40 (2010).

198. Glaser, S. et al. Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia. *Gene. Dev.* 26, 120-125 (2012).

199. Boisvert-Adamo, K., Longmate, W., Abel, E.V. & Aplin, A.E. Mcl-1 is required for melanoma cell resistance to anoikis. *Mol. Cancer Res.* 7, 549-56 (2009).

200. Boersma, M.D. et al. Evaluation of diverse alpha/beta-backbone patterns for functional alpha-helix mimicry: analogues of the Bim BH3 domain. *J. Am. Chem. Soc.* 134, 315-23 (2012).

201. LaBelle, J.L. et al. A stapled BIM BH3 helix overcomes the apoptotic resistance of refractory hematologic cancers. *J. Clin. Invest.* 122, 2018-2031 (2013).

202. Stewart, M.L., Fire, E., Keating, A.E. & Walensky, L.D. The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. *Nat. Chem. Biol.* 6, 595-601 (2010).

203. Bardwell, P.D. et al. The Bcl-2 family antagonist ABT-737 significantly inhibits multiple animal models of autoimmunity. *J. Immunol.* 182, 7482-9 (2009).

204. Carrington, E.M. et al. BH3 mimetics antagonizing restricted prosurvival Bcl-2 proteins represent another class of selective immune modulatory drugs. *P. Natl. Acad. Sci. U. S. A.* 107, 10967-71 (2010).

205. Lee, E.F. et al. Discovery and molecular characterization of a Bcl-2-regulated cell death pathway in schistosomes. *P. Natl. Acad. Sci. U. S. A.* 108, 6999-7003 (2011).

206. Gavathiotis, E., Reyna, D.E., Bellairs, J.A., Leshchiner, E.S. & Walensky, L.D. Direct and selective small-molecule activation of proapoptotic BAX. *Nat. Chem. Biol.* 8, 639-45 (2012).

207. Hyman, B.T. & Yuan, J. Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology. *Nat. Rev. Neurosci.* 13, 395-406 (2012).

208. Kale, J., Liu, Q., Leber, B. & Andrews, D.W. Shedding light on apoptosis at subcellular membranes. *Cell* 151, 1179-84 (2012).

209. Lovell, J.F. et al. Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax. *Cell* 135, 1074-84 (2008).

210. Shamas-Din, A., Kale, J., Leber, B. & Andrews, D.W. Mechanisms of action of Bcl-2 family proteins. *Cold Spring Harbor Perspect. Biol.* 5, a008714 (2013).

211. Karbowski, M., Norris, K.L., Cleland, M.M., Jeong, S.Y. & Youle, R.J. Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 443, 658-652 (2006).

212. He, C. et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature* 481, 511-5 (2012).

213. Chen, Y.B. et al. Bcl-xL regulates mitochondrial energetics by stabilizing the inner membrane potential. *J Cell Biol* 195, 263-76 (2011).

214. Verhagen, A.M. et al. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing inhibitor of apoptosis (IAP) proteins. *Cell* 102, 43-53 (2000).

215. Du, C., Fang, M., Li, Y., Li, L. & Wang, X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* 102, 33-42 (2000).

216. Dai, H., Meng, X.W., Lee, S.H., Schneider, P.A. & Kaufmann, S.H. Context-dependent Bcl-2/Bak interactions regulate lymphoid cell apoptosis. *J Biol Chem* 284, 18311-22 (2009).

217. Lee, E.F. et al. Crystal structure of ABT-737 complexed with Bcl-x(L): implications for selectivity of antagonists of the Bcl-2 family. *Cell Death Differ.* 14, 1711-1713 (2007).