TIMELINE

Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies

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Abstract | The 'hallmarks of cancer' are generally accepted as a set of genetic and epigenetic alterations that a normal cell must accrue to transform into a fully malignant cancer. It follows that therapies designed to counter these alterations might be effective as anti-cancer strategies. Over the past 30 years, research on the BCL-2-regulated apoptotic pathway has led to the development of small-molecule compounds, known as 'BH3-mimetics', that bind to pro-survival BCL-2 proteins to directly activate apoptosis of malignant cells. This Timeline article focuses on the discovery and study of BCL-2, the wider BCL-2 protein family and, specifically, its roles in cancer development and therapy.

In the past 30 years, cell death has become a major field of investigation, building to a crescendo with the recent award of 'Breakthrough Therapy Designation' from the US Food and Drug Administration (FDA) to ABT-199 (also known as venetoclax), a selective inhibitor of the anti-apoptotic protein BCL-2, in recognition of its promise as a treatment for patients with chemotherapy-resistant chronic lymphocytic leukaemia (CLL)[1].

Although research on cell death extends back more than 150 years, until the late 1980s it remained an esoteric subject. Today, however, it is a major research field, with more than 20,000 new publications on apoptosis or programmed cell death appearing each year. The explosion in interest was sparked by research on one protein, BCL-2, as experiments on BCL-2 showed that mechanisms for cell death are highly conserved throughout the evolution of animals, and because chromosomal translocations that activate the *BCL2* gene are associated with malignant disease in humans. Thus, the identification of BCL-2 as an inhibitor of cell death marked recognition of the first component of a cell death mechanism in any organism, and established a new hallmark of cancer[2] — evasion of cell death (apoptosis) (FIG. 1).

Over the past three decades, research in hundreds of laboratories has identified and characterized at least 16 members of the BCL-2 protein family, and categorized them into three functional groups that each bear one or more BCL-2 homology (BH) domains (FIG. 2). These are the pro-survival BCL-2 family members (including BCL-2 itself), the multi-BH-domain pro-apoptotic members (such as BAX and BAK) and the pro-apoptotic 'BH3-only' proteins (such as BIM (also known as BCL2L11) and PUMA (also known as BBC3)). Many of the upstream pathways that control these proteins have been elucidated, as well as the effector processes triggered by their activation that are the ultimate cause of cell demolition. Reviews that provide detailed in-depth discussion of BCL-2-regulated apoptosis signalling at a molecular level are available[3–9]. Although non-apoptotic roles for BCL-2 family members have been investigated, including roles in autophagy, endoplasmic reticulum (ER) calcium dynamics and the unfolded protein response, their importance in normal physiology and cancer remains unclear, and is beyond the scope of this article (for a review on these topics, see REF. 10). This Timeline article focuses on key advances in our understanding of the function of the BCL-2 protein family in cell death, in the development of cancer, and as targets in cancer therapy.

Early studies on apoptosis

In their 1972 paper that adopted the word 'apoptosis' to describe a physiological process of cellular suicide, Kerr and colleagues[11] recognized the presence of apoptotic cells in tissue sections of certain human cancers. Accordingly, they proposed that increasing the rate of apoptosis of neoplastic cells relative to their rate of production could potentially be therapeutic. However, interest in cell death and its role in cancer languished until the late 1980s, when genetic abnormalities that prevented cell death were directly linked to malignancy in humans.

Until the early 1980s, most oncogenes were discovered as genes carried by transforming retroviruses (for example, v-*myc*, v-*src* and v-*abl*), genes located at recurrent chromosomal translocation break points (for example, *BCR–ABL* and *MYC*), or genes that could transfer oncogenic properties from malignant cells to non-malignant ones (for example, mutant RAS)[12]. Whereas the normal counterparts of these oncogenes promoted cell proliferation in a controlled manner, when they were dysregulated in cancers, they caused uncontrolled cell growth and proliferation.

BCL2: a novel class of oncogene

The discovery of BCL-2 started with the association of t(14;18) chromosomal translocations with human follicular lymphoma by Fukuhara and Rowley[13]. This enabled others to clone the chromosomal break point, and subsequently the cDNA, of a gene on chromosome 18, which was termed *BCL2* for B cell leukaemia or lymphoma gene number 2 (REFS 14–18).

The strong association of translocations involving *BCL2* with follicular lymphoma suggested that it was an oncogene, but

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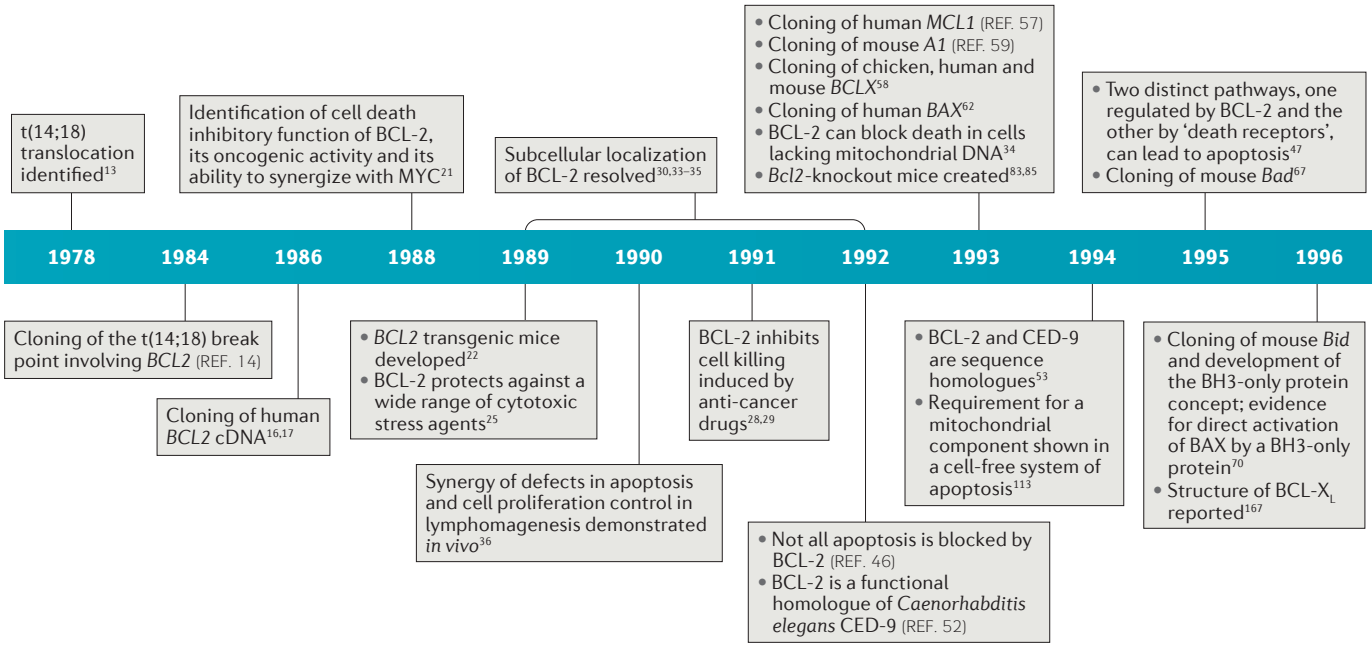

Figure 1 | **Timeline of key discoveries.** This timeline describes key discoveries related to the identification of BCL-2, determination of its function, its role and that of other BCL-2 family members in cancer, and efforts to target BCL-2 family members for the treatment of cancer. FDA, Food and Drug Administration; MCL1, myeloid cell leukaemia 1.

did not constitute proof, and the amino acid sequence did not provide clues to its function. Because expression of several known oncogenes, including SV40 large T antigen, v-*abl* and v-*fms*, enabled interleukin-3 (IL-3)-dependent mouse myeloid FDC-P1 cells to grow in the absence of that cytokine, and to form tumours in mice (see, for example, REFS 19,20), a *BCL2* expression construct was transduced into these cells and they were cultured without cytokine. Although the BCL-2-expressing cells did not proliferate in the absence of IL-3, unlike the parental cells, they failed to die when IL-3 was removed; however, when it was restored (even after several weeks), they began to proliferate once more[21].

These experiments revealed that BCL-2 did not affect cell proliferation, but promoted cell survival by preventing the death of growth factor-dependent cells cultured without a cytokine. Korsmeyer and colleagues[22] extended these findings by showing that *BCL2* transgenic mice accumulate excess B lymphocytes and that these cells are protected from spontaneous death in culture. Further studies from numerous groups showed that overexpression of BCL-2 was able to block apoptosis, triggered by diverse cellular stresses, in cell lines and in primary cells of many types[23–29].

Although BCL-2 was originally reported to reside on the cytosolic face of intracellular membranes[30,31], subsequent articles suggested that it is localized to the inner mitochondrial membrane[26] or the plasma membrane[32]. This was resolved by Monaghan *et al.*[33], Jacobson *et al.*[34] and Lithgow *et al.*[35] who showed that the first reports were correct, and that BCL-2 resides on the outer mitochondrial membrane, the ER membrane and the nuclear envelope, with the bulk of the protein exposed to the cytoplasm in all of these locations.

Collectively, these findings revealed that BCL-2 was unlike other oncogenic proteins known at the time, as it did not stimulate cell growth or proliferation, but promoted tumorigenesis by enabling cells that would normally undergo programmed cell death to survive. This abnormal cell survival facilitated the acquisition of additional oncogenic lesions to drive neoplastic transformation[36–38]. BCL-2 thus became the first component of the cell death machinery to be cloned and recognized, and it became the archetype of a new class of oncogenes, the inhibitors of cell death.

Although overexpression of BCL-2 enabled growth factor-dependent cell lines (for example, FDC-P1) to survive in the absence of cytokine, when these cells were injected into mice, they did not form tumours, suggesting that inhibition of cell death alone was not sufficient to render a cell fully transformed. In 1983, Land *et al.*[39] had shown that transformation of fibroblasts required expression not only of a RAS oncogene, but also of *MYC*. Furthermore, in a patient, Croce *et al.*[40] had observed the transformation of a follicular lymphoma bearing a *BCL2* translocation into acute pre-B cell leukaemia after acquisition of a second chromosomal translocation involving *MYC*. To test directly whether BCL-2 could synergize with MYC in neoplastic transformation, a retroviral vector bearing *BCL2* was introduced into bone marrow cells from pre-leukaemic *Eμ-Myc* transgenic mice. Cells expressing both *BCL2* and *MYC*, but not those expressing either oncogene alone, gave rise to immortalized cell lines *in vitro* that caused lymphoma when transplanted into irradiated mice[21]. This synergy between inhibition of cell death and dysregulated cell proliferation in tumorigenesis was confirmed and extended *in vivo* by generating *Eμ-Bcl2;Eμ-Myc* bi-transgenic mice, which rapidly succumbed to highly aggressive lymphoma with a stem cell phenotype[36]. This synergy was explained when it was shown that cells respond to overexpression of MYC when they are stressed (for example, as a result of limiting levels of growth factors) by undergoing apoptosis through a mechanism that BCL-2 can block[41–43].

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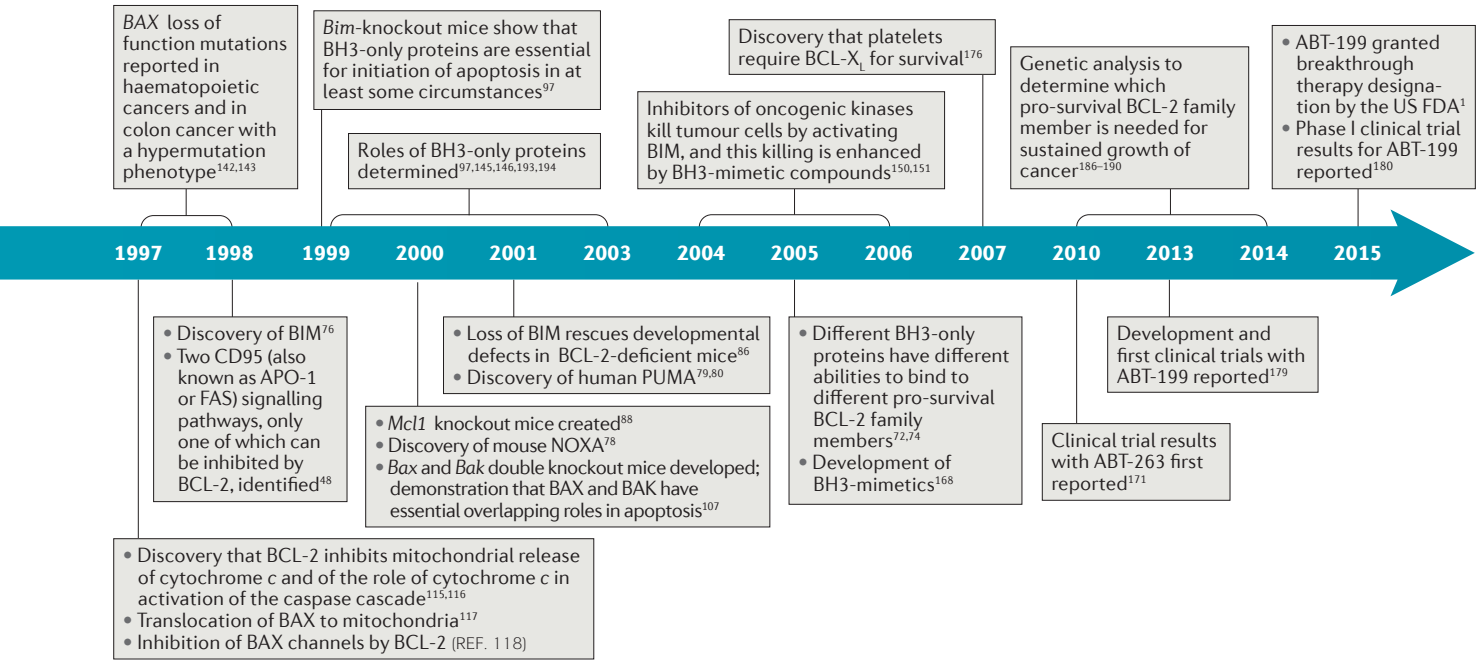

Although some early studies using BCL-2 overexpression in cell lines suggested that it might promote cell growth and proliferation[23,44], investigations of transgenic mice overexpressing BCL-2 in B cells, T cells or both demonstrated beyond doubt that BCL-2 specifically inhibits cell death and does not promote proliferation[22,28,29,45]. Furthermore, studies with these transgenic mice confirmed *in vitro* studies[25] showing that BCL-2 not only inhibited apoptosis owing to deprivation of growth factors but also protected cells from a broad range of cytotoxic stimuli, importantly including diverse anti-cancer drugs[22,27–29]. Studies such as these showed that BCL-2 acts at the convergence of several upstream apoptosis-inducing signalling pathways. Nevertheless, it soon became apparent that BCL-2 did not control all types of cell death. For example, BCL-2 did not prevent the killing of cells targeted by cytotoxic T cells (mediated by perforin and granzymes)[46], and it did not inhibit apoptosis of primary lymphocytes triggered by ligation of the 'death receptor' FAS[47], even though in other cell types, including certain tumours, BCL-2 is able to block FAS-induced apoptosis[48,49] (see below for further discussion).

Conservation of cell death mechanisms

Until BCL-2 was recognized to be an inhibitor of cell death, little was known of the genetics of apoptosis in mammalian cells, but research on programmed cell death in invertebrates was progressing rapidly, largely through the power of genetics in the nematode *Caenorhabditis elegans*. Sulston and Horvitz[50] had shown that the fate of 131 of the 1,090 somatic cells formed during development is to undergo programmed cell death. Moreover, by classic forward genetic approaches, they had shown that around a dozen genes were needed for operation of this process[51] and, by performing crosses, they could demonstrate that these genes acted in a hierarchy. Many seemed to be specific for cell death and had no other role, but their full characterization awaited cloning of the genes.

In 1992, Vaux *et al.*[52] described the effects of expressing human *BCL2* in *C. elegans*. In worms that expressed *BCL2*, the number of developmentally programmed cell deaths was markedly reduced. This meant that the human BCL-2 protein was able to engage with the worm's cell death machinery, implying that the processes of apoptosis (of mammalian cells) and programmed cell death (in *C. elegans*) were implemented by the same molecular mechanism, one that had been conserved for more than 500 million years of evolution. The effect of human *BCL2* expression most closely resembled that of a gain-of-function mutation in a *C. elegans* gene termed *ced-9*, and suggested that these genes were functionally homologous. The subsequent cloning and sequencing of the *ced-9* gene proved that this was indeed the case[53]. Furthermore, as *ced-9* was known to act upstream of *ced-3* and *ced-4*, it seemed likely that BCL-2 would somehow act to negatively control the products of the mammalian homologues of these genes.

Cloning of *ced-3* showed that it encoded a latent cysteine protease that, when activated, caused programmed cell death[54], which implied that BCL-2 would act like CED-9 to prevent activation of caspases. Although this is indeed the case, it is important to note that there are major differences in the regulation of apoptosis between mammals and nematodes. In the worm, CED-9 directly inhibits CED-4, the activator of the caspase CED-3, and mitochondrial factors are not required for cell killing. In contrast, in mammals, anti-apoptotic BCL-2 acts by inhibiting the pro-apoptotic proteins BAX and BAK, for which there are no homologues in *C. elegans*. BAX and BAK promote cell death by causing mitochondrial outer membrane permeabilization (MOMP; see below), enabling the release of cytochrome *c*, which activates the CED-4 homologue apoptotic peptidase activating factor 1 (APAF1) in the cytosol to cause activation of caspase 9 and the caspase cascade[55].

In the mammalian and even overall vertebrate context, although there are a few exceptions, in most cases orthologues of BCL-2 family members can be used interchangeably. This has enabled rapid progress owing to the reproducibility of findings in experiments using human and mouse cells.

The BCL-2 family expands

When they cloned BCL-2, Cleary *et al.*[17] noticed that it resembled BHRF1, a product of the Epstein–Barr virus and, since then, many further viruses have

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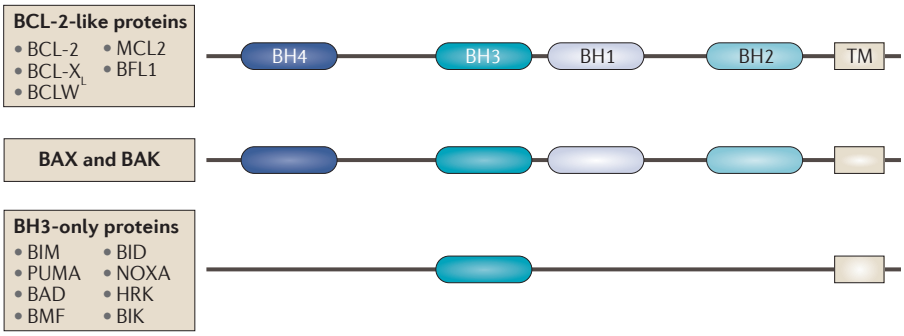

Figure 2 | **The BCL-2 family comprises three subsets of interacting family members.** The schematic shows the presence of BCL-2 homology (BH) domains, defined by sequence similarity to BCL-2, that facilitate subclassification of the BCL-2 family members into the three major subgroups of proteins. In the case of the BH3-only proteins the BH3 domain acts as a ligand domain to facilitate interaction with the other subgroups. Many pro-apoptotic as well as anti-apoptotic BCL-2 family members also have a transmembrane (TM) domain to facilitate association with the outer mitochondrial membrane; the exceptions are BFL1, BAD, BID, PUMA and BMF. All the human BCL-2 family members depicted here have direct mouse homologues of the same name, except for BFL1 which is the human homologue of mouse A1. HRK, Harakiri.

been found to carry *BCL2*-like genes.[56] Importantly, because some of these viruses are implicated in cancer, it is possible that their BCL-2-related proteins contribute to tumorigenesis and may thus constitute therapeutic targets (see below). The first non-viral pro-survival *BCL2*-like genes to be identified were *MCL1* (myeloid cell leukaemia 1), *Bclx* (also known as *Bcl2l1*) and *A1* (also known as *Bcl2a1* and called *BFL1* in humans). *MCL1* was identified in 1993 in a screen for genes induced by phorbol 12-myristate 13-acetate (PMA) in myeloid leukaemia cells[57]. In the same year, the *Bclx* gene, which encodes the pro-survival BCL-X_L protein (and the rarely detected shorter splice variant, BCL-X_S), was discovered by low-stringency hybridization, initially in chickens, and then in mammals[58]. *A1* was identified as a gene induced by granulocyte–macrophage colony-stimulating factor (GM-CSF) in myeloid cells[59]. *BCLW* (also known as *BCL2L2*) joined the pro-survival BCL-2 family members when it was cloned in 1996 (REF. 60), and mouse *Bcl2l10* (also known as *Diva* and *Boo*; the human homologue is called *BCLB*) was cloned in 1998 (REF. 61).

Korsmeyer and colleagues[62] identified BAX as a protein that co-immunoprecipitated with BCL-2, and found that the two proteins shared similar amino acid sequences within the BH domains. Surprisingly, BAX was found to promote, rather than inhibit, apoptosis when overexpressed[62]. This was the first discovery that the BCL-2 family contains both pro-survival and pro-apoptotic members, and that they regulate cell death by physically interacting with each other. The other multi-BH-domain pro-apoptotic BCL-2 family member, BAK, was cloned in 1995 (REFS 63,64), and the highly related BOK (the function of which is still unclear[65]) was identified in 1997 (REF. 66).

BAD was discovered as a BCL-2-binding protein in 1995 using the yeast two-hybrid system and λ phage expression screening[67]. Despite initial controversy about the number and type of BH domains present in BAD, subsequent analysis showed that it has just a BH3 domain[68]. BAD and BIK (also described in 1995 (REF. 69)) thus became the prototypic members of a novel subclass of the BCL-2 family that are now called the BH3-only proteins. Korsmeyer's laboratory also described BID, which, after being cleaved by caspase 8 to generate the active truncated form, tBID, binds not only to BCL-2 to inhibit it but also to BAX to activate it. This provided the first example of direct activation of pro-apoptotic BAX by a BH3-only protein[70] (FIG. 3). Mutations in the BH3 domain of BID abrogated its pro-apoptotic function as well as its ability to interact with BCL-2 and BAX. Later biochemical and genetic studies revealed that although all BH3-only proteins can bind to pro-survival BCL-2 family members, leading to the indirect activation of BAX and BAK, some — for example, tBID, BIM and PUMA — can also bind directly to and activate BAX and BAK[71–74].

Other BH3-only proteins include Harakiri (HRK)[75], BIM[76], BMF[77], and the p53 target genes *NOXA* (also known as *PMAIP1*)[78] and *PUMA*[79,80]. Several other proteins (for example, BNIP3 and NIX (also known as BNIP3L)) have been found to have sequences that resemble BH3 domains, but their ability to bind to and regulate pro-survival BCL-2 proteins or pro-apoptotic BAX and BAK has not been established, and some clearly function in non-apoptotic processes[81,82]. These and other proteins containing certain BH domains — for example, BCLG (also known as BCL2L14) and BFK (also known as BCL2L15) — are not further discussed here.

BCL-2 family regulation of apoptosis

Starting in the early 1990s, the individual and overlapping functions of BCL-2 family members and the consequences of disrupted regulation of apoptosis were revealed by the generation of gene knockout mice. *Bcl2*$^{-/-}$ mice completed embryonic development but succumbed to polycystic kidney disease early in life[83–85]. Elevated rates of apoptosis were evident in the lymphoid organs, which were reduced in size, and the mice became prematurely grey owing to loss of melanocytes. These defects could all be rescued by concomitant loss of pro-apoptotic BIM[86]. *Bclx*-deficient mice died around embryonic day 13.5 (E13.5) owing to increased apoptosis of erythroid and neuronal cells[87]. Complete loss of *Mcl1* caused embryonic lethality before blastocyst implantation at E3.5 (REF. 88), and studies with conditional knockout strains revealed that MCL1 is crucial for the survival of many cell types, including cardiomyocytes[89,90], neurons[91], haematopoietic stem or progenitor cells[92,93] and immature as well as mature lymphoid cell subsets[94–96].

Mice lacking the BH3-only protein BIM had increased numbers of lymphocytes, which were resistant to diverse apoptotic stimuli, including cytokine deprivation, abnormal calcium flux and ER stress[97,98]. Many ageing *Bim*$^{-/-}$ mice developed a fatal systemic lupus erythematosus (SLE)-like autoimmune disease with severe glomerulonephritis and autoantibodies against a range of self-antigens[97], reminiscent of the pathologies seen in *BCL2* transgenic mice several years earlier[45]. Moreover, aged mice lacking both BIM and PUMA presented with signs of lymphoid neoplasms more frequently than mice lacking only BIM or PUMA[99], demonstrating the overlapping tumour-suppressive function of these BH3-only proteins. Deletion of the *Bim* gene accelerated lymphoma development in *Eμ-Myc* transgenic mice more than deletion of genes for any other BH3-only protein[100]. Notably, loss[101] or silencing[102] of *BIM*

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is frequently found in human cancers: for example, mantle cell lymphoma and renal carcinoma.

BID-deficient mice were normal in the absence of stress, but their hepatocytes were resistant to FAS-induced apoptosis[103]. This, together with previous biochemical investigations[104,105] and later genetic studies[106], confirmed the type I/type II model[47], that the death receptor FAS can cause apoptosis by two different pathways, one involving BID, and the other independent of BID. In type I cells, such as lymphocytes, enough caspase 8 is activated that apoptosis is not prevented by overexpression of BCL-2. Conversely, in type II cells, such as hepatocytes and pancreatic-β cells, FAS-induced apoptosis depends on caspase-mediated cleavage of BID to generate tBID, which can then activate the intrinsic apoptotic pathway by activating BAX and BAK directly as well as by neutralizing BCL-2-like pro-survival proteins[104,105].

In 2000, mice were generated that were deficient for both BAX and BAK[107]. Remarkably, although mice lacking either BAX or BAK alone have only minor abnormalities (most notably, a defect in spermatogenesis in *Bax*$^{-/-}$ males[108]), the *Bax*$^{-/-}$;*Bak*$^{-/-}$ animals exhibited developmental abnormalities, including persistence of interdigital webs, imperforate vagina and excess neuronal cells in certain areas of the brain[107]. Although most *Bax*$^{-/-}$;*Bak*$^{-/-}$ mice died soon after birth, the few surviving animals developed lymphadenopathy, SLE-like autoimmune disease and lymphoid neoplasms when old[109,110].

Cells from the *Bax*$^{-/-}$;*Bak*$^{-/-}$ mice proved to be resistant to many apoptotic stimuli, including enforced expression of BH3-only proteins[107,111,112], demonstrating that BAX and BAK have essential (and largely overlapping) roles in unleashing the effector phase of mitochondrial apoptosis. Perhaps even more surprisingly, because some *Bax*$^{-/-}$;*Bak*$^{-/-}$ mice survived into adulthood, this showed that there is not an absolute requirement for BAX- and BAK-dependent apoptosis during embryonic development of the mouse.

Mechanism of BAX and BAK killing

In 1994, Newmeyer *et al.*[113] used a cell-free system to show that a mitochondrial component was required for the induction of apoptosis. Wang and colleagues[114] found that during apoptosis, cytochrome *c* was released from the mitochondria and could promote caspase activation *in vitro*. Furthermore, BCL-2 could prevent the release of cytochrome *c*[115,116]. How BCL-2 achieved this was revealed by the discoveries that BAX moves from the cytosol to the mitochondria during apoptosis[117] and, once there, can oligomerize to form channels that allow the release of cytochrome *c*[118]. Although the release of cytochrome *c* was necessary for APAF1-mediated activation of caspase 9 and downstream effector caspases, it is important to note that activation of BAX and BAK is usually sufficient to cause the death of the cell, even of those cells that lack APAF1 or caspase 9 (REFS 119,120). Thus, in mammalian cells, BAX or BAK activation constitutes the point of no return in apoptosis signalling, and the caspase cascade is crucial for cellular demolition.

Structural analysis of single proteins[121,122] and complexes, such as BIM bound to BCL-X_L (REF. 123), revealed how the various members of the BCL-2 family interact at the molecular level and how BAX and BAK must change shape to cause MOMP and apoptosis[124,125]. These interactions take place mostly on or within intracellular — for example, mitochondrial — membranes, and innovative experiments using fluorescence resonance energy transfer (FRET) helped to clarify the topology of BCL-2 protein family members on membranes[126]. According to the 'embedded together' model, pro-apoptotic and anti-apoptotic BCL-2 family proteins interact in membranes and undergo conformational changes that either result in membrane permeabilization by BAX or BAK (apoptotic death) or prevent it (cell survival)[127].

BCL-2 family deregulation in cancer

In addition to its activation by the t(14;18) chromosomal translocation in follicular lymphoma, amplification of the *BCL2* gene has been identified in some cases of diffuse large B cell lymphoma[128] and small-cell lung carcinoma[129] (FIG. 4). Furthermore, most cases of CLL overexpress BCL-2 because they have deleted or silenced miR-15a and/or miR-16.1, microRNAs (miRNAs) that normally suppress *BCL2* expression[130].

Somatic copy number alterations (SCNAs) of *BCLX* and *MCL1* (gain) and *BOK* (loss) have been detected in a substantial proportion of human cancers[131]. Moreover, whole-genome mRNA expression analyses and western blotting have revealed that a multitude of human cancers present with elevated levels of BCL-X_L. This is thought to enhance chemotherapy resistance,

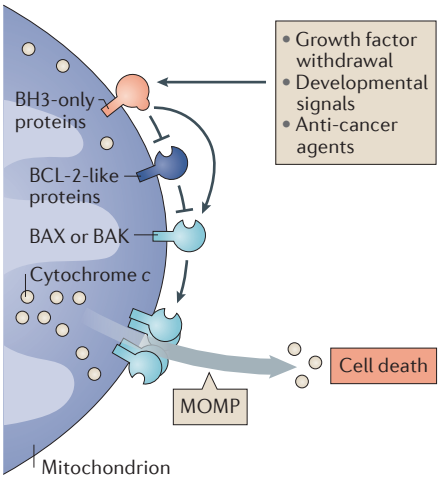

Figure 3 | **BCL-2-regulated apoptosis signalling.** Cytotoxic stimuli, such as growth factor deprivation or anti-cancer drugs, activate pro-apoptotic BH3-only proteins through transcriptional or post-transcriptional processes. Some BH3-only proteins (for example, BAD) initiate apoptosis signalling indirectly, by binding to pro-survival BCL-2 proteins, thereby preventing them from keeping pro-apoptotic BAX and BAK in check. Other BH3-only proteins (for example, truncated BID (tBID), BIM and PUMA) can also initiate apoptosis directly (see main text for mechanism), by binding to BAX or BAK. Activated BAX and BAK cause mitochondrial release (mitochondrial outer membrane permeabilization (MOMP)) of apoptogenic factors (for example, cytochrome c) into the cytoplasm where they promote activation of caspases, the proteases that mediate cell demolition.

for example, in subsets of breast cancer[132], neuroblastoma[133], colorectal cancer[134], gastric adenoma and carcinoma[135], hepatocellular carcinoma[136] and prostate cancer[137]. In a broad range of cancers, high levels of BCL-X_L or MCL1 have been ascribed to the loss or silencing of miRNAs that normally attenuate their expression, such as let-7 to target *BCLX*[138] or miR-29, miR-125 and miR-193 to target *MCL1* (REFS 139,140).

Mutations predicted to compromise the pro-apoptotic members of the family have also been observed in human cancer. Homozygous deletion of *BIM* has been found in ~20% of cases of human mantle cell lymphoma[101]. In addition, epigenetic silencing of *BIM* or *PUMA* was reported in several cancers, including renal cell carcinoma and Burkitt lymphoma[102,141]. Moreover, frameshift mutations in *BAX* were found in colon cancers with a hypermutation phenotype[142] and loss-of-function mutations in *BAX* were detected in certain haematopoietic cancers[143].

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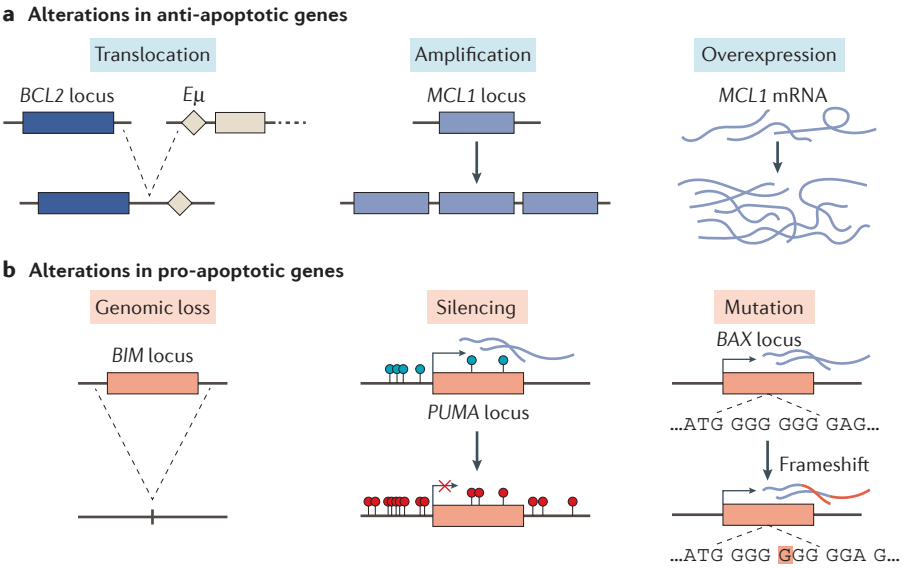

Figure 4 | **Mechanisms of BCL-2 family deregulation in human cancer.** Alterations of both anti-apoptotic and pro-apoptotic genes through genetic or epigenetic mechanisms have been observed in cancer. **a** | Increased levels of anti-apoptotic BCL-2 family members can occur by chromosomal translocations (for example, of *BCL2*), somatic gene copy number amplifications (for example, of myeloid cell leukaemia 1 (*MCL1*)) or through overexpression (for example, *MCL1*). **b** | Loss of pro-apoptotic BCL-2 family members can occur by genomic deletion (for example, of *BIM*), gene silencing due to hypermethylation (for example, of *PUMA*) or mutation (for example, frameshift mutation in *BAX*). The figure shows only some examples of these mechanisms and is not meant to be an exhaustive list.

BH3-only proteins in cancer therapy

The role of the BCL-2-regulated apoptotic pathway in the response to anti-cancer therapeutics was first recognized when *BCL2*-transfected cell lines or non-transformed lymphoid cells from *BCL2* transgenic mice were found to be profoundly resistant to a broad range of cytotoxic insults, including gamma-radiation and several chemotherapeutic drugs (for example, etoposide or dexamethasone)[25,28,29,144]. Cells from *Bax*$^{-/-}$;*Bak*$^{-/-}$ mice are profoundly resistant to a broad range of anti-cancer therapies[107], and distinct BH3-only proteins were found to be necessary for cell killing by various agents. PUMA (and to a lesser extent NOXA) is required for the killing of normal as well as cancerous cells by therapeutic concentrations of DNA damage-inducing drugs — for example, etoposide or cyclophosphamide — that act at least in part through p53 (REFS 145–149). BIM also has a role in DNA damage-induced cell killing (indirectly activated by p53 and possibly also through a p53-independent pathway), as well as apoptosis induced by taxol, histone deacetylase inhibitors and glucocorticoids (the latter in a manner overlapping with PUMA)[97,147].

BIM is also required for the killing of diverse cancer cells by inhibitors of oncogenic kinases, such as treatment of chronic myeloid leukaemia (CML) cells with the BCR–ABL inhibitor imatinib[150,151], treatment of lung cancer cells with one of the epidermal growth factor receptor (EGFR) inhibitors gefitinib or erlotinib[152–154] and treatment of *BRAF*-mutant melanoma or colon carcinoma cells with inhibitors of MEK or BRAF[155,156]. A polymorphism in the *BIM* gene that affects splicing and is found in certain Asian populations was reported to diminish the therapeutic responses of CML to imatinib and of lung cancers to gefitinib or erlotinib[157].

These experiments showed that, at clinically achievable doses, many cancer therapeutic agents do not kill cells directly, but cause stresses that are detected by the cells, which then respond by undergoing apoptosis. Overall, cancer cells are genetically unstable and hence more fragile than normal cells, and studies in which cells are exposed to peptides that mimic pro-apoptotic BH3-only proteins have led to the concept of cancer cells being 'primed for death' (REFS 158,159). Tumour cells that express high levels of cell death inhibitors, such as BCL-2 or MCL1, should be chemoresistant, and therefore drugs that target these cell survival molecules might increase the sensitivity of such tumour cells to chemotherapy or even kill them as single agents.

BH3-mimetics for cancer therapy

As it became clear that BCL-2-like proteins promoted the survival of tumour cells, it was apparent that drugs that inhibited these proteins might be useful therapeutically. Perhaps because of the historical challenges associated with drugging protein–protein interactions, early drug development programmes focused on inhibiting BCL-2 expression through the use of antisense oligonucleotides[160]. Despite initial promise, subsequent studies have revealed that much of the activity observed with these compounds probably derived from their ability to induce interferons rather than their ability to repress BCL-2 expression[161]. Certain drugs identified through compound library screening, including gossypol, apogossypol and obatoclax, that were reported to inhibit BCL-2 and some of its pro-survival homologues kill not only control but also *Bax*$^{-/-}$;*Bak*$^{-/-}$ cells[162–166]. Therefore, at least some of their activity seems to be off-target, and these compounds have not progressed in clinical trials.

The 3D structure of BCL-X_L, both on its own[167] and in complex with a BH3 peptide from BAK[121], enabled a different approach to be taken. Fesik, Rosenberg and co-workers[168] developed compounds to mimic the function of BH3-only proteins (BH3-mimetics) with the goal of bypassing the block in apoptosis signalling that exists in many tumour cells — for example, due to mutations in *TP53* that prevented induction of pro-apoptotic BH3-only proteins. ABT-737, which binds to and inhibits BCL-2, BCL-X_L and BCLW, much like a BH3 peptide, was the 'first-in-class' of such compounds. *In vitro* and *in vivo*, ABT-737 killed certain cancer cell lines as a single agent, and could kill other cancer cell lines when it was combined with standard chemotherapeutic drugs[168]. Studies using cells from *Bax*$^{-/-}$;*Bak*$^{-/-}$ mice confirmed that ABT-737 and its clinically used successor ABT-263 (also known as navitoclax)[169] kill in a BAX- and BAK-dependent (that is, on-target) manner[165,170].

In clinical trials (TABLE 1), ABT-263 as a single agent significantly reduced tumour burden in most patients with CLL[171,172], and in preclinical studies in combination with other conventional treatments it showed efficacy in several

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Table 1 | **BH3-mimetics undergoing clinical trials for cancer indications**

BH3-mimetic	Alternative name	Targets	Therapy	Indication	Clinical trial stage
ABT-263	Navitoclax	BCL-2, BCL-X_L and BCLW	Single agent	Chronic lymphocytic leukaemia	Phase I/II
				Cutaneous T cell lymphoma	Phase I/II
				Follicular lymphoma	Phase I/II
				Indolent lymphoma	Phase I/II
				Mantle cell lymphoma	Phase I/II
				Non-Hodgkin lymphoma	Phase I/II
				Peripheral T cell lymphomas	Phase I/II
			Combination*	Prostate cancer	Phase II
				Colon cancer	Phase I/II
				Melanoma	Phase I/II
				Non-small-cell lung cancer	Phase I/II
				Pancreatic cancer	Phase I/II
				Rectal cancer	Phase I/II
				Skin cancer	Phase I/II
				Small-cell lung cancer	Phase I/II
				Chronic lymphocytic leukaemia	Phase I
				Diffuse large B cell lymphoma	Phase I
				Follicular lymphoma	Phase I
				Hepatocellular carcinoma	Phase I
				Hodgkin lymphoma	Phase I
				Lymphoblastic lymphoma	Phase I
				Lymphoma	Phase I
				Non-Hodgkin lymphoma	Phase I
				Other haematological disorders	Phase I
ABT-199	Venetoclax	BCL-2	Single agent	Chronic lymphocytic leukaemia	Phase III
				Acute myeloid leukaemia	Phase I/II
				Diffuse large B cell lymphoma	Phase I
				Follicular lymphoma	Phase I
				Lymphoma	Phase I
				Mantle cell lymphoma	Phase I
				Multiple myeloma	Phase I
				Non-Hodgkin lymphoma	Phase I
			Combination*	Chronic lymphocytic leukaemia	Phase III
				B cell non-Hodgkin lymphoma	Phase I/II
				Diffuse large B cell lymphoma	Phase I/II
				Follicular lymphoma	Phase II
				Non-Hodgkin lymphoma	Phase II
S-055746	None	BCL-2	Single agent	Haematological malignancies including myelodysplasia	Phase I
PNT-2258	None	BCL-2	Single agent	Diffuse large B cell lymphoma	Phase II
				Follicular lymphoma	Phase II
				Non-Hodgkin lymphoma	Phase II

Data compiled from the Global Data database: http://healthcare.globaldata.com/ (accessed July 2015). *Combination with standard of care therapies.

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additional cancers, including certain types of breast cancer[173–175]. However, as BCL-X_L is crucial for the survival of platelets[176,177], BAX- and BAK-mediated thrombocytopenia limits the dosing of ABT-263 in patients, and this will probably also be the case for BCL-X_L-selective BH3-mimetics[178].

For the treatment of cancers that depend on BCL-2, the BCL-2-specific BH3-mimetic ABT-199 was developed. Because it does not target BCL-X_L, ABT-199 does not reduce platelet lifespan[179] and is therefore better tolerated than ABT-263. This compound has proved highly efficacious and tolerable in a Phase I trial[180] and has rapidly progressed into Phase III clinical trials as a single agent for the treatment of patients with relapsed or refractory lymphoid malignancies (particularly CLL). ABT-199 is also being investigated in combination with other anti-cancer therapies (TABLE 1), such as anti-CD20 monoclonal antibodies (for example, rituximab, ofatumumab and obinutuzumab), and is expected to reduce tumour burden as reported for ABT-263 (REF. 181). These trials are supported by preclinical studies in tissue culture and with transplanted tumours in mice, which showed that BCL-2-targeting BH3-mimetics augment the killing of certain haematological cancers[179,182,183] as well as breast cancers[184] elicited by conventional chemotherapeutics or targeted therapies[185].

As MCL1 is abnormally overexpressed (for example, owing to SCNA) in many cancers[131] and has been proved to be necessary for the sustained survival and growth of diverse types of tumour[186–190], high-affinity MCL1-specific BH3-mimetic compounds are eagerly awaited, as both research tools and drugs. However, caution will have to be taken with the use of MCL1 inhibitors, because this pro-survival BCL-2 family member is essential for the survival of many normal cell types, including cardiomyocytes and neuronal cells[89–93,96].

Combinations of BH3-mimetics with standard chemotherapeutics, particularly those inducing DNA damage, are likely to cause substantial side effects, because both drugs will affect not only the malignant but also the normal cells. Combinations of BH3-mimetics with drugs that only (or at least preferentially) affect the cancer cells, such as CD20 antibodies[181] or inhibitors of oncogenic kinases, such as imatinib, gefitinib, erlotinib or vemurafenib[191], provide a promising strategy for the selective killing of cancer cells while minimizing bystander killing of normal cells. Although for all combinations the therapeutic window will have to be determined in clinical trials, *in vitro* tests on cancer cells, such as 'BH3 profiling' might enable the effectiveness of combining BH3-mimetics and conventional chemotherapeutics to be predicted[158,159,192].

Conclusions and perspectives

More than 30 years have passed since the cloning of the t(14;18) chromosomal break point in human follicular lymphoma, and the naming of BCL-2 by Tsujimoto *et al.*[14] Since then, researchers have shown that the role of BCL-2 is to inhibit cell death, have identified many cell death effectors and have uncovered a complex web that regulates apoptosis. Inhibitors of BCL-2 and some of its relatives (the BH3-mimetic drugs) are currently showing great promise in clinical trials.

Nevertheless, challenges remain. Although the clinical trial results (particularly for ABT-199) look encouraging, as yet no BH3-mimetic drug has been approved for clinical use. Once approved, determination of the optimal use and the best combinations with other conventional or targeted therapies will take time. If drug resistance develops, the mechanisms will have to be elucidated. BH3-mimetic drugs that specifically inhibit MCL1, BFL1 or pathogen-encoded pro-survival BCL-2 family members might prove effective for use in the treatment of cancer, and autoimmune diseases, as well as infectious diseases, but the therapeutic window for each compound will have to be determined carefully. Identification of the upstream signalling mechanisms that control the expression and function of BCL-2 family members might provide further drug targets.

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1. AbbVie Media Room. Venetoclax receives breakthrough therapy designation in relapsed or refractory chronic lymphocytic leukemia in previously treated patients with the 17p deletion genetic mutation. http://abbvie.mediaroom.com/2015-05-06-Investigational-Medicine-Venetoclax-Receives-Breakthrough-Therapy-Designation-in-Relapsed-or-Refractory-Chronic-Lymphocytic-Leukemia-in-Previously-Treated-Patients-with-the-17p-Deletion-Genetic-Mutation (updated 6 May 2015).
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Competing interests statement
The authors declare competing interests: see Web version for details.

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