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BCL-2: Long and winding path from discovery to therapeutic target

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ABSTRACT

In 1988, the BCL-2 protein was found to promote cancer by limiting cell death rather than enhancing proliferation. This discovery set the wheels in motion for an almost 30 year journey involving many international research teams that has recently culminated in the approval for a drug, ABT-199/venetoclax/Venclexta that targets this protein in the treatment of cancer. This review will describe the long and winding path from the discovery of this protein and understanding the fundamental process of apoptosis that BCL-2 and its numerous homologues control, through to its exploitation as a drug target that is set to have significant benefit for cancer patients.

Contents

1. Introduction

Kerr, Wyllie and Curry first coined the term apoptosis in 1972 to describe the phenomenon of programmed cell death that they had observed in diverse cell types [1]. In this seminal article, it was established that apoptosis plays a key physiological role in normal tissue development and homeostasis, but also during the regression of tumours. At that time, however, little was known about the mechanisms by which a cell could essentially commit suicide. Since then, an understanding of the apoptotic machinery that controls these cell fate decisions has been an active area of research, and we now understand in exquisite detail the roles of the BCL-2 family of proteins as the master regulators of apoptosis [2–5]. Evasion of apoptosis is recognised as a hallmark of cancer cells [6], and therapies that target this survival pathway are now making their way into the clinic [7,8]. This review summarises the discoveries of the BCL-2 family that have led to the development of a targeted BCL-2 inhibitor, ABT-199/venetoclax, that has recently received Federal Drug Administration (FDA) approval for the

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treatment of a poor prognosis subset of chronic lymphocytic leukaemia (CLL) patients with 17p chromosomal deletion. Along this journey we hope to identify the lessons learned from targeting BCL-2, and how future new therapies may follow the same route as ABT-199/venetoclax.

2. BCL-2: a driver of tumour development

Human BCL-2 was the first pro-survival protein to be discovered in any species, and the namesake of the family of proteins that regulate apoptosis. The discovery of BCL-2, however, came not from studying cell death, but from studying cancer. Its very name, B Cell Lymphoma/Leukaemia 2, is derived from this fact. The *BCL-2* gene was identified by Tsujimoto and colleagues [9] from their studies of the t(14;18) chromosomal translocation, which is found in almost all cases of follicular lymphomas [10–13]. They, and others [14,15], identified that the translocation fused the immunoglobulin heavy chain (IgH) joining region on chromosome 14 with the novel oncogene *BCL-2* on chromosome 18 and that the IgH enhancer element could affect the expression of *BCL-2* in these lymphoma cells. Accordingly, *BCL-2* mRNA expression in t (14; 18) chromosomal translocation bearing lymphoma cells was found to be significantly higher than in normal B cells [16,17].

The function of this novel oncogene, however, was a complete mystery. The sequence of the *BCL-2* gene had no homology to any of the known oncogenes of the time (e.g. *MYC*, *RAS*, *SRC*, *ABL*) [14]. The only protein that BCL-2 did share homology with was an equally enigmatic protein from Epstein-Barr Virus (EBV), known as BHRF1 [18]. Biochemical studies performed described BCL-2 as a 26 kDa protein that associated with cell membranes, but its cellular function remained elusive [19,20]. By studying *BCL-2* mRNA expression levels in non-transformed cells, it was found that it is highly expressed in pre-B cells and at lower levels in mature B cells [16]. Notably, it is during the pre-B stage that the *BCL-2* gene is subject to the chromosomal translocation with the *IgH* gene locus, due to errors that occur during VDJ recombination to produce a functional IgH chain [21,22]. However, in addition to pre-B cells, high levels of *BCL-2* mRNA expression were also noted in T cells [16]. Furthermore, *BCL-2* transcripts were elevated in response to mitogenic stimulation in both B and T lymphocytes [23].

Cloning of the murine homologue of *BCL-2* in 1987 [24] enabled expression studies to be carried out in mice. Consistent with the human studies that had associated prominent *BCL-2* expression with lymphocytes [16], *Bcl-2* in mice was most strongly expressed in the spleen and thymus. However, appreciable levels of *Bcl-2* mRNA could also be detected in the brain, heart, kidney and liver, with notably higher expression in newborn mice for these non-haematopoietic organs [24]. Mouse *Bcl-2* also appeared to act in the same manner as its human homologue in cancer, given that high levels of its expression were associated with pre-B and follicular lymphomas, but not with tumours originating from pro-B cells or plasma cells [25].

The advent of molecular technologies during the 1970s and 1980s were pivotal to the studies on the function of *BCL-2*. Research performed by Peter Nowell's group represented the first use of gene expression vectors to study *BCL-2* function [26]. They reported that ectopic expression of *BCL-2* in a fibroblast cell line did not cause the morphological changes that were observed when such cells were transfected with vectors encoding another membrane-associated protein, mutant RAS. However, transplantation of the *BCL-2*-transfected cells into mice did produce tumours. Whilst this supported the tumorigenicity of overexpressed *BCL-2*, the study still lacked the mechanistic understanding for why this occurred. It was not until 1988, with the work of Vaux, Cory and Adams, that at last a pro-survival role of *BCL-2* was revealed [27]. The groundbreaking experiment itself was remarkably simple: they transfected IL-3-dependent haematopoietic progenitor cell lines with retroviral constructs to overexpress *BCL-2*, removed the essential growth factor, and then counted the cells. *BCL-2*-transfected cells went into cell cycle arrest in the absence of the growth factor, but importantly, though the cells did not proliferate, they did not die, whereas all control cells died within 48–72 hours.

This was a paradigm shift – cancer cells do not only have the capacity to proliferate uncontrollably, but they can also *survive* uncontrollably. It was subsequently shown that expression of *BCL-2* could protect cells from a range of cellular stresses, such as heat shock and diverse cytotoxic agents such as methotrexate, etoposide and γ-irradiation [28,29]. Moreover, the pro-survival activity of BCL-2 was not restricted to IL-3-dependent myeloid progenitor cells, but was seen when lymphoid or haematopoietic cell lines were deprived of other essential growth factors, such as granulocyte macrophage colony stimulating factor (GM-CSF) or IL-4 [30]. BCL-2 over-expression also protected non-transformed B and T cells from transgenic mice against spontaneous cell death in culture [29,31–34].

However, in contrast to the Nowell group study with fibroblasts [26], BCL-2 over-expressing IL-3 dependent myeloid progenitor cells injected into mice failed to produce tumours [27,28,35]. *Eμ-Bcl-2* transgenic mice were generated to mimic the t(14;18) chromosomal translocation seen in follicular lymphoma within the whole animal [29,31,33]. Despite clear accumulation of B lymphoid cells and a profound survival advantage of the transgenic B cells in culture [29,31,33,36], these mice did not develop lymphomas unless they were aged [37,38]. Even at an advanced age (1–2 years) the incidence of tumours was low (5–10%) and they did not resemble the follicular lymphoma characteristic of the t(14;18) chromosomal translocation in humans. Later studies with *Vav-Bcl-2* transgenic mice [39], which over-express higher levels of BCL-2 in all haematopoietic cells, would eventually recapitulate a follicular lymphoma phenotype, even though this was also only observed with age [40]. These studies suggested that the survival advantage conferred by BCL-2 over-expression enabled some cells to acquire other oncogenic lesions that would cause them to eventually become fully transformed. The proof of this concept was provided by the generation of doubly transgenic mice for *Bcl-2* in combination with another oncogene, *c-Myc (Eμ-Myc/Bcl-2)* [41], by utilising the well-characterised *Eμ-Myc* model of pre-B/B cell lymphoma development [42,43]. The combination of enhanced proliferation, caused by *c-Myc*, with evasion of apoptosis elicited by *Bcl-2* led to stunningly rapid lymphoma development, with all mice succumbing before 45 days of age.

Thus the deregulated expression of *Bcl-2*, such as that seen in t(14;18)-chromosomal translocation-carrying lymphomas, was oncogenic in that it was *permissive*; its pro-survival activity enabled nascent neoplastic cells to survive long enough to gain other advantageous oncogenic mutations, for example in genes like *c-Myc*, that ultimately led to their full malignant transformation. Since these landmark studies, BCL-2 over-expression has been observed in a wide variety of lymphomas, leukaemias (most notably chronic lymphocytic leukaemia (CLL), see below), as well as in other types of cancer [44]. Evasion of apoptosis, via BCL-2 over-expression or otherwise, was acknowledged as a hallmark of cancer [6] and it is this wide relevance to cancer in general that holds so much promise for the future of targeted therapies against BCL-2 and its pro-survival relatives (see below).

3. Mapping the route: understanding the function of the BCL-2 family

Since the identification of the founding member BCL-2 [45], 16

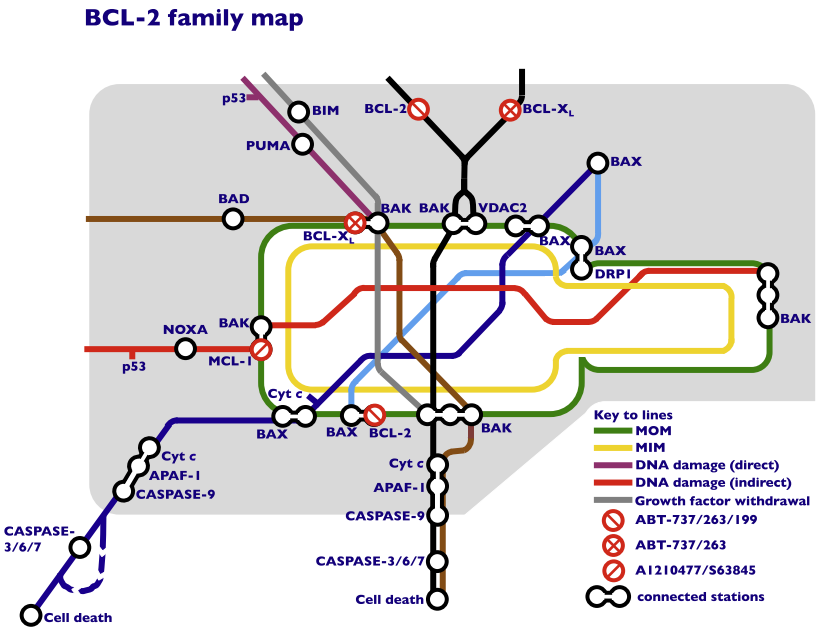

Fig. 1. Map of the key interactions of the BCL-2 family of proteins. Following exposure to an apoptotic stimulus, including growth factor withdrawal or DNA damage, BH3-only proteins are upregulated and/or activated. BH3-only proteins can initiate cell death either by binding to and inhibiting the pro-survival proteins (*indirect* line) or by binding and activating BAK or BAX (*direct* line). Once BAK or BAX are activated they self-associate on the mitochondrial outer membrane (*MOM*) to form complexes (*connected stations*) that damage the MOM, leading to the release of cytochrome *c*. Cytochrome *c* forms the apoptosome complex with APAF-1 and CASPASE-9 leading to downstream activation of CASPASE-3, 6, and 7 that co-ordinate dismantling of the cell. Activated caspases can feedback onto the mitochondria to further disable the organelle and expedite cell death. BAK and BAX connect with other mitochondrial proteins including VDAC2 and DRP1 (dynamin-related protein 1) [153–155], which provide additional control of their subcellular localisation and apoptotic activity. BH3-mimetic compounds bind to specific pro-survival BCL-2 proteins to block their pro-survival function. *MIM*, mitochondrial inner membrane.

additional BCL-2 family members have been identified due to their sequence conservation of the so-called BCL-2 homology (BH) domains 1–4 (reviewed in Ref. [2]). Although diverse functions of the BCL-2 proteins have been proposed, including anti-oxidant defence, calcium signalling, autophagy and mitochondrial dynamics (reviewed in Ref. [46]), the predominant (if not exclusive) role of the family is to regulate cell survival by regulating the integrity of the mitochondrial outer membrane (MOM), which if breached sounds the death knell for a cell. Not only does this MOM breach disable mitochondrial metabolism it also allows the release of apoptogenic factors, such as cytochrome *c* and Smac/DIABLO. When released, these factors activate caspases, aspartate-specific proteases that co-ordinate the destruction of the dying cell [47–53].

The selective, dynamic and complex interactions between the two pro-apoptotic subgroups (the BH3-only apoptosis initiators such as BIM, BID, PUMA and BAD and the multi-BH domain apoptosis effectors BAK, BAX and possibly BOK) and the pro-survival members of the BCL-2 family determine cellular fate in response to stress signals (Fig. 1). The ultimate goal of the family is to either inhibit (BCL-2, MCL-1, BCL-X_L, BFL-1/A1, BCL-W, BCL-B) or to induce the activation of BAK and BAX [54]. Although the details of the complex interactions that govern this process are still not fully resolved and are likely to be highly context-dependent, genetic and structural evidence supports that BAK and BAX can be activated by direct interaction with BH3-only proteins as well as by BH3-only protein-mediated neutralisation of the pro-survival family members [55–58]. Whether direct interaction with BH3-only proteins is essential for BAK/BAX activation is unclear. Although compound deletion of the main activating BH3-only proteins BIM, BID and PUMA impacts on mouse development [59], this does not fully phenocopy BAK/BAX deficiency [60], nor does this finding imply mechanism. Additionally, a recent study has shown that ectopic expression of BAK or BAX was able to kill a cell engineered to be completely devoid of BH3-only proteins [61]. It thus appears that the pro-survival BCL-2 proteins guard against BAK/BAX activity by two routes: by inhibiting BH3-only protein interactions or by limiting BAK/BAX self-association once activated [62–64]. Thus the effectiveness of any small-molecules to inhibit the pro-survival activity of BCL-2 proteins relies on their ability to block both routes. Once BAK and BAX are activated they undergo a series of conformation changes that facilitate their self-association to form an “apoptotic pore” that damages the MOM. The details of these structural changes and the consequent homo-oligomerisation of BAK and BAX are beginning to be delineated and have been reviewed elsewhere [65]. The role of the enigmatic third putative executioner protein, BOK, is still unclear with so far limited evidence to support a functional redundancy with BAK and BAX *in vivo* [66,67]. However, recent evidence has suggested that BOK mediates mitochondrial damage in response to endoplasmic reticulum stress possibly independent of regulation by other BCL-2 family proteins [68].

A common feature of the tertiary structures of the pro-survival BCL-2 proteins that is shared with BAK and BAX is a hydrophobic surface groove (Fig. 2) [69]. Mutagenesis and structural studies have highlighted this groove as key in the interaction of these proteins with the BH3 domain from other family members [56,57,70,71]. The groove has conserved hydrophobic pockets compatible with hydrophobic residues in the BH3 domain ligand. These hydrophobic interactions determine the specificity of the interactions with BH3-only proteins exhibiting a clear distinction in their affinity for specific pro-survival proteins, some being promiscuous (BIM, BID, PUMA) whilst others selective for BCL-2, BCL-X_L and BCL-W (BAD) or MCL-1 and BFL-1/A1 (NOXA) [72]. Specific BH3-only proteins are key in the response of cells to chemotherapeutic agents, for example PUMA and NOXA in response to DNA

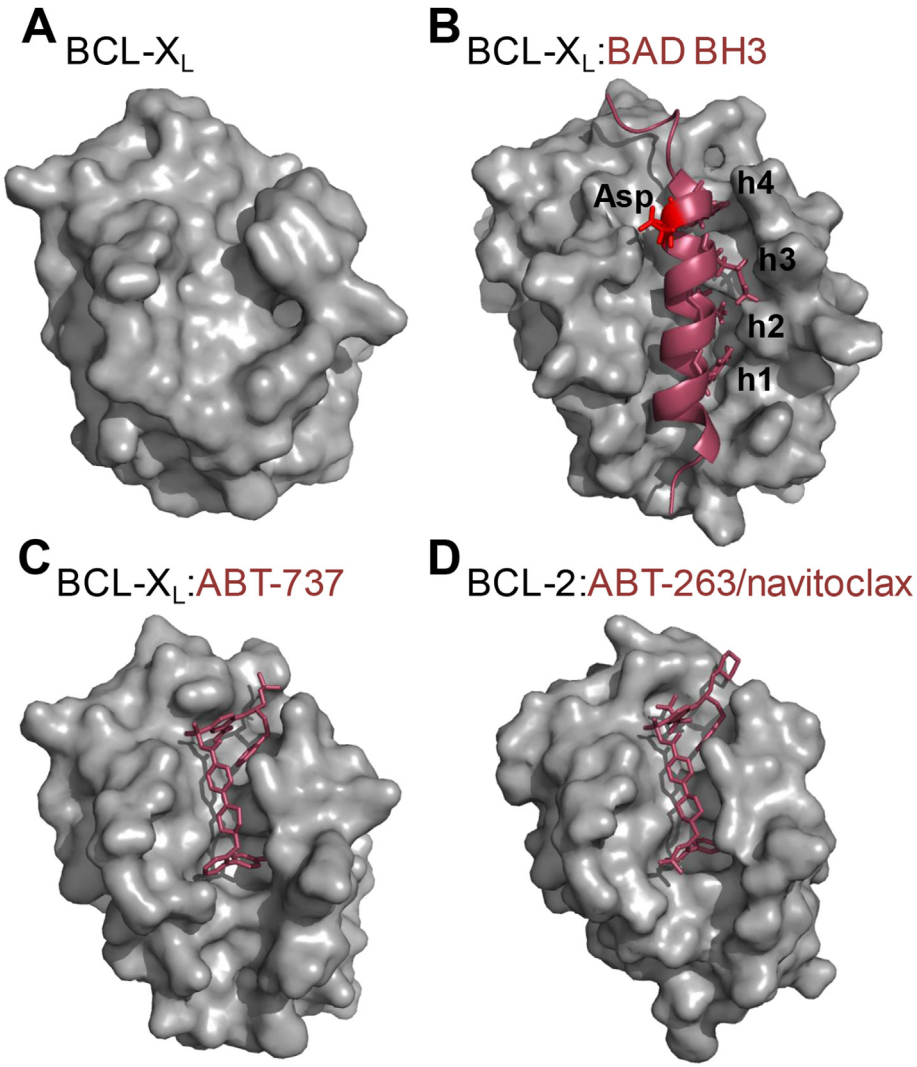

Fig. 2. Rational targeting of the BCL-X$_L$ hydrophobic groove. A. Surface representation structure of BCL-X$_L$ (PDB 1MAZ [156]). **B**. Surface representation of the structure of BCL-X$_L$ (*grey*) bound with the BH3 peptide from BAD (*pink*) (PDB 1G5J [71]). Hydrophobic residues (*stick*) and a charged aspartate (*red stick*) in the BAD BH3 are key in its specific interaction with hydrophobic pockets in the BCL-X$_L$ groove. **C**. Surface representation of the structure of BCL-X$_L$ (*grey*) in combination with ABT-737 (*pink*) (PDB 2YXJ [157]). **D**. Surface representation of the structure of BCL-2 (*grey*) in complex with ABT-263/navitoclax (*pink*) (PDB 4LVT [106]. Key interactions of the small molecules mimic those of BAD docking to the BCL-X$_L$ groove.

damaging agents [73] and BIM in response to taxanes [74]. The understanding that the hydrophobic groove of the pro-survival BCL-2 family members is critical for interactions with activated BH3-only proteins identified the hydrophobic groove as an important site for drug targeting to manipulate cancer cell apoptosis. This understanding of the interactions of BH3-only proteins and pro-survival BCL-2 proteins at the atomic level was fundamental to generating "BH3 mimetic" drugs, as will be discussed below.

4. En route to discovering antagonists of BCL-2

So the bags were packed, the oven was off and we were ready to go. Right? Not so fast. The basic premise that such a broad hydrophobic protein:protein interface could be targeted by a small molecule at all was questioned, with such interfaces largely perceived as "undruggable". So the journey could have been over before it had even begun. However, early compound library screening identified a number of apoptosis inducers that were subsequently reported to bind to pro-survival BCL-2 proteins and disrupt their interactions, suggesting that specific targeting of these protein interactions was indeed a feasible strategy [7]. So the journey to discover BH3-mimetics with the ability to efficiently and specifically antagonise pro-survival BCL-2 proteins began in earnest.

Route 1: Library screening- The first potential BCL-2 antagonist discovered was gossypol, a polyphenolic compound isolated from cotton seed. Identified in a natural compound library screen as an inducer of cell death, gossypol, in particular the (−) enantiomer (AT-101), was reported to bind BCL-2, BCL-X$_L$, and MCL-1 [75]. Preclinical evidence supported the notion that gossypol could potently induce cell death *in vitro* and suggested that it could limit tumour growth *in vivo* [75,76]. However, subsequent studies questioned whether the binding of BCL-2 proteins is the route of its cytotoxic action given that gossypol could kill cells in a BAK/BAX-independent fashion [77], whilst BAK/BAX-deficiency renders cells resistant to diverse apoptosis inducers including BH3-only protein over-expression [78,79]. In clinical trials gossypol lacked efficacy in patients with small cell lung cancer (SCLC) and its development was further hampered by toxicity issues that were

likely not related to apparent binding to pro-survival BCL-2 proteins [80].

Another natural compound, the indole bipyrrole small molecule obatoclax (GX15-070), was likewise identified in a natural compound library screen and was, perhaps uniquely, found to inhibit the interactions of most if not all of the pro-survival BCL-2 proteins (BFL-1/A1 was not tested in the initial studies) rather than just a restricted subset [81,158]. Preclinical studies suggested promising synergy between obatoclax and commonly used chemotherapeutic drugs *in vitro* and *in vivo,* supporting its progression to clinical trials [82]. Given the pan-BCL-2 inhibitor activity of the drug, one might expect significant on-target toxicity, but, surprisingly, obatoclax was well tolerated in Phase I trials [83–85]. Despite this, the drug displayed limited efficacy in Phase II trials of SCLC [86] and Hodgkin's lymphoma [87], most likely due to its relatively low affinity (~0.5 μM) for pro-survival BCL-2 proteins. Additionally, the route of the cytotoxic action of obatoclax and its anti-tumour activity in preclinical studies is most likely not related to binding BCL-2 proteins, as, like gossypol, the drug efficiently kills cells that are devoid of both BAK and BAX [77]. Thus, to date, compound library screening has identified a number of interesting apoptosis-inducing agents with potential tumoricidal effects. Although most of these are not *bona fide* BH3-mimetics with cell killing activity that is dependent on BAK and BAX and hence reliant on the mitochondrial (also called BCL-2-regulated, intrinsic, stress-induced) apoptotic pathway, engineered derivatives with improved affinity and selectivity for distinct BCL-2 family members may indeed prove useful in the laboratory and potentially also in the clinic.

Route 2: Rational drug design- An alternative approach using structure-activity relationship (SAR) and nuclear magnetic resonance (NMR)-based fragment screening to engineer small molecule ligands that bind to the hydrophobic groove of BCL-X_L has yielded the most advanced series of small molecule BCL-2 pro-survival protein antagonists to date. This rational design approach could in theory exploit the differences in BH3-only protein binding to pro-survival proteins and thereby provide more specific (and potentially less toxic) antagonists either as clinical agents or important research tools. Structure-guided medicinal chemistry of the initial lead compounds that had relatively low affinities for BCL-2-like proteins and poor bioavailability (e.g. high serum binding) led to the development of ABT-737, a potent inhibitor of BCL-2, BCL-X_L (IC50 < 1 nM and <0.5 nM for BCL-2 and BCL-X_L, respectively) and BCL-W, but negligible binding to MCL-1 or BFL-1/A1 (>1 μM) [88]. As such, its binding profile mimics that of the BH3-only protein BAD. Consistent with this binding profile, ABT-737 could induce apoptosis *in vitro* particularly in cells that were devoid of MCL-1 [77,88]. Importantly, the cell killing activity of this drug was completely dependent on BAK/BAX, thus distinguishing it from the putative BCL-2 antagonists mentioned above. Excitingly, this compound exhibited single-agent activity in mouse models of certain cancers following intra-peritoneal administration [88]. Although its lack of oral bioavailability precluded its development as a clinical agent, ABT-737 has proven an immensely valuable research tool providing researchers with significant insight into the molecular control of cell death in both cancerous as well as normal cells. Its use has revealed that some tumour cells are "addicted" to BCL-2, BCL-X_L and/or BCL-W pro-survival proteins whilst others rely on MCL-1 and/or BFL-1/A1 for their sustained survival and expansion. *In vitro* and *in vivo* studies with ABT-737 found it effective as a single agent in certain contexts, but in many cancers efficient cell killing was only seen when ABT-737 was combined with standard chemotherapeutic agents [88] or with inhibitors of oncogenic kinases such as those targeting BCR-ABL [89], MEK [90] or EGFR [91]. These studies indicate that blocking a single or even a group of pro-survival BCL-2 proteins is often not sufficient to induce cell death and to achieve a significant anti-tumour effect. This highlights that the real power of these agents in the clinic is likely to be in combination with other anti-cancer drugs, with targeting of a cancer-specific "Achilles heel" (e.g. using inhibitors of oncogenic kinases) likely the safest as in such drug combinations only the BH3-mimetic would be expected to cause collateral damage to healthy tissues [92].

5. Are we there yet? clinical trials with ABT-263 (navitoclax) and ABT-199 (venetoclax)

ABT-737 performed exceptionally in preclinical testing, but was unable to enter the clinic due to its poor solubility and bioavailability. Medicinal chemistry modifications of ABT-737 led to the development of ABT-263 that retained the specific binding profile and subnanomolar affinities for BCL-2, BCL-X_L and BCL-W whilst having improved bioavailability and drug likeness characteristics [93]. The significant anti-tumour activity of ABT-263, and ABT-737 before it, in animal models provided exciting support for the progression of these small molecules into the clinic.

Three Phase I clinical trials were conducted in parallel for ABT-263 (navitoclax) in patients with relapsed or refractory disease of various different tumour types. CLL was a natural place to start, as the overexpression of BCL-2 is highly characteristic of this disease [94]. A second trial included a broader spectrum of leukaemias/lymphomas, such as follicular lymphoma, diffuse large B cell lymphoma and mantle cell lymphoma, though still mostly consisted of CLL patients [95]. The third trial focused on SCLC [96], for which ABT-263/navitoclax had shown particular promise in pre-clinical studies [93,97]. All three trials demonstrated anti-tumour efficacy from BCL-2/BCL-X_L/BCL-W inhibition, and the drug was deemed safe and tolerable to progress to Phase II clinical trials. To date, however, only one Phase II trial has been completed for the treatment of relapsed SCLC [98]. Navitoclax performed surprisingly poorly in this trial given the effects seen in preclinical xenograft models, with only 2.6% of patients achieving a partial response. The conclusion from this study was that navitoclax should be considered for combination treatments to enhance the cytotoxicity of other anti-cancer chemotherapies. Indeed, despite the success of its younger sibling ABT-199/venetoclax (see below), ABT-263/navitoclax has not yet disappeared from the clinic. Currently, it is being evaluated in combination with gemcitabine [99], rituximab [100,101], erlotinib [102] and irinotecan [103] in haematopoietic and solid malignancies.

Aside from navitoclax's relatively poor performance as a single agent, there were two additional issues that arose from the initial clinical trials that warranted the development of a compound specific for BCL-2 alone. Firstly, high-grade thromobocytopenia induced by treatment with ABT-263/navitoclax was common and thereby limited the dose that could be administered. This was not unexpected, as it had been noted in prior animal studies [93] and could be explained by the dependency of platelets on BCL-X_L for their survival [104,105]. Moreover, the fact that navitoclax does not selectively inhibit BCL-2 also caused problems in terms of efficacy. Patient responses were much more varied than had been observed in preclinical experiments, and this was attributed to ABT-263/navitoclax binding to BCL-2, BCL-X_L or BCL-W rather than only the target that is critical for the survival of the cancer cells.

To resolve these complications, a compound was derived ABT-199/venetoclax that only bound BCL-2 [106]. As predicted, this compound had limited effect on platelet levels in mice, but retained its single-agent cytotoxic activity against tumours that were dependent on BCL-2 [106,107].

The results of the first clinical trial with ABT-199/venetoclax in

patients with relapsed CLL were recently reported [101]. The effect of specifically inhibiting BCL-2 was remarkable, with an overall response rate of 79% and complete remission occurring for 20% of the 116 patient cohort. Importantly, the reduction in disease burden was proven to be due to apoptosis of CLL cells [108]. Furthermore, the efficacy of this drug in CLL was not affected by p53 mutations or loss [108], which are frequent in relapsed or chemotherapy-resistant CLL [109] and many other cancers. The FDA of the United States and the Medicines and Healthcare Products Regulatory Agency UK have recently approved ABT-199/venetoclax for use in patients with relapsed CLL carrying 17p chromosomal loss (the region containing the *p53* gene). ABT-199/venetoclax has also recently been granted FDA breakthrough status for use in combination with hypomethylating agents in patients with acute myeloid leukaemia (AML). Given the emerging pre-clinical data reporting efficacy in diverse tumours including acute lymphoblastic leukaemia [110] and neuroblastoma [111] either as a monotherapy or in combination, we anticipate further approvals for ABT-199/venetoclax in the coming years, both in the United States and elsewhere, for a broad range of cancers.

6. Roadblocks encountered with ABT-199/venetoclax as an anti-cancer therapy

Although generally well tolerated, ABT-199/venetoclax was not without undesirable side effects in patients. The most severe toxic effect of ABT-199/venetoclax was tumour lysis syndrome, essentially occurring because the drug was too effective at killing the tumour cells, which led to the tragic death of one patient who had been treated with a high dose of 1200 mg and the halting of the trial. The study was resumed after due delay and tumour lysis syndrome in patients was subsequently managed by prophylactic measures, such as commencing treatment with lower doses of the drug. Most patients were able to resume taking ABT-199/venetoclax upon the resolution of these symptoms [101].

The second most common adverse effect was neutropenia [101]. This was manageable to a certain extent by co-treatment with granulocyte colony stimulating factor (G-CSF), but further studies are required to determine whether patients who have received previous chemotherapy are more susceptible to neutropenia, or whether this is a general effect of BCL-2 inhibition alone.

Another important focus of future work will be to understand the biology of the tumours in the ~20% of CLL patients who did not respond to ABT-199/venetoclax therapy, and also those of the ~40% of CLL patients whose disease progressed and even became more aggressive. It has been demonstrated in *in vitro* studies that overexpression of other pro-survival proteins (e.g. MCL-1 and BCL-X_L) confers resistance to ABT-737 [112,113] and therefore presumably also to ABT-199/venetoclax. Accordingly, upregulation of MCL-1 and BFL-1, which are not targets of ABT-737, was observed in resistant cell lines that were generated by long-term exposure to this BH3 mimetic [114]. This would also constitute a logical escape route for a tumour under pressure by ABT-199/venetoclax. Interestingly, overexpression of BCL-X_L or BCL-W was also able to render lymphoma cells resistant to ABT-737 despite them also being targets of this BH3-mimetic [113]. These findings suggest that resistance to a BCL-2 inhibitor could in theory be conferred by any of its pro-survival relatives. Overall, the mechanisms that are responsible for poor responsiveness to ABT-199/venetoclax in non-responding patients remain to be elucidated, and such insight might provide clues to what combination strategies could be employed to restore responsiveness.

Being able to predict which patients will benefit from ABT-199/venetoclax is another area of uncertainty. *In vitro* testing of ABT-199/venetoclax on CLL cells from patients was not reliably predictive of the patients' responses to this drug [108]. A method known as “BH3 profiling” has been proposed as an alternative *in vitro* assay to predict patient responses, as it has been shown to predict clinical response to chemotherapy for a range of cancer types [115]. BH3 profiling essentially measures the potential of a tumour cell's mitochondria to undergo permeabilisation in response to BH3 peptides derived from various BH3-only proteins. This potential to undergo apoptosis is termed “mitochondrial priming”, i.e. how susceptible the mitochondria are to induce the apoptotic programme based on which pro-survival and pro-apoptotic proteins are present [115]. A higher level of mitochondrial priming in CLL cells from patients enrolled in the ABT-199/venetoclax clinical trial was somewhat predictive of the patients' responses [108]. Hence, this test may be more informative than *in vitro* cell death assays. A potential limitation of this assay is that in studying the mitochondria in isolation, as BAX is largely cytosolic unless apoptotic signals direct it to the mitochondria, it may be biased towards BAK-dependent killing and the pro-survival proteins that protect from it (largely MCL-1 and BCL-X_L) [116]. A third approach to assess BCL-2 family mRNA levels has also been proposed [117]. In this study, no correlations could be found between the expression of mRNAs encoding individual pro-survival proteins, or pro-apoptotic BH3-only proteins, and the response to ABT-737 in tumour cell lines. However, the ratio of *Mcl-1* and *Bfl-1* transcripts relative to *Bcl-2* mRNA proved to be predictive of the response of tumour cells to ABT-737. A caveat to this is that, given the potential for post-translational regulation of BCL-2 family proteins, levels of mRNA transcript are not always predictive of the levels of protein. Whether the latter approaches will be applicable to predict a clinical response remains to be determined. However, as it is the reservoir of free or unoccupied pro-survival BCL-2-like protein that governs whether a cell succumbs to apoptotic stimuli, any reliable diagnostic assay will have to assess protein complexes rather than just protein expression levels. Overall, reliably predictive biomarkers from cancer patients who respond or do not respond to ABT-199/venetoclax are lacking and research into this area will be valuable as clinical trials continue and this drug is used routinely in the clinic.

7. Where next? alternative routes to induce apoptosis

Given the (albeit protracted) success in developing small molecule inhibitors of BCL-2, an obvious target is now MCL-1, particularly as a number of tumours are specifically dependent on MCL-1 for their development as well as their sustained survival and growth; this includes multiple myeloma, AML and MYC-driven lymphoma [118–121]. Structure-based design led to the development of a small molecule, A-1210477, that bound MCL-1 with nanomolar affinity and specifically disrupted interactions of MCL-1 with NOXA [122,123]. The drug was found to kill mouse embryonic fibroblasts in a BAK/BAX-dependent fashion, although killing of certain breast cancer lines was reliant on BAK alone [123,124]. A-1210477 induced apoptosis of multiple myeloma cells and non-small cell lung cancer (NSCLC) cells as a single agent, and synergised with ABT-263 in a panel of tumour cell lines, including non-Hodgkin's lymphoma, breast and gastric cancer lines [124–126]. Whether the drug is capable of inducing cancer cell death and limiting tumour growth *in vivo* has yet to be shown.

Deletion of MCL-1 in mice results in early embryonic lethality, the most profound phenotype caused by the loss of any of the pro-survival BCL-2 family members [127]. Also T and B cells, as well as plasma cells are reliant on MCL-1 for survival [128,129]. Thus on-target toxicity is an understandable concern that dampened enthusiasm for, and may have brought a premature end to the journey of blocking MCL-1 as a therapeutic strategy in cancer.

However, recent preclinical studies with a new MCL-1 inhibitor developed by the pharmaceutical company Servier suggests that small molecule inhibition of MCL-1 can indeed be tolerated at doses efficacious for limiting the growth of MCL-1-dependent tumours *in vivo* [130]. S63845 was developed from an NMR-based fragment library screen and was found to selectively and potently (K_d of 0.19 nM by surface plasmon resonance) bind MCL-1. This drug efficiently killed a panel of tumour lines as single agent *in vitro* and did so in a BAK/BAX-dependent (i.e. on target) fashion. Remarkably, S63845 exhibited potent anti-tumour effects as a single agent *in vivo* in multiple myeloma xenograft models, *Eμ-Myc* mouse lymphoma and human AML xenografts. The drug could also markedly diminish the growth of certain solid tumours (NSCLC, breast, melanoma) both *in vitro* and *in vivo*, particularly when combined with inhibitors of oncogenic kinases (e.g. the mutant B-RAF inhibitor vemurafinib/Zelboraf). Importantly, S63845 was efficacious at killing tumour cells at doses that caused only limited or no toxicity in healthy tissues. These exciting new findings suggest that the roadblock in targeting MCL-1 in cancer therapy may have been lifted and paves the way for clinical trials.

As has been discussed previously, tumour escape can also occur via increased expression of the pro-survival proteins that are not targeted by a particular BH3 mimetic [130]. Accordingly, insensitivity to the MCL-1 inhibitor, S63845, could in several tumour cell lines be attributed to significant expression of *BCL-X* mRNA. In addition, both BCL-X_L and BFL-1 have been implicated in the resistance of cancer cells to standard chemotherapeutic agents and not only BCL-2-targeting BH3 mimetics. Both *MCL-1* and *BCL-X* gene amplifications due to somatically acquired gene copy-number alterations were observed in a broad range of tumours [131]. Furthermore, a strong correlation was found with *BCL-X* mRNA levels and resistance to cytotoxic agents [132], thus indicating the need to develop BCL-X_L-specific inhibitors for combination therapies. Indeed, future work with ABT-263/navitoclax and BCL-X_L-selective BH3 mimetics such as WEHI-539 and A-1331852 [133–135] may be beneficial in many cancers that are refractory to standard chemotherapeutic regimens. BFL-1 overexpression has been associated with chemotherapy resistance [136,137], and also metastatic disease [138,139]. BFL-1 is predominantly expressed in haematopoietic cells [140] and so BFL-1 has been particularly associated with certain leukaemias and lymphomas, but perhaps surprisingly also melanoma [141]. Structure-based design studies for the development of BFL-1 inhibitors have not yet been published, but screening for small molecules that can inhibit BFL-1 activity have provided proof-of-concept that BFL-1 inhibition can kill certain B cell lymphoma lines [142].

Finally, some cancers are driven by oncogenic viruses that encode pro-survival BCL-2 homologues, such as BHRF1 in EBV, which causes lymphoma in immune-suppressed organ transplant recipients, certain types of T cell lymphoma and nasopharyngeal carcinoma. If it can be demonstrated that BHRF1 is essential for the survival of these cancer cells, its targeting by BH3 mimetic compounds should be endeavoured.

To induce tumour cell apoptosis, an alternative strategy might be to directly activate the process, to press the accelerator rather than to take the foot off the brakes. This can be potentially achieved by mimicking the activating interaction of certain BH3-only proteins on BAK and/or BAX. The Korsmeyer and Walensky labs have reported activation of BAX using BH3 peptides stabilised with a hydrocarbon staple to improve stability and affinity [143]. Interestingly, these stapled peptides are thought to bind and activate BAX, but not BAK, at a site independent of the canonical hydrophobic groove activation site [144], and were shown to induce cell killing *in vitro* and also anti-tumour effects *in vivo* [145]. Although the *in vivo* utility of such hydrocarbon stapled BH3 peptides has been questioned [146], the first small molecule agonist of BAX, BAM-7, that could induce BAX-dependent apoptosis *in vitro* was identified by an *in silico* screening targeting this alternative activation site [147]. Computational docking approaches have also identified small molecule agonists with of BAX that promote BAX association with the MOM to induce cell death in culture and they had some ability to limit tumour growth *in vivo* [148,149].

Although to date no small molecule activators of BAK have been reported, we recently reported the antibody-mediated activation of BAK that allows us to selectively activate BAK [150] and so may provide an important opportunity to fine-tune the induction of apoptosis in a clinical setting. However, the utility of such antibody-based reagents as anti-cancer therapeutics is going to be determined by their efficient delivery into cells and their intracellular stability. Emerging technologies in antibody delivery and intrabody generation may facilitate this [151].

Given that BAK and BAX are both expressed in most tissues, one might predict that systemic administration of a BAK/BAX activating drug would be a recipe for disaster in terms of toxicity to healthy tissues. However, the observation that BAX or BAK specific peptide mimetics can seemingly be tolerated (at least in mice) at doses that limit tumour growth suggests this may not necessarily be the case [145]. A cancer cell is often characterised by upregulation of pro-survival BCL-2 proteins yet despite this they are often more sensitive to pro-survival BCL-2-like protein inhibition compared with normal cells. This is likely due to the concurrent upregulated expression of BH3-only proteins such as BIM, which occupy the expanded pro-survival proteins. Consequently, although pro-survival proteins are abundant, unoccupied pro-survival proteins are often limiting in cancer cells, thus making them less tolerant of additional disturbances in BCL-2 proteins, for example with the addition of a BH3-mimetic drug. In a normal cell, the pool of pro-survival proteins unoccupied by BH3-only proteins can sequester activated BAK/BAX. In contrast, in a cancer cell the pool of unoccupied pro-survival proteins is limited thus they are less able to constrain activated BAX/BAK from self-associating, causing MOM permeabilisation and killing the cell. This “priming” of cancer cells may thus provide a rationale for a therapeutic window whereby directly activating BAK/BAX selectively will kill tumour cells without massive collateral damage to healthy tissues.

Recently, an alternative site to the hydrophobic binding groove on pro-survival BCL-2 proteins has been proposed as a potential target site. Targeting the BCL-2 BH4 domain with a small molecule, BDA-366, was found to induce a conformation change to convert BCL-2 from a pro-survival protein into a pro-apoptotic protein [152]. This small molecule was found to kill lung cancer lines *in vitro* and limited the growth of lung cancer xenografts in the absence of any toxicity. *In vivo* activity was even more pronounced when combined with an mTOR inhibitor. This approach may represent a novel way to exploit the upregulation of BCL-2 in tumours [152].

Whether these new directions (drugs or drug targets) will progress to the clinic remains to be seen. However, given the recent, albeit long awaited, success of targeting BCL-2 with ABT-199/venetoclax, stemming from the basic understanding of its role in controlling apoptosis in cancer cells, suggests that we are set to embark on an exciting new journey with direct and selective targeting of the apoptotic machinery in cancer as the final destination.

Competing interest statement

The authors would like to declare that their employer, the Walter and Eliza Hall Institute was a partner of Genentech and Abbvie Pharmaceuticals in the development of ABT-199, and a partner of Servier in the development of S63845. The Walter and Eliza Hall Institute received milestone payments from Genentech

and AbbVie for the development of BH3 mimetic drugs and expects royalties from sales of ABT-199.

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References

[1] J.F.R. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[2] R.J. Youle, A. Strasser, The BCL-2 protein family: opposing activities that mediate cell death, Nat. Rev. Mol. Cell. Biol. 9 (2008) 47–59.
[3] P.E. Czabotar, G. Lessene, A. Strasser, J.M. Adams, Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy, Nat. Rev. Mol. Cell. Biol. 15 (2014) 49–63.
[4] B. Leber, J. Lin, D.W. Andrews, Still embedded together binding to membranes regulates Bcl-2 protein interactions, Oncogene 29 (2010) 5221–5230.
[5] F. Llambi, D.R. Green, Apoptosis and oncogenesis: give and take in the BCL-2 family, Curr. Opin. Genet. Dev. 21 (2011) 12–20.
[6] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[7] P.E. Czabotar, G. Lessene, Bcl-2 family proteins as therapeutic targets, Curr. Pharm. Des. 16 (2010) 3132–3148.
[8] K.A. Sarosiek, A. Letai, Directly targeting the mitochondrial pathway of apoptosis for cancer therapy using BH3 mimetics - recent successes, current challenges and future promise, FEBS J. 283 (2016) 3523–3533.
[9] Y. Tsujimoto, L.R. Finger, J. Yunis, P.C. Nowell, C.M. Croce, Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation, Science 226 (1984) 1097–1099.
[10] S. Fukuhara, J.D. Rowley, D. Variakojis, H.M. Golomb, Chromosome abnormalities in poorly differentiated lymphocytic lymphoma, Cancer Res. 39 (1979) 3119–3128.
[11] G. Manolov, Y. Manolova, Marker band in one chromosome 14 from Burkitt lymphomas, Nature 237 (1972) 33–34.
[12] J.J. Yunis, M.M. Oken, M.E. Kaplan, K.M. Ensrud, R.R. Howe, A. Theologides, Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma, N. Engl. J. Med. 307 (1982) 1231–1236.
[13] P.H. Levine, R.R. Connelly, F.W. McKay, Burkitt's lymphoma in the USA: cases reported to the American Burkitt Lymphoma Registry compared with population-based incidence and mortality data, IARC Sci. Publ. (1985) 217–224.
[14] A. Bakhshi, J.P. Jensen, P. Goldman, J.J. Wright, O.W. McBride, A.L. Epstein, S.J. Korsmeyer, Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around J_H on chromosome 14 and near a transcriptional unit on 18, Cell 41 (1985) 899–906.
[15] M.L. Cleary, J. Sklar, Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 7439–7443.
[16] W.B. Graninger, M. Seto, B. Boutain, P. Goldman, S.J. Korsmeyer, Expression of *Bcl*-2 and *Bcl*-2-Ig fusion transcripts in normal and neoplastic cells, J. Clin. Invest. 80 (1987) 1512–1515.
[17] M. Seto, U. Jaeger, R.D. Hockett, W. Graninger, S. Bennett, P. Goldman, S.J. Korsmeyer, Alternative promoters and exons, somatic mutation and deregulation of the *Bcl*-2-Ig fusion gene in lymphoma, EMBO J. 7 (1988) 123–131.
[18] M.L. Cleary, S.D. Smith, J. Sklar, Cloning and structural analysis of cDNAs for *bcl*-2 and a hybrid *bcl*-2/immunoglobulin transcript resulting from the t(14;18) translocation, Cell 47 (1986) 19–28.
[19] Y. Tsujimoto, C.M. Croce, Analysis of the structure, transcripts, and protein products of *bcl*-2, the gene involved in human follicular lymphoma, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 5214–5218.
[20] Y. Tsujimoto, N. Ikegaki, C.M. Croce, Characterization of the protein product of *bcl*-2, the gene involved in human follicular lymphoma, Oncogene 2 (1987) 3–7.
[21] Y. Tsujimoto, J. Gorham, J. Cossman, E. Jaffe, C.M. Croce, The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining, Science 229 (1985) 1390–1393.
[22] A. Bakhshi, J.J. Wright, W. Graninger, M. Seto, J. Owens, J. Cossman, J.P. Jensen, P. Goldman, S.J. Korsmeyer, Mechanism of the t(14;18) chromosomal translocation: structural analysis of both derivative 14 and 18 reciprocal partners, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 2396–2400.
[23] J.C. Reed, Y. Tsujimoto, J.D. Alpers, C.M. Croce, P.C. Nowell, Regulation of *bcl*-2 proto-oncogene expression during normal human lymphocyte proliferation, Science 236 (1987) 1295–1299.
[24] M. Negrini, E. Silini, C. Kozak, Y. Tsujimoto, C.M. Croce, Molecular analysis of *mbcl*-2: structure and expression of the murine gene homologous to the human gene involved in follicular lymphoma, Cell 49 (1987) 455–463.
[25] N. Gurfinkel, T. Unger, D. Givol, J.F. Mushinski, Expression of the *bcl*-2 gene in mouse B lymphocytic cell lines is differentiation stage specific, Eur. J. Immunol. 17 (1987) 567–570.
[26] J.C. Reed, M. Cuddy, T. Slabiak, C.M. Croce, P.C. Nowell, Oncogenic potential of *bcl*-2 demonstrated by gene transfer, Nature 336 (1988) 259–261.
[27] D.L. Vaux, S. Cory, J.M. Adams, *Bcl*-2 gene promotes haemopoietic cell survival and cooperates with c-*myc* to immortalize pre-B cells, Nature 335 (1988) 440–442.
[28] Y. Tsujimoto, Stress-resistance conferred by high level of *bcl*-2 alpha protein in human B lymphoblastoid cell, Oncogene 4 (1989) 1331–1336.
[29] A. Strasser, A.W. Harris, S. Cory, *Bcl*-2 transgene inhibits T cell death and perturbs thymic self-censorship, Cell 67 (1991) 889–899.
[30] G. Nuñez, L. London, D. Hockenbery, M. Alexander, J.P. McKearn, S.J. Korsmeyer, Deregulated *Bcl*-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines, J. Immunol. 144 (1990) 3602–3610.
[31] A. Strasser, A.W. Harris, D.L. Vaux, E. Webb, M.L. Bath, J.M. Adams, S. Cory, Abnormalities of the immune system induced by dysregulated *bcl*-2 expression in transgenic mice, Curr. Top. Microbiol. Immunol. 166 (1990) 175–181.
[32] A. Strasser, S. Whittingham, D.L. Vaux, M.L. Bath, J.M. Adams, S. Cory, A.W. Harris, Enforced *BCL2* expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 8661–8665.
[33] T.J. McDonnell, N. Deane, F.M. Platt, G. Nuñez, U. Jaeger, J.P. McKearn, S.J. Korsmeyer, *bcl*-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation, Cell 57 (1989) 79–88.
[34] C.L. Sentman, J.R. Shutter, D. Hockenbery, O. Kanagawa, S.J. Korsmeyer, *bcl*-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes, Cell 67 (1991) 879–888.
[35] G. Nuñez, M. Seto, S. Seremitis, D. Ferrero, F. Grignani, S.J. Korsmeyer, R. Dalla-Favera, Growth- and tumor-promoting effects of deregulated *BCL2* in human B-lymphoblastoid cells, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 4589–4593.
[36] T.J. McDonnell, G. Nuñez, F.M. Platt, D. Hockenbery, L. London, J.P. McKearn, S.J. Korsmeyer, Deregulated *Bcl*-2-immunoglobulin transgene expands a resting but responsive immunoglobulin M and D-expressing B-cell population, Mol. Cell. Biol. 10 (1990) 1901–1907.
[37] T.J. McDonnell, S.J. Korsmeyer, Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18), Nature 349 (1991) 254–256.
[38] A. Strasser, A.W. Harris, S. Cory, The *bcl*-2 oncogene regulates lymphocyte survival and potentiates lymphomagenesis, in: M.L.A.D. Watters (Ed.), Cell Death: the Cellular and Molecular Basis of Apoptosis, Harwood Academic Publishers, 1993, pp. 167–177.
[39] S. Ogilvy, D. Metcalf, C.G. Print, M.L. Bath, A.W. Harris, J.M. Adams, Constitutive bcl-2 expression throughout the hematopoietic compartment affects multiple lineages and enhances progenitor cell survival, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 14943–14948.
[40] A. Egle, A.W. Harris, M.L. Bath, L. O'Reilly, S. Cory, VavP-*Bcl2* transgenic mice develop follicular lymphoma preceded by germinal center hyperplasia, Blood 103 (2004) 2276–2283.
[41] A. Strasser, A.W. Harris, M.L. Bath, S. Cory, Novel primitive lymphoid tumours induced in transgenic mice by cooperation between *myc* and *bcl*-2, Nature 348 (1990) 331–333.
[42] A.W. Harris, C.A. Pinkert, M. Crawford, W.Y. Langdon, R.L. Brinster, J.M. Adams, The Eμ-*myc* transgenic mouse: a model for high-incidence spontaneous lymphoma and leukemia of early B cells, J. Exp. Med. 167 (1988) 353–371.
[43] J.M. Adams, A.W. Harris, C.A. Pinkert, L.M. Corcoran, W.S. Alexander, S. Cory, R.D. Palmiter, R.L. Brinster, The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice, Nature 318 (1985) 533–538.
[44] Y. Soini, P. Pääkkö, V.-P. Lehto, Histopathological evaluation of apoptosis in cancer, Am. J. Pathol. 153 (1998) 1041–1053.
[45] Y. Tsujimoto, J. Cossman, E. Jaffe, C.M. Croce, Involvement of the *bcl*-2 gene in human follicular lymphoma, Science 228 (1985) 1440–1443.
[46] J.M. Hardwick, L. Soane, Multiple functions of BCL-2 family proteins, Cold Spring Harb. Perspect. Biol. 5 (2013).
[47] D.R. Green, G. Kroemer, The pathophysiology of mitochondrial cell death, Science 305 (2004) 626–629.
[48] A. Strasser, L. O'Connor, V.M. Dixit, Apoptosis signaling, Ann. Rev. Biochem. 69 (2000) 217–245.
[49] A.M. Verhagen, P.G. Ekert, M. Pakusch, J. Silke, L.M. Connolly, G.E. Reid, R.L. Moritz, R.J. Simpson, D.L. Vaux, Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing inhibitor of apoptosis (IAP) proteins, Cell 102 (2000) 43–53.

[50] C. Du, M. Fang, Y. Li, L. Li, X. Wang, Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition, Cell 102 (2000) 33–42.
[51] R.M. Kluck, E. Bossy-Wetzel, D.R. Green, D.D. Newmeyer, The release of cytochrome *c* from mitochondria - a primary site for Bcl-2 regulation of apoptosis, Science 275 (1997) 1132–1136.
[52] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (1997) 479–489.
[53] X. Liu, C.N. Kim, J. Yang, R. Jemmerson, X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c, Cell 86 (1996) 147–157.
[54] M.C. Wei, W.X. Zong, E.H. Cheng, T. Lindsten, V. Panoutsakopoulou, A.J. Ross, K.A. Roth, G.R. MacGregor, C.B. Thompson, S.J. Korsmeyer, Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death, Science 292 (2001) 727–730.
[55] M.C. Wei, T. Lindsten, V.K. Mootha, S. Weiler, A. Gross, M. Ashiya, C.B. Thompson, S.J. Korsmeyer, tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c, Genes Dev. 14 (2000) 2060–2071.
[56] P.E. Czabotar, D. Westphal, G. Dewson, S. Ma, C. Hockings, W.D. Fairlie, E.F. Lee, S. Yao, A.Y. Robin, B.J. Smith, D.C. Huang, R.M. Kluck, J.M. Adams, P.M. Colman, Bax crystal structures reveal how BH3 domains activate bax and nucleate its oligomerization to induce apoptosis, Cell 152 (2013) 519–531.
[57] T. Moldoveanu, C.R. Grace, F. Llambi, A. Nourse, P. Fitzgerald, K. Gehring, R.W. Kriwacki, D.R. Green, BID-induced structural changes in BAK promote apoptosis, Nat. Struct. Mol. Biol. 20 (2013) 589–597.
[58] H. Kim, H.C. Tu, D. Ren, O. Takeuchi, J.R. Jeffers, G.P. Zambetti, J.J. Hsieh, E.H. Cheng, Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis, Mol. Cell 36 (2009) 487–499.
[59] D. Ren, H.C. Tu, H. Kim, G.X. Wang, G.R. Bean, O. Takeuchi, J.R. Jeffers, G.P. Zambetti, J.J. Hsieh, E.H. Cheng, BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program, Science 330 (2010) 1390–1393.
[60] T. Lindsten, A.J. Ross, A. King, W. Zong, J.C. Rathmell, H.A. Shiels, E. Ulrich, K.G. Waymire, P. Mahar, K. Frauwirth, Y. Chen, M. Wei, V.M. Eng, D.M. Adelman, M.C. Simon, A. Ma, J.A. Golden, G. Evan, S.J. Korsmeyer, G.R. MacGregor, C.B. Thompson, The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues, Mol. Cell 6 (2000) 1389–1399.
[61] K.L. O'Neill, K. Huang, J. Zhang, Y. Chen, X. Luo, Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane, Genes Dev. 30 (2016) 973–988.
[62] B. Leber, J. Lin, D.W. Andrews, Embedded together: the life and death consequences of interaction of the Bcl-2 family with membranes, Apoptosis 12 (2007) 897–911.
[63] G. Dewson, R.M. Kluck, Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis, J. Cell. Sci. 122 (2009) 2801–2808.
[64] F. Llambi, T. Moldoveanu, S.W.G. Tait, L. Bouchier-Hayes, J. Temirov, L.L. McCormick, C.P. Dillon, D.R. Green, A unified model of mammalian BCL-2 protein family interactions at the mitochondria, Mol. Cell 44 (2011) 517–531.
[65] D. Westphal, R.M. Kluck, G. Dewson, Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis, Cell. Death Differ. 21 (2014) 196–205.
[66] F. Ke, P. Bouillet, T. Kaufmann, A. Strasser, J. Kerr, A.K. Voss, Consequences of the combined loss of BOK and BAK or BOK and BAX, Cell. Death Dis. 4 (2013) e650.
[67] F. Ke, A. Voss, J.B. Kerr, L.A. O'Reilly, L. Tai, N. Echeverry, P. Bouillet, A. Strasser, T. Kaufmann, BCL-2 family member BOK is widely expressed but its loss has only minimal impact in mice, Cell. Death Differ. 19 (2012) 915–925.
[68] F. Llambi, Y.M. Wang, B. Victor, M. Yang, D.M. Schneider, S. Gingras, M.J. Parsons, J.H. Zheng, S.A. Brown, S. Pelletier, T. Moldoveanu, T. Chen, D.R. Green, BOK is a non-canonical BCL-2 family effector of apoptosis regulated by ER-associated degradation, Cell 165 (2016) 421–433.
[69] A.M. Petros, E.T. Olejniczak, S.W. Fesik, Structural biology of the Bcl-2 family of proteins, Biochim. Biophys. Acta 1644 (2004) 83–94.
[70] M. Sattler, H. Liang, D. Nettesheim, R.P. Meadows, J.E. Harlan, M. Eberstadt, H.S. Yoon, S.B. Shuker, B.S. Chang, A.J. Minn, C.B. Thompson, S.W. Fesik, Structure of Bcl-x_L-Bak peptide complex: recognition between regulators of apoptosis, Science 275 (1997) 983–986.
[71] A.M. Petros, D.G. Nettseheim, Y. Wang, E.T. Olejniczak, R.P. Meadows, J. Mack, K. Swift, E.D. Matayoshi, H. Zhang, C.B. Thompson, S.W. Fesik, Rationale for Bcl-x_L/Bad peptide complex formation from structure, mutagenesis, and biophysical studies, Protein Sci. 9 (2000) 2528–2534.
[72] L. Chen, S.N. Willis, A. Wei, B.J. Smith, J.I. Fletcher, M.G. Hinds, P.M. Colman, C.L. Day, J.M. Adams, D.C.S. Huang, Differential targeting of pro-survival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function, Mol. Cell 17 (2005) 393–403.
[73] A. Villunger, E.M. Michalak, L. Coultas, F. Mullauer, G. Bock, M.J. Ausserlechner, J.M. Adams, A. Strasser, p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa, Science 302 (2003) 1036–1038.
[74] P. Bouillet, D. Metcalf, D.C.S. Huang, D.M. Tarlinton, T.W.H. Kay, F. Köntgen, J.M. Adams, A. Strasser, Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity, Science 286 (1999) 1735–1738.
[75] C.L. Oliver, M.B. Miranda, S. Shangary, S. Land, S. Wang, D.E. Johnson, (-)-Gossypol acts directly on the mitochondria to overcome Bcl-2- and Bcl-X(L)-mediated apoptosis resistance, Mol. Cancer Ther. 4 (2005) 23–31.
[76] R.M. Mohammad, S. Wang, A. Aboukameel, B. Chen, X. Wu, J. Chen, A. Al-Katib, Preclinical studies of a nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-X(L) [(-)-gossypol] against diffuse large cell lymphoma, Mol. Cancer Ther. 4 (2005) 13–21.
[77] M.F. van Delft, A.H. Wei, K.D. Mason, C.J. Vandenberg, L. Chen, P.E. Czabotar, S.N. Willis, C.L. Scott, C.L. Day, S. Cory, J.M. Adams, A.W. Roberts, D.C. Huang, 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 (2006) 389–399.
[78] W.X. Zong, T. Lindsten, A.J. Ross, G.R. MacGregor, C.B. Thompson, BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak, Genes Dev. 15 (2001) 1481–1486.
[79] E.H. Cheng, M.C. Wei, S. Weiler, R.A. Flavell, T.W. Mak, T. Lindsten, S.J. Korsmeyer, BCL-2, BCL-x_L sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis, Mol. Cell 8 (2001) 705–711.
[80] M.Q. Baggstrom, Y. Qi, M. Koczywas, A. Argiris, E.A. Johnson, M.J. Millward, S.C. Murphy, C. Erlichman, C.M. Rudin, R. Govindan, C. Mayo Phase, C. California, A phase II study of AT-101 (Gossypol) in chemotherapy-sensitive recurrent extensive-stage small cell lung cancer, J. Thorac. Oncol. 6 (2011) 1757–1760.
[81] M. Konopleva, J. Watt, R. Contractor, T. Tsao, D. Harris, Z. Estrov, W. Bornmann, H. Kantarjian, J. Viallet, I. Samudio, M. Andreeff, Mechanisms of antileukemic activity of the novel Bcl-2 homology domain-3 mimetic GX15-070 (obatoclax), Cancer Res. 68 (2008) 3413–3420.
[82] P. Perez-Galan, G. Roue, N. Villamor, E. Campo, D. Colomer, The BH3-mimetic GX15-070 synergizes with bortezomib in mantle cell lymphoma by enhancing Noxa-mediated activation of Bak, Blood 109 (2007) 4441–4449.
[83] A.D. Schimmer, S. O'Brien, H. Kantarjian, J. Brandwein, B.D. Cheson, M.D. Minden, K. Yee, F. Ravandi, F. Giles, A. Schuh, V. Gupta, M. Andreeff, C. Koller, H. Chang, S. Kamel-Reid, M. Berger, J. Viallet, G. Borthakur, A phase I study of the pan bcl-2 family inhibitor obatoclax mesylate in patients with advanced hematologic malignancies, Clin. Cancer Res. 14 (2008) 8295–8301.
[84] S.M. O'Brien, D.F. Claxton, M. Crump, S. Faderl, T. Kipps, M.J. Keating, J. Viallet, B.D. Cheson, Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia, Blood 113 (2009) 299–305.
[85] P.K. Paik, C.M. Rudin, A. Brown, N.A. Rizvi, N. Takebe, W. Travis, L. James, M.S. Ginsberg, R. Juergens, S. Markus, L. Tyson, S. Subzwari, M.G. Kris, L.M. Krug, A phase I study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in solid tumor malignancies, Cancer Chemother. Pharmacol. 66 (2010) 1079–1085.
[86] P.K. Paik, C.M. Rudin, M.C. Pietanza, A. Brown, N.A. Rizvi, N. Takebe, W. Travis, L. James, M.S. Ginsberg, R. Juergens, S. Markus, L. Tyson, S. Subzwari, M.G. Kris, L.M. Krug, A phase II study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in relapsed small cell lung cancer, Lung Cancer 74 (2011) 481–485.
[87] Y. Oki, A. Copeland, F. Hagemeister, L.E. Fayad, M. Fanale, J. Romaguera, A. Younes, Experience with obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist in patients with relapsed or refractory classical Hodgkin lymphoma, Blood 119 (2012) 2171–2172.
[88] T. Oltersdorf, S.W. Elmore, A.R. Shoemaker, R.C. Armstrong, D.J. Augeri, B.A. Belli, M. Bruncko, T.L. Deckwerth, J. Dinges, P.J. Hajduk, M.K. Joseph, S. Kitada, S.J. Korsmeyer, A.R. Kunzer, A. Letai, C. Li, M.J. Mitten, D.G. Nettesheim, S. Ng, P.M. Nimmer, J.M. O'Connor, A. Oleksijew, A.M. Petros, J.C. Reed, W. Shen, S.K. Tahir, C.B. Thompson, K.J. Tomaselli, B. Wang, M.D. Wendt, H. Zhang, S.W. Fesik, S.H. Rosenberg, An inhibitor of Bcl-2 family proteins induces regression of solid tumours, Nature 435 (2005) 677–681.
[89] J. Kuroda, S. Kimura, M. Andreeff, E. Ashihara, Y. Kamitsuji, A. Yokota, E. Kawata, M. Takeuchi, R. Tanaka, Y. Murotani, Y. Matsumoto, H. Tanaka, A. Strasser, T. Maekawa, ABT-737 is a useful component of combinatory chemotherapies for chronic myelogenous leukaemias with diverse drug resistance mechanisms, Br. J. Haematol. 140 (2008) 181–190.
[90] M.S. Cragg, E.S. Jansen, M. Cook, C. Harris, A. Strasser, C.L. Scott, 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 (2008) 3651–3659.
[91] M.S. Cragg, J. Kuroda, H. Puthalakath, D.C.S. Huang, A. Strasser, Gefitinib-induced killing of NSCLC cell lines expressing mutant *EGFR* requires bim and can Be enhanced by BH3 mimetics, PLoS Med. 4 (2007) 1681–1689.
[92] M.S. Cragg, C. Harris, A. Strasser, C.L. Scott, Unleashing the power of inhibitors of oncogenic kinases through BH3 mimetics, Nat. Rev. Cancer 9 (2009) 321–326.
[93] C. Tse, A.R. Shoemaker, J. Adickes, M.G. Anderson, J. Chen, S. Jin, E.F. Johnson, K.C. Marsh, M.J. Mitten, P. Nimmer, L. Roberts, S.K. Tahir, Y. Xiao, X. Yang, H. Zhang, S. Fesik, S.H. Rosenberg, S.W. Elmore, ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor, Cancer Res. 68 (2008) 3421–3428.
[94] A.W. Roberts, J.F. Seymour, J.R. Brown, W.G. Wierda, T.J. Kipps, S.L. Khaw, D.A. Carney, S.Z. He, D.C. Huang, H. Xiong, Y. Cui, T.A. Busman,

E.M. McKeegan, A.P. Krivoshik, S.H. Enschede, R. Humerickhouse, 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. Of. J. Am. Soc. Clin. Oncol. 30 (2012) 488–496.
[95] W.H. Wilson, O.A. O'Connor, M.S. Czuczman, A.S. LaCasce, J.F. Gerecitano, J.P. Leonard, A. Tulpule, K. Dunleavy, H. Xiong, Y.L. Chiu, Y. Cui, T. Busman, S.W. Elmore, S.H. Rosenberg, A.P. Krivoshik, S.H. Enschede, R.A. Humerickhouse, Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity, Lancet Oncol. 11 (2010) 1149–1159.
[96] L. Gandhi, D.R. Camidge, M. Ribeiro de Oliveira, P. Bonomi, D. Gandara, D. Khaira, C.L. Hann, E.M. McKeegan, E. Litvinovich, P.M. Hemken, C. Dive, S.H. Enschede, C. Nolan, Y.L. Chiu, T. Busman, H. Xiong, A.P. Krivoshik, R. Humerickhouse, G.I. Shapiro, C.M. Rudin, Phase I study of Navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors, J. Clin. Oncol. Of. J. Am. Soc. Clin. Oncol. 29 (2011) 909–916.
[97] A.R. Shoemaker, M.J. Mitten, J. Adickes, S. Ackler, M. Refici, D. Ferguson, A. Oleksijew, J.M. O'Connor, B. Wang, D.J. Frost, J. Bauch, K. Marsh, S.K. Tahir, X. Yang, C. Tse, S.W. Fesik, S.H. Rosenberg, S.W. Elmore, Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models, Clin. Cancer Res. 14 (2008) 3268–3277.
[98] C.M. Rudin, C.L. Hann, E.B. Garon, M. Ribeiro de Oliveira, P.D. Bonomi, D.R. Camidge, Q. Chu, G. Giaccone, D. Khaira, S.S. Ramalingam, M.R. Ranson, C. Dive, E.M. McKeegan, B.J. Chyla, B.L. Dowell, A. Chakravartty, C.E. Nolan, N. Rudersdorf, T.A. Busman, M.H. Mabry, A.P. Krivoshik, R.A. Humerickhouse, G.I. Shapiro, L. Gandhi, Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer, Clin. Cancer Res. 18 (2012) 3163–3169.
[99] J.M. Cleary, C.M. Lima, H.I. Hurwitz, A.J. Montero, C. Franklin, J. Yang, A. Graham, T. Busman, M. Mabry, K. Holen, G.I. Shapiro, H. Uronis, A phase I clinical trial of navitoclax, a targeted high-affinity Bcl-2 family inhibitor, in combination with gemcitabine in patients with solid tumors, Invest New Drugs 32 (2014) 937–945.
[100] D.C. Phillips, Y. Xiao, L.T. Lam, E. Litvinovich, L. Roberts-Rapp, A.J. Souers, J.D. Leverson, Loss in MCL-1 function sensitizes non-Hodgkin's lymphoma cell lines to the BCL-2-selective inhibitor venetoclax (ABT-199), Blood Cancer J. 5 (2015) e368.
[101] A.W. Roberts, M.S. Davids, J.M. Pagel, B.S. Kahl, S.D. Puvvada, J.F. Gerecitano, T.J. Kipps, M.A. Anderson, J.R. Brown, L. Gressick, S. Wong, M. Dunbar, M. Zhu, M.B. Desai, E. Cerri, S. Heitner Enschede, R.A. Humerickhouse, W.G. Wierda, J.F. Seymour, Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia, N. Engl. J. Med. 374 (2016) 311–322.
[102] A.W. Tolcher, P. LoRusso, J. Arzt, T.A. Busman, G. Lian, N.S. Rudersdorf, C.A. Vanderwal, W. Kirschbrown, K.D. Holen, L.S. Rosen, Safety, efficacy, and pharmacokinetics of navitoclax (ABT-263) in combination with erlotinib in patients with advanced solid tumors, Cancer Chemother. Pharmacol. 76 (2015) 1025–1032.
[103] A.W. Tolcher, P. LoRusso, J. Arzt, T.A. Busman, G. Lian, N.S. Rudersdorf, C.A. Vanderwal, J.F. Waring, J. Yang, K.D. Holen, L.S. Rosen, Safety, efficacy, and pharmacokinetics of navitoclax (ABT-263) in combination with irinotecan: results of an open-label, phase 1 study, Cancer Chemother. Pharmacol. 76 (2015) 1041–1049.
[104] K.D. Mason, M.R. Carpinelli, J.I. Fletcher, J.E. Collinge, A.A. Hilton, S. Ellis, P.N. Kelly, P.G. Ekert, D. Metcalf, A.W. Roberts, D.C. Huang, B.T. Kile, Programmed a nuclear cell death delimits platelet life span, Cell 128 (2007) 1173–1186.
[105] K.U. Wagner, E. Claudio, E.B. Rucker 3rd, G. Riedlinger, C. Broussard, P.L. Schwartzberg, U. Siebenlist, L. Hennighausen, Conditional deletion of the Bcl-x gene from erythroid cells results in hemolytic anemia and profound splenomegaly, Development 127 (2000) 4949–4958.
[106] A.J. Souers, J.D. Leverson, E.R. Boghaert, S.L. Ackler, N.D. Catron, J. Chen, B.D. Dayton, H. Ding, S.H. Enschede, W.J. Fairbrother, D.C. Huang, S.G. Hymowitz, S. Jin, S.L. Khaw, P.J. Kovar, L.T. Lam, J. Lee, H.L. Maecker, K.C. Marsh, K.D. Mason, M.J. Mitten, P.M. Nimmer, A. Oleksijew, C.H. Park, C.M. Park, D.C. Phillips, A.W. Roberts, D. Sampath, J.F. Seymour, M.L. Smith, G.M. Sullivan, S.K. Tahir, C. Tse, M.D. Wendt, Y. Xiao, J.C. Xue, H. Zhang, R.A. Humerickhouse, S.H. Rosenberg, S.W. Elmore, ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets, Nat. Med. 19 (2013) 202–208.
[107] M. Vogler, D. Dinsdale, M.J. Dyer, G.M. Cohen, ABT-199 selectively inhibits BCL2 but not BCL2L1 and efficiently induces apoptosis of chronic lymphocytic leukaemic cells but not platelets, Br. J. Haematol. 163 (2013) 139–142.
[108] M.A. Anderson, J. Deng, J.F. Seymour, C. Tam, S.Y. Kim, J. Fein, L. Yu, J.R. Brown, D. Westerman, E.G. Si, I.J. Majewski, D. Segal, S.L. Heitner Enschede, D.C. Huang, M.S. Davids, A. Letai, A.W. Roberts, The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism, Blood 127 (2016) 3215–3224.
[109] D.A. Landau, S.L. Carter, P. Stojanov, A. McKenna, K. Stevenson, M.S. Lawrence, C. Sougnez, C. Stewart, A. Sivachenko, L. Wang, Y. Wan, W. Zhang, S.A. Shukla, A. Vartanov, S.M. Fernandes, G. Saksena, K. Cibulskis, B. Tesar, S. Gabriel, N. Hacohen, M. Meyerson, E.S. Lander, D. Neuberg, J.R. Brown, G. Getz, C.J. Wu, Evolution and impact of subclonal mutations in chronic lymphocytic leukemia, Cell 152 (2013) 714–726.
[110] L.M. High, B. Szymanska, U. Wilczynska-Kalak, N. Barber, R. O'Brien, S.L. Khaw, I.B. Vikstrom, A.W. Roberts, R.B. Lock, The Bcl-2 homology domain 3 mimetic ABT-737 targets the apoptotic machinery in acute lymphoblastic leukemia resulting in synergistic in vitro and in vivo interactions with established drugs, Mol. Pharmacol. 77 (2010) 483–494.
[111] L.T. Bate-Eya, I.J. den Hartog, I. van der Ploeg, L. Schild, J. Koster, E.E. Santo, E.M. Westerhout, R. Versteeg, H.N. Caron, J.J. Molenaar, M.E. Dolman, High efficacy of the BCL-2 inhibitor ABT199 (venetoclax) in BCL-2 high-expressing neuroblastoma cell lines and xenografts and rational for combination with MCL-1 inhibition, Oncotarget 7 (2016) 27946–27958.
[112] R.W. Rooswinkel, B. van de Kooij, M. Verheij, J. Borst, Bcl-2 is a better ABT-737 target than Bcl-xL or Bcl-w and only Noxa overcomes resistance mediated by Mcl-1, Bfl-1, or Bcl-B, Cell. Death Dis. 3 (2012) e366.
[113] D. Merino, S.L. Khaw, S.P. Glaser, D.J. Anderson, L.D. Belmont, C. Wong, P. Yue, M. Robati, B. Phipson, W.D. Fairlie, E.F. Lee, K.J. Campbell, C.J. Vandenberg, S. Cory, A.W. Roberts, M.J. Ludlam, D.C. Huang, P. Bouillet, 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 (2012) 5807–5816.
[114] D. Yecies, N.E. Carlson, J. Deng, A. Letai, Acquired resistance to ABT-737 in lymphoma cells that up-regulate MCL-1 and BFL-1, Blood 115 (2010) 3304–3313.
[115] T. Ni Chonghaile, K.A. Sarosiek, T.T. Vo, J.A. Ryan, A. Tammareddi, G. Moore Vdel, J. Deng, K.C. Anderson, P. Richardson, Y.T. Tai, C.S. Mitsiades, U.A. Matulonis, R. Drapkin, R. Stone, D.J. Deangelo, D.J. McConkey, S.E. Sallan, L. Silverman, M.S. Hirsch, D.R. Carrasco, A. Letai, Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy, Science 334 (2011) 1129–1133.
[116] K.G. Wolter, Y.T. Hsu, C.L. Smith, A. Nechushtan, X.G. Xi, R.J. Youle, Movement of Bax from the cytosol to mitochondria during apoptosis, J. Cell. Biol. 139 (1997) 1281–1292.
[117] S. Al-Harbi, B.T. Hill, S. Mazumder, K. Singh, J. Devecchio, G. Choudhary, L.A. Rybicki, M. Kalaycio, J.P. Maciejewski, J.A. Houghton, A. Almasan, An antiapoptotic BCL-2 family expression index predicts the response of chronic lymphocytic leukemia to ABT-737, Blood 118 (2011) 3579–3590.
[118] S.P. Glaser, E.F. Lee, E. Trounson, P. Bouillet, A. Wei, W.D. Fairlie, D.J. Izon, J. Zuber, A.R. Rappaport, M.J. Herold, W.S. Alexander, S.W. Lowe, L. Robb, A. Strasser, Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia, Genes Dev. 26 (2012) 120–125.
[119] G.L. Kelly, S. Grabow, S.P. Glaser, L. Fitzsimmons, B.J. Aubrey, T. Okamoto, L.J. Valente, M. Robati, L. Tai, W.D. Fairlie, E.F. Lee, M.S. Lindstrom, K.G. Wiman, D.C. Huang, P. Bouillet, M. Rowe, A.B. Rickinson, M.J. Herold, A. Strasser, Targeting of MCL-1 kills MYC-driven mouse and human lymphomas even when they bear mutations in p53, Genes Dev. 28 (2014) 58–70.
[120] B. Koss, J. Morrison, R.M. Perciavalle, H. Singh, J.E. Rehg, R.T. Williams, J.T. Opferman, Requirement for antiapoptotic MCL-1 in the survival of BCR-ABL B-lineage acute lymphoblastic leukemia, Blood 122 (2013) 1587–1598.
[121] S. Grabow, A.R. Delbridge, L.J. Valente, A. Strasser, MCL-1 but not BCL-XL is critical for the development and sustained expansion of thymic lymphoma in p53-deficient mice, Blood 124 (2014) 3939–3946.
[122] M. Bruncko, L. Wang, G.S. Sheppard, D.C. Phillips, S.K. Tahir, J. Xue, S. Erickson, S. Fidanze, E. Fry, L. Hasvold, G.J. Jenkins, S. Jin, R.A. Judge, P.J. Kovar, D. Madar, P. Nimmer, C. Park, A.M. Petros, S.H. Rosenberg, M.L. Smith, X. Song, C. Sun, Z.F. Tao, X. Wang, Y. Xiao, H. Zhang, C. Tse, J.D. Leverson, S.W. Elmore, A.J. Souers, Structure-guided design of a series of MCL-1 inhibitors with high affinity and selectivity, J. Med. Chem. 58 (2015) 2180–2194.
[123] J.D. Leverson, H. Zhang, J. Chen, S.K. Tahir, D.C. Phillips, J. Xue, P. Nimmer, S. Jin, M. Smith, Y. Xiao, P. Kovar, A. Tanaka, M. Bruncko, G.S. Sheppard, L. Wang, S. Gierke, L. Kategaya, D.J. Anderson, C. Wong, J. Eastham-Anderson, M.J. Ludlam, D. Sampath, W.J. Fairbrother, I. Wertz, S.H. Rosenberg, C. Tse, S.W. Elmore, A.J. Souers, Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax), Cell. Death Dis. 6 (2015) e1590.
[124] Y. Xiao, P. Nimmer, G.S. Sheppard, M. Bruncko, P. Hessler, X. Lu, L. Roberts-Rapp, W.N. Pappano, S.W. Elmore, A.J. Souers, J.D. Leverson, D.C. Phillips, MCL-1 is a key determinant of breast Cancer cell survival: validation of MCL-1 dependency utilizing a highly selective small molecule inhibitor, Mol. Cancer Ther. 14 (2015) 1837–1847.
[125] D.C. Phillips, Y. Xiao, L.T. Lam, E. Litvinovich, L. Roberts-Rapp, A.J. Souers, J.D. Leverson, Loss in MCL-1 function sensitizes non-Hodgkin's lymphoma cell lines to the BCL-2-selective inhibitor venetoclax (ABT-199), Blood Cancer J. 6 (2016) e403.
[126] E.A. Punnoose, J.D. Leverson, F. Peale, E.R. Boghaert, L.D. Belmont, N. Tan, A. Young, M. Mitten, E. Ingalla, W.C. Darbonne, A. Oleksijew, P. Tapang, P. Yue, J. Oeh, L. Lee, S. Maiga, W.J. Fairbrother, M. Amiot, A.J. Souers, D. Sampath, Expression profile of BCL-2, BCL-XL, and MCL-1 predicts pharmacological response to the BCL-2 selective antagonist venetoclax in multiple myeloma models, Mol. Cancer Ther. 15 (2016) 1132–1144.
[127] J.L. Rinkenberger, S. Horning, B. Klocke, K. Roth, S.J. Korsmeyer, Mcl-1 deficiency results in peri-implantation embryonic lethality, Genes Dev. 14 (2000) 23–27.
[128] J.T. Opferman, A. Letai, C. Beard, M.D. Sorcinelli, C.C. Ong, S.J. Korsmeyer, Development and maintenance of B and T lymphocytes requires

antiapoptotic MCL-1, Nature 426 (2003) 671–676.
[129] V. Peperzak, I. Vikstrom, J. Walker, S.P. Glaser, M. LePage, C.M. Coquery, L.D. Erickson, K. Fairfax, F. Mackay, A. Strasser, S.L. Nutt, D.M. Tarlinton, Mcl-1 is essential for the survival of plasma cells, Nat. Immunol. 14 (2013) 290–297.
[130] A. Kotschy, Z. Szlavik, J. Murray, J. Davidson, A.L. Maragno, G.L. Toumelin-Braizat, M. Chanrion, G.L. Kelly, J.-N. Gong, D. Moujalled, A. Bruno, M. Csekei, A. Paczal, Z. Szabo, S. Sipos, G. Radics, A. Proszenyak, B. Balint, L. Ondi, G. Blasko, A. Robertson, A. Surgenor, P. Dokurno, I. Chen, N. Matassova, J. Smith, C. Pedder, C. Graham, A. Studeny, G. Lysiak-Auvity, A.-M. Girard, F. Gravé, D. Segal, C.D. Riffkin, G. Pomilio, L.C.A. Galbraith, B.J. Aubrey, M.S. Brennan, M.J. Herold, C. Chang, G. Guasconi, N. Cauquil, F. Melchiore, N. Guigal-Stephan, B. Lockhart, F. Colland, J.A. Hickman, A.W. Roberts, D.C.S. Huang, A.H. Wei, A. Strasser, G. Lessene, O. Geneste, The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models, Nature (2016). Published online 19/10/16.
[131] R. Beroukhim, C.H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J.S. Boehm, J. Dobson, M. Urashima, K.T. Mc Henry, R.M. Pinchback, A.H. Ligon, Y.J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M.S. Lawrence, B.A. Weir, K.E. Tanaka, D.Y. Chiang, A.J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F.J. Kaye, H. Sasaki, J.E. Tepper, J.A. Fletcher, J. Tabernero, J. Baselga, M.S. Tsao, F. Demichelis, M.A. Rubin, P.A. Janne, M.J. Daly, C. Nucera, R.L. Levine, B.L. Ebert, S. Gabriel, A.K. Rustgi, C.R. Antonescu, M. Ladanyi, A. Letai, L.A. Garraway, M. Loda, D.G. Beer, L.D. True, A. Okamoto, S.L. Pomeroy, S. Singer, T.R. Golub, E.S. Lander, G. Getz, W.R. Sellers, M. Meyerson, The landscape of somatic copy-number alteration across human cancers, Nature 463 (2010) 899–905.
[132] S.A. Amundson, T.G. Myers, D. Scudiero, S. Kitada, J.C. Reed, A.J. Fornace Jr., An informatics approach identifying markers of chemosensitivity in human cancer cell lines, Cancer Res. 60 (2000) 6101–6110.
[133] G. Lessene, P.E. Czabotar, B.E. Sleebs, K. Zobel, K.N. Lowes, J.M. Adams, J.B. Baell, P.M. Colman, K. Deshayes, W.J. Fairbrother, J.A. Flygare, P. Gibbons, W.J. Kersten, S. Kulasegaram, R.M. Moss, J.P. Parisot, B.J. Smith, I.P. Street, H. Yang, D.C. Huang, K.G. Watson, Structure-guided design of a selective BCL-X(L) inhibitor, Nat. Chem. Biol. 9 (2013) 390–397.
[134] R.M. Brady, A. Vom, M.J. Roy, N. Toovey, B.J. Smith, R.M. Moss, E. Hatzis, D.C. Huang, J.P. Parisot, H. Yang, I.P. Street, P.M. Colman, P.E. Czabotar, J.B. Baell, G. Lessene, De-novo designed library of benzoylureas as inhibitors of BCL-XL: synthesis, structural and biochemical characterization, J. Med. Chem. 57 (2014) 1323–1343.
[135] J.D. Leverson, D.C. Phillips, M.J. Mitten, E.R. Boghaert, D. Diaz, S.K. Tahir, L.D. Belmont, P. Nimmer, Y. Xiao, X.M. Ma, K.N. Lowes, P. Kovar, J. Chen, S. Jin, M. Smith, J. Xue, H. Zhang, A. Oleksijew, T.J. Magoc, K.S. Vaidya, D.H. Albert, J.M. Tarrant, N. La, L. Wang, Z.F. Tao, M.D. Wendt, D. Sampath, S.H. Rosenberg, C. Tse, D.C. Huang, W.J. Fairbrother, S.W. Elmore, A.J. Souers, Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy, Sci. Transl. Med. 7 (2015) 279ra240.
[136] A.A. Morales, A. Olsson, F. Celsing, A. Osterborg, M. Jondal, L.M. Osorio, High expression of bfl-1 contributes to the apoptosis resistant phenotype in B-cell chronic lymphocytic leukemia, Int. J. Cancer 113 (2005) 730–737.
[137] A. Olsson, M. Norberg, A. Okvist, K. Derkow, A. Choudhury, G. Tobin, F. Celsing, A. Osterborg, R. Rosenquist, M. Jondal, L.M. Osorio, Upregulation of bfl-1 is a potential mechanism of chemoresistance in B-cell chronic lymphocytic leukaemia, Br. J. Cancer 97 (2007) 769–777.
[138] I.C. Park, S.H. Lee, D.Y. Whang, W.S. Hong, S.S. Choi, H.S. Shin, T.B. Choe, S.I. Hong, Expression of a novel Bcl-2 related gene, Bfl-1, in various human cancers and cancer cell lines, Anticancer Res. 17 (1997) 4619–4622.
[139] A.I. Riker, S.A. Enkemann, O. Fodstad, S. Liu, S. Ren, C. Morris, Y. Xi, P. Howell, B. Metge, R.S. Samant, L.A. Shevde, W. Li, S. Eschrich, A. Daud, J. Ju, J. Matta, The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis, BMC Med. Genomics 1 (2008) 13.
[140] E.Y. Lin, A. Orlofsky, M.S. Berger, M.B. Prystowsky, Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to *bcl-2*, J. Immunol. 151 (1993) 1979–1988.
[141] M. Vogler, BCL2A1: the underdog in the BCL2 family, Cell. Death Differ. 19 (2012) 67–74.
[142] A.L. Mathieu, O. Sperandio, V. Pottiez, S. Balzarin, A. Herledan, J.O. Elkaim, M.L. Fogeron, C. Piveteau, S. Dassonneville, B. Deprez, B.O. Villoutreix, N. Bonnefoy, F. Leroux, Identification of small inhibitory molecules targeting the Bfl-1 anti-apoptotic protein that alleviates resistance to ABT-737, J. Biomol. Screen 19 (2014) 1035–1046.
[143] L.D. Walensky, K. Pitter, J. Morash, K.J. Oh, S. Barbuto, J. Fisher, E. Smith, G.L. Verdine, S.J. Korsmeyer, A stapled BID BH3 helix directly binds and activates BAX, Mol. Cell 24 (2006) 199–210.
[144] E. Gavathiotis, M. Suzuki, M.L. Davis, K. Pitter, G.H. Bird, S.G. Katz, H.C. Tu, H. Kim, E.H. Cheng, N. Tjandra, L.D. Walensky, BAX activation is initiated at a novel interaction site, Nature 455 (2008) 1076–1081.
[145] L.D. Walensky, A.L. Kung, I. Escher, T.J. Malia, S. Barbuto, R.D. Wright, G. Wagner, G.L. Verdine, S.J. Korsmeyer, Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix, Science 305 (2004) 1466–1470.
[146] T. Okamoto, K. Zobel, A. Fedorova, C. Quan, H. Yang, W.J. Fairbrother, D.C. Huang, B.J. Smith, K. Deshayes, P.E. Czabotar, Stabilizing the pro-apoptotic BimBH3 helix (BimSAHB) does not necessarily enhance affinity or biological activity, ACS Chem. Biol. 8 (2013) 297–302.
[147] E. Gavathiotis, D.E. Reyna, J.A. Bellairs, E.S. Leshchiner, L.D. Walensky, Direct and selective small-molecule activation of proapoptotic BAX, Nat. Chem. Biol. 8 (2012) 639–645.
[148] M. Xin, R. Li, M. Xie, D. Park, T.K. Owonikoko, G.L. Sica, P.E. Corsino, J. Zhou, C. Ding, M.A. White, A.T. Magis, S.S. Ramalingam, W.J. Curran, F.R. Khuri, X. Deng, Small-molecule Bax agonists for cancer therapy, Nat. Commun. 5 (2014) 4935.
[149] G. Zhao, Y. Zhu, C.O. Eno, Y. Liu, L. Deleeuw, J.A. Burlison, J.B. Chaires, J.O. Trent, C. Li, Activation of the proapoptotic Bcl-2 protein Bax by a small molecule induces tumor cell apoptosis, Mol. Cell. Biol. 34 (2014) 1198–1207.
[150] S. Iyer, K. Anwari, A.E. Alsop, W.S. Yuen, D.C. Huang, J. Carroll, N.A. Smith, B.J. Smith, G. Dewson, R.M. Kluck, Identification of an activation site in Bak and mitochondrial Bax triggered by antibodies, Nat. Commun. 7 (2016) 11734.
[151] A.L. Marschall, S. Dubel, T. Boldicke, Specific in vivo knockdown of protein function by intrabodies, MAbs 7 (2015) 1010–1035.
[152] B. Han, D. Park, R. Li, M. Xie, T.K. Owonikoko, G. Zhang, G.L. Sica, C. Ding, J. Zhou, A.T. Magis, Z.G. Chen, D.M. Shin, S.S. Ramalingam, F.R. Khuri, W.J. Curran, X. Deng, Small-molecule Bcl2 BH4 antagonist for lung Cancer therapy, Cancer Cell 27 (2015) 852–863.
[153] E.H. Cheng, T.V. Sheiko, J.K. Fisher, W.J. Craigen, S.J. Korsmeyer, VDAC2 inhibits BAK activation and mitochondrial apoptosis, Science 301 (2003) 513–517.
[154] S.B. Ma, T.N. Nguyen, I. Tan, R. Ninnis, S. Iyer, D.A. Stroud, M. Menard, R.M. Kluck, M.T. Ryan, G. Dewson, Bax targets mitochondria by distinct mechanisms before or during apoptotic cell death: a requirement for VDAC2 or Bak for efficient Bax apoptotic function, Cell. Death Differ. 21 (2014) 1925–1935.
[155] M. Karbowski, Y.J. Lee, B. Gaume, S.Y. Jeong, S. Frank, A. Nechushtan, A. Santel, M. Fuller, C.L. Smith, R.J. Youle, Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis, J. Cell. Biol. 159 (2002) 931–938.
[156] S.W. Muchmore, M. Sattler, H. Liang, R.P. Meadows, J.E. Harlan, H.S. Yoon, D. Nettesheim, B.S. Chang, C.B. Thompson, S.-L. Wong, S.-C. Ng, S.W. Fesik, X-ray and NMR structure of human Bcl-x_L, an inhibitor of programmed cell death, Nature 381 (1996) 335–341.
[157] E.F. Lee, P.E. Czabotar, B.J. Smith, K. Deshayes, K. Zobel, P.M. Colman, W.D. Fairlie, Crystal structure of ABT-737 complexed with Bcl-x(L): implications for selectivity of antagonists of the Bcl-2 family, Cell. Death Differ. 14 (2007) 1711–1713.
[158] M. Nguyen, R.C. Marcellus, A. Roulston, et al., Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1–mediated resistance to apoptosis, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19512–19517.