Decoding and unlocking the BCL-2 dependency of cancer cells

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Abstract | Cancer cells are subject to many apoptotic stimuli that would kill them were it not for compensatory prosurvival alterations. BCL-2-like (BCL-2L) proteins contribute to such aberrant behaviour by engaging a network of interactions that is potent at promoting survival but that is also fragile: inhibition of a restricted number of interactions may suffice to trigger cancer cell death. Currently available and novel compounds that inhibit these interactions could be efficient therapeutic agents if this phenotype of BCL-2L dependence was better understood at a molecular, cellular and systems level and if it could be diagnosed by relevant biomarkers.

Apoptotic threshold
The amount of stress that has to be imposed on a given cell population to trigger apoptotic death.

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doi:10.1038/nrc3538
Publsihed online 20 June 2013

Escape from apoptosis is an almost systematic hallmark of cancer cells that contributes to tumour progression and to drug resistance. In terms of developing strategies to kill cancer cells, there are two ways of envisioning this anti-apoptotic phenotype: that cancer cells are universally deficient for apoptosis and that alternative modes of cell death need to be exploited; or that understanding how apoptosis is bypassed will enable the identification of therapeutic targets in the mechanisms that allow cancer cell survival. Alterations in the activity of BCL-2 family members are frequently found in cancer cells and these contribute to an increased apoptotic threshold. Anti-apoptotic proteins of this family allow cancer cells to survive various stressful environments and cell death signals, such as those induced by antitumor therapy, the tumour microenvironment or oncogene signalling. Based on this assumption, BCL-2-like (BCL-2L) proteins represent a molecular vulnerability because inhibition of their survival activity may suffice to selectively eliminate cancer cells.

We now have at our disposal a solid framework for understanding not only how cancer cells might be locked into a BCL-2L-protein-dependent state, but also how to unlock this. The use of the initial inhibitory compounds that target BCL-2L proteins in preclinical studies and in early clinical trials has shown that the concept of 'BCL-2L dependence' (BOX 1) is sufficiently correct to raise hopes of a useful therapy, but not sufficiently well circumscribed to enable substantial clinical efficacy. Here we discuss available data and the key issues that novel compounds, or novel therapeutic strategies, will have to tackle to fully exploit BCL-2L dependence.

The lock: survival by BCL-2 family proteins

Apoptosis is a programmed form of cell death that has been conserved throughout evolution. It is executed by a cascade of caspase activation[1] that is triggered by the extrinsic or intrinsic (mitochondrial) apoptotic pathways. The intrinsic pathway is characterized by mitochondrial outer membrane permeabilization (MOMP) and is regulated by the BCL-2 family of proteins (BOX 2) through a network of mutually exclusive, and not functionally equivalent, interactions between anti- and pro-apoptotic members. Multidomain pro-apoptotic proteins, such as BAX and BAK, are absolutely required for MOMP. They are synthesized in an essentially inactive form yet acquire the ability to insert into mitochondrial membranes, oligomerize and trigger MOMP by themselves after a series of partially characterized conformational changes, which are referred to as their 'activation'[2]. This activation is favoured by diverse proteinaceous and non-proteinaceous factors, including by a subset of activator BH3-only proteins (such as BIM (also known as BCL-2L11), BID and PUMA (encoded by *BBC3*) that can directly interact with BAX or BAK[3–6]. Anti-apoptotic proteins (such as BCL-2, BCL-X_L (also known as BCL-2L1) or MCL1) prevent BAX or BAK activation and activity by binding to them and/or by binding to activator BH3-only proteins in complexes that are preferentially, if not exclusively, located at the mitochondria. The maintenance of these interactions is required for cells to survive. This is finely regulated by 'sensitizer' BH3-only proteins (such as BAD and NOXA (encoded by *PMAIP1*) that cannot activate BAX or BAK but that compete with these and with activator BH3-only proteins for binding to anti-apoptotic proteins (FIG. 1).

Caspase activation
A caspase multimerization-induced conformational change leading to auto-catalytic processing and activation of caspases, which are a family of cysteine active proteases.

BH3 mimetics
Compounds that interfere with the prosurvival function of anti-apoptotic BCL-2 family members by interacting with their BH3-binding groove and competing with (some) pro-apoptotic BCL-2 members for binding.

The balance between the pro- and anti-apoptotic activities of BCL-2 family members is, in many cancers, tipped towards survival by genetic or epigenetic changes, signalling pathway alterations and post-translational modifications. For example, increased expression of BCL-2, BCL-X_L and MCL1 has been reported in human haematopoietic cancers[7,8] and in some solid tumours[9], whereas the *BOK* and *BBC3* genes are frequently deleted in human cancers[9]. These alterations in the BCL-2 network provide a selective advantage to cancer cells by allowing them to survive various stressful environments, cell stress phenotypes and/or cell death signals that directly ensue from oncogenic signalling[10]. Cancer cells may be addicted to these survival mechanisms to counteract death signals that persist during tumour progression, as indicated by: numerous genetically engineered mouse models[11–15]; the observation that most human tumour samples exhibit enhanced MOMP sensitivity to peptide-based inhibitors of BCL-2 homologues compared with most normal tissues[16]; and that human cancer cell death can be induced by the downregulation of survival proteins (or single-agent treatments with BH3 mimetics, as described below), in classical two-dimensional[9,17] or in three-dimensional[18,19] cultures. Mechanistic investigations of BCL-2L dependence *in vitro* gave support to the idea that BCL-2 homologues maintain survival (at least in part) by preventing activator BH3-only proteins and BAX and/or BAK from triggering apoptosis[17,20].

The key: exploiting BCL-2L dependence

Core protein–protein interactions engaged by BCL-2 proteins are tractable therapeutic targets. The interactions between BCL-2 family members that regulate cancer cell survival rely on the binding of the BH3 domain of pro-apoptotic members to a hydrophobic groove at the surface of an anti-apoptotic protein. When this occurs, the short (about 25 amino-acid-long) contiguous BH3 domain adopts an amphipathic α-helix structure. As the activation of pro-apoptotic family members, such as BAX and BAK, leads to exposure of their BH3 domain, it increases their interaction with anti-apoptotic BCL-2L proteins[2].

Studies using synthetic peptides encompassing BH3 domains showed that these domains promote apoptosis[21,22] but that distinct peptides have distinct effects. BAD, NOXA and HRK BH3 peptides antagonize specific subsets of anti-apoptotic BCL-2L proteins (FIG. 1), whereas BID, BIM and PUMA (in some studies) BH3 peptides trigger BAX and BAK activation in addition to interacting with all known anti-apoptotic BCL-2L proteins. The functional distinction between activator and sensitizer BH3-only proteins thus essentially relies on functional differences between their short BH3 domains. Importantly, the ability of BH3 peptides to activate BAX or BAK and/or inhibit BCL-2 homologues can be used to foretell the response of cancer cells to apoptotic stimuli through a technique called BH3 profiling (BOX 3). Rapid MOMP in response to specific BH3 peptides in such assays is indicative of the existence, at the mitochondria, of complexes that are crucially involved in maintaining survival. It is, by inference, indicative of an acute BCL-2L dependence and/or a low apoptotic threshold.

Key elements in the binding of BH3 domains to anti-apoptotic BCL-2L proteins involve four conserved hydrophobic residues that are exposed on one face of the BH3 α-helix. As shown by NMR spectroscopy and X-ray crystallography, these four residues form electrostatic interactions with four hydrophobic pockets (P1–P4) in the hydrophobic groove made by the BH1, BH2 and BH3 domains of anti-apoptotic proteins. Regions on either side of the BH3 motif are also involved in these interactions[23–26]. Crystal or solution structures are known for five prosurvival family members bound with various BH3 peptides (Protein Data Bank entries 1PQ1, 1PQ0, 2NL9, 1WSX and 1BXL)[27–30]. Informative representations of the respective hydrophobic grooves, including the P1 to P4 positions, are shown in REF. 31. The BH3-binding pockets of anti-apoptotic proteins share many features owing to common sequences in their BH1, BH2 and BH3 domains. These similarities, in addition to plasticity that allows for mutual adjustments in the conformation of both partners on binding, account for the promiscuous binding of BH3-only proteins, such as BIM and PUMA, to anti-apoptotic BCL-2L proteins.

The selectivity of these interactions relies on differences in a few key residues within both the BH3 domain and the BH3-binding groove. The presence of an additional residue in the BH1 domain of MCL1 results in a conserved arginine residue — which is involved in a hydrogen-bonding interaction with an aspartic acid residue in BH3 ligands — being less exposed in MCL1 than in BCL-X_L[31]. The first two hydrophobic residues of BH3 ligands are more constricted and less contiguous in the MCL1 complex than in the BCL-X_L complex because, on binding, the third α-helix in MCL1 is better formed than that of BCL-X_L. Moreover, distinctive differences in the BH3 domain of MCL1 (adjacent to the conserved glycine and aspartic acid residues) remodel the binding site for the fourth hydrophobic residue of BH3 ligands.

Key points

- The balance between pro- and anti-apoptotic activities of BCL-2 family members is tipped towards survival in many cancer cells, thus allowing them to survive various stressful environments, tumour stress phenotypes and/or oncogene-induced death signals.
- As death signals may persist during tumour progression, cancer cells may be addicted to these survival mechanisms and be in a state of dependence on 'BCL-2-like' (BCL-2L) anti-apoptotic proteins.
- Survival of 'BCL-2L-dependent' cancer cells relies on the maintenance of protein–protein complexes in which the BH3 domain of some pro-apoptotic BCL-2 family members engages a hydrophobic groove at the surface of anti-apoptotic BCL-2L proteins.
- Structural characterization of the BH3-binding interface of anti-apoptotic BCL-2L proteins has led to the identification of small-molecule BH3 mimetics that disrupt key interactions and promote cancer cell apoptosis by on-target effects.
- A dual BCL-2 and BCL-X_L inhibitor and a specific BCL-2 inhibitor have shown clinical activity in haematological malignancies. The dual inhibitor induces dose-limiting thrombocytopenia owing to BCL-X_L inhibition.
- Finely-tuned inhibition of BCL-X_L and of MCL1 in cancer cells by new and selective drugs remains a challenge and a necessity.
- Understanding the exact effects of inhibitors on endogenous (membrane-localized) complexes, identifying predictive biomarkers for drug efficacy and circumscribing the global biological effects of these compounds is also required.

Box 1 | **BCL-2L dependence**

A dependence on BCL-2-like (BCL-2L) proteins describes a cellular state in which a limited number of prosurvival BCL-2 homologues (such as BCL-X_L and MCL1) are required for sustained cancer cell viability and tumour growth. Conceptually, BCL-2L protein dependence evokes oncogene dependence and can thus be investigated by experimental paradigms that have previously been used to explore what establishes oncogene addiction and how cells can escape from it. Multiple and complex non-cell-autonomous and cell-autonomous death signals encountered by cancer cells may affect dependence on BCL-2L proteins. Many candidate pathways exist, but arguably integrated functional approaches are required to diagnose whether or not dependence prevails. Mechanistically, dependence on BCL-2L proteins is understood to rely on anti-apoptotic BCL-2L proteins counteracting self-sufficient death signals that kill cells when these survival proteins are absent or inhibited. Pathways leading to increased expression of anti-apoptotic BCL-2L proteins will establish BCL-2L dependence, but this will only be biologically important if other pathways concomitantly trigger death signals that favour the activation of multi-domain pro-apoptotic proteins, making anti-apoptotic BCL-2L proteins necessary for survival. Experimentally, BCL-2L dependence can be evaluated by RNA interference or by the integrative functional test called BH3 profiling (BOX 3). BCL-2L protein dependence corresponds to cellular sensitivity to BH3 mimetics used as single agents.

Interactions in addition to those reported above might also intervene and influence the outcome of BH3 binding. A recent study showed that a PUMA BH3 peptide induces a partial unfolding of two α-helices within BCL-X_L. This conformational change is due to the presence of a tryptophan residue in the flanking sequence of the PUMA BH3 domain that is not found in other BH3 domains[32].

A conformational change in BAX and BAK was also reported on binding of BIM and BID BH3 peptides to a canonical BH3-binding hydrophobic groove in the BAX and BAK structures[33]. This favours BAX or BAK oligomerization and triggers apoptotic activity. Immunoprecipitation and surface plasmon resonance assays hint that additional sites contribute to this binding as the BH3 domain of BID and PUMA also engage BAX through its amino-terminal α1 helix[5]. A structural study using a stapled peptide BIM BH3 domain showed that this interaction site might not only be formed by the α1 helix but also by the α2, α4 and α6 helices of BAX[34].

Structural investigations of BH3-mediated interactions provided the necessary insight to design BCL-2-inhibitory compounds, to explain the selectivity of these compounds against subsets of BCL-2 family members and to predict that anti-apoptotic proteins might be inhibited by BH3 mimetics without interfering with the binding of activator BH3 domains to structurally distinct sites in BAX and BAK. They also facilitated the development of a direct BAX activator[35]. The latter compound might be poorly potent in cells in which anti-apoptotic proteins (that can inhibit active BAX) are in excess of BAX[36], and the rationale for its selectivity against cancer cells needs to be clarified. From here onwards, our Review focuses on inhibitors of anti-apoptotic BCL-2L proteins for which there is evidence of therapeutic potential.

A panorama of drugs and addictions. Targeting BCL-2 family members with small molecules is challenging because the BH3-binding site is shallow and does not form an easily tractable pocket. Different strategies were used to discover molecules that are able to engage specific interactions with anti-apoptotic proteins. Stapled BH3 peptides provided a straightforward approach and gave interesting tools for preclinical investigation or secondary screens, but none of these initial molecules have entered clinical trials to date. Numerous small-molecule BH3 mimetics have been characterized (TABLE 1). Most of them bind their targets with poor affinity and/or trigger cell death independently from the mitochondrial apoptotic pathway, thus suggesting that they have effects beyond BCL-2L protein inhibition. They may be useful clinically, but not because they target BCL-2L proteins specifically.

By contrast, two small-molecule BH3 mimetics that bind with high affinity (<1 μM) to BCL-2, BCL-X_L and BCL-W (also known as BCL-2L2) but not to MCL1 or

Box 2 | **The BCL-2 family**

The BCL-2 family consists of interacting proteins that have a major role in the regulation of mitochondrial outer membrane permeabilization (MOMP) and apoptosis (FIG. 1). The BCL-2 family is composed of three functionally distinct groups: anti-apoptotic proteins (such as BCL-2, BCL-X_L, MCL1, BCL-W and BCL-2A1), multi-domain pro-apoptotic proteins (BAX, BAK and BOK) and BH3-only pro-apoptotic proteins (such as BIM, BID, PUMA, BAD, HRK, NOXA and BMF). Anti-apoptotic proteins share four BCL-2 homology domains (BH1–4). They preferentially localize at the mitochondrial outer membrane (MOM) owing to a hydrophobic carboxyl-terminal end, but they also localize at other subcellular membranes or are present in the cytosol. Multi-domain pro-apoptotic proteins BAX and BAK harbour only three BH domains (1–3) even though their first helix near the amino terminus resembles a BH4 domain. They are synthesized as essentially inactive proteins that localize either in the cytosol (for BAX) or at the mitochondria (for BAK). On activation, they acquire the ability to translocate to mitochondrial membranes, insert into mitochondrial membranes, oligomerize and trigger MOMP by themselves (that is, in the absence of any other BCL-2 family member), through processes that still need to be fully characterized. BAX and BAK have an essential role in MOMP and their combined absence renders most cells resistant to MOMP and cell death induction[101]. BOK, the physiological expression of which is more restricted than that of BAX and BAK, is less well studied, and it remains to be established whether it can functionally substitute for BAX and BAK under certain conditions or whether it functions as a regulator. BH3-only proteins function upstream of BAX and BAK[102]. Depending on the presence of specific targeting domains in their sequence, they can (for BIM and PUMA) or cannot (for BAD) interact with mitochondria by themselves. Their pro-apoptotic activities are regulated at the transcriptional, translational and post-translational levels and their function requires their ability to interact with anti-apoptotic proteins and/or, in the case of the subset of 'activator BH3-only proteins' (BIM, BID and PUMA), with BAX or BAK. The BCL-2 family database provides state-of-the-art information regarding the structure, regulation and activities of BCL-2 family members across species.

Stapled peptide
A peptide in which helicity is stabilized by the introduction of an intramolecular hydrocarbon linker between crucial residues. This modification protects the peptide from proteolytic degradation and enhances its ability to cross cell membranes.

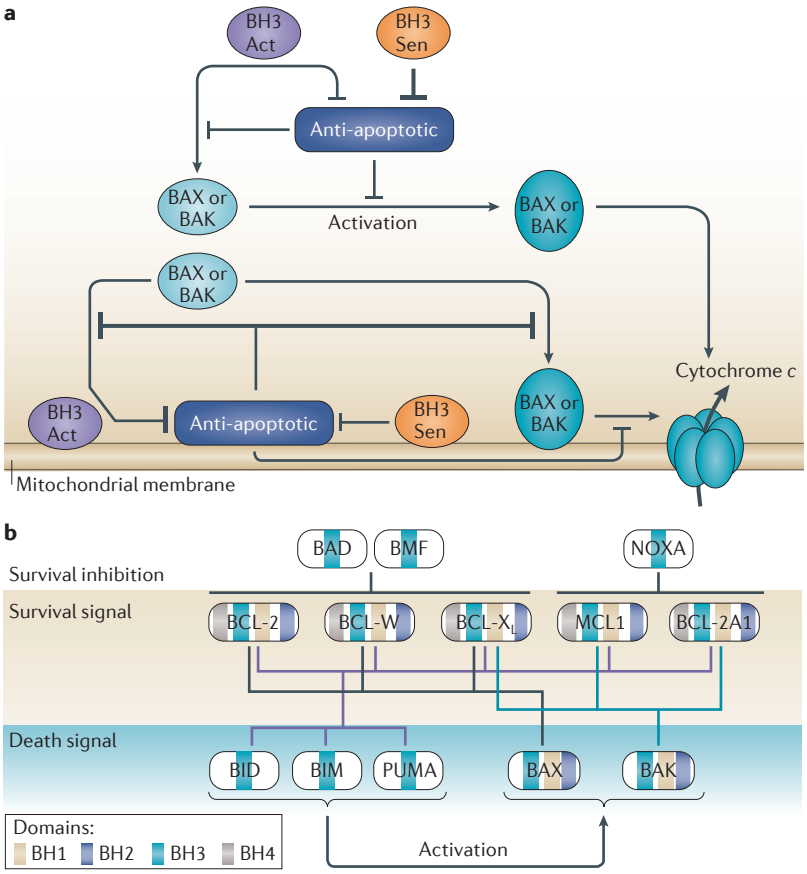

Figure 1 | **Regulation of mitochondrial outer membrane permeabilization and cell survival by interacting BCL-2 family members.** **a** | Regulation of BAX and BAK activation occurs through a dynamic network of diverse, functionally distinct interacting partners that are in equilibrium and are only partly exchangeable. Schematically, activator BH3-only proteins ('BH3 Act' in the figure) can directly interact with BAX and BAK to initiate conformational changes and activation (shown by the darker cyan colour). Anti-apoptotic proteins ('anti-apoptotic' in the figure) prevent these events by sequestering activator BH3-only proteins and/or by interacting with BAX and BAK. A subset of sensitizer BH3-only proteins ('BH3 Sen' in the figure) cannot activate BAX and BAK directly and instead prevent anti-apoptotic proteins from interacting with BAX, BAK and activator BH3-only proteins. Our ligand–receptor perception of BCL-2 family member-mediated regulation of mitochondrial outer membrane permeabilization (MOMP) derives from studies carried out in solution that have provided valid, but incomplete, biophysical and structural information. Indeed, mitochondrial membranes actively contribute to the molecular mechanisms involved, as protein–membrane interactions promote dynamic conformational and functional changes. Membrane-embedded anti-apoptotic proteins can inhibit BAX or BAK activation by competing with membrane-localized activator BH3-only proteins for binding to inactive BAX and they can prevent BAX and BAK oligomerization and subsequent pore formation by functioning as dominant-negative forms of BAX or BAK. Membrane proteins that are not BCL-2 family members (not shown here) can also intervene in these processes[103,104]. **b** | Validated interactions between BCL-2 family members are represented by connecting lines. The BCL-2 homology (BH) domains that are present in each family member are also indicated. The expression of BAX or BAK (which are proteins that have the BH1–3 domains) increases the likelihood of cell death, and coincident expression of activator BH3-only ligands (such as BID, BIM and PUMA) is potentially detrimental to the cell because these interact with BAX and BAK and activate them. This generates a 'death signal' in the absence of anti-apoptotic proteins (such as BCL-2 and BCL-X_L, which contain all four BH domains). BCL-2 and BCL-X_L provide a 'survival signal' that involves interactions with activator BH3-only proteins and with BAX and BAK. Inhibition of this survival signal ('survival inhibition' in the figure) can occur through the interaction of sensitizer BH3-only proteins (such as BAD, BMF and NOXA) with anti-apoptotic proteins. In the absence of anti-apoptotic proteins, sensitizer BH3-only proteins exert no intrinsic pro-apoptotic activity.

BCL-2A1) are of particular interest. ABT-737 (REF. 37) and ABT-263 (also known as navitoclax)[38], both developed by Abbott Laboratories (now Abbvie), are closely related chemically and have identical biological properties. They displace (some) pro-apoptotic proteins from BCL-2 and BCL-X_L and require BAX or BAX for cell killing, thus confirming an on-target effect. The crystal structure of BCL-X_L bound by ABT-737 showed that the selectivity of this compound is based on two hydrophobic pockets (P2 and P4) within the BH3-binding groove[39]. A less flexible structure in the MCL1 P2 pocket might account for the much lower affinity of ABT-737 for MCL1.

Functionally, these compounds exploit BCL-2L dependence, whether this state is fuelled by antitumor therapy, tumour stress phenotypes or oncogenic signals. ABT-737 and ABT-263 have synergistic toxicity with conventional chemotherapeutics and radiation. There is a strong rationale for combining them with cytotoxic drugs, such as histone deacetylase inhibitors that can increase the expression of BIM[40] or genotoxic treatments that can increase PUMA expression[41]. Cells undergoing prolonged mitotic arrest (for example, in response to paclitaxel treatment) were shown to be sensitive to ABT-737 (REF. 42). However, there is a risk that the combination of a BH3 mimetic with chemotherapy increases the toxicity of the chemotherapy, thus the use of targeted therapies (see below) seems to be more appropriate.

These compounds affect the viability of cancer cells that exhibit typical tumour stress phenotypes[43]. The survival of polyploid cells (a recognized prominent source of aneuploidy in cancer) was shown to be BCL-X_L dependent and to be significantly affected by ABT-737 treatment[44]. During tumour growth, cells also encounter conditions of low oxygen and nutrients that influence the BCL-2 network of proteins and render cells sensitive to ABT-737 (REFS 45,46). Acidosis, which is a characteristic feature of the tumour microenvironment, was also shown to enhance the response to ABT-737 (REF. 47).

ABT-737 exhibits single-agent activity against some tumour cells from B cell lymphomas, chronic lymphocytic leukaemias (CLLs), acute myeloid leukaemias (AMLs), acute lymphoblastic leukaemias (ALLs), a subgroup of multiple myeloma and small-cell lung cancers (SCLCs)[37]. This single-agent activity may directly ensue from constitutive death signals that are induced by some oncogenes (FIG. 2). For example, deregulated MYC triggers pro-apoptotic signals and favours MOMP[10]. Thus, cancer cells with increased MYC expression require a compensatory survival signal that can be provided by the overexpression of BCL-2, BCL-X_L or MCL1. (Some highly aggressive lymphomas harbour both a *MYC* (t8;14) and a *BCL2* (t14;18) translocation[48], and two-thirds of cancers carrying *MCL1* or *BCL2L1* amplifications also carry amplifications in the chromosomal region encoding *MYC*[9].) BCL-2L dependence is probably also important in cells that are driven by dominant oncogenes other than MYC. In non-small-cell lung cancer (NSCLC) cell lines, gene expression signatures of ABT-737 sensitivity correspond to gene expression signatures of addiction to RAS[49]. Moreover, RAS-mutant cells were occasionally reported to rely (partly or completely) on ABT-737 targets for their survival[50,51]. This implies that

Oncogene addiction
A status in which a cancer cell seems to have acquired an exquisite dependence on an activated oncogene for proliferation or survival.

cancer cells can be sensitive to BH3 mimetics even when their driving oncogenic pathway (such as RAS) has overtly anti-apoptotic effects. Indeed, some of the anti-apoptotic signals that are involved can mitigate BCL-2L dependence, for example by downregulating the expression of activator BH3-only proteins. Yet, other anti-apoptotic signals may favour such a dependence by enhancing the expression of anti-apoptotic proteins while leaving intact the expression of BH3 activators that are driven by pro-apoptotic oncogenic pathways, such as active MYC. Therefore, the anti-apoptotic phenotype of cancer cells may result from the blocking of oncogene-induced death signals by compensatory, oncogene-induced survival signals in such a way that a reliance on BCL-2L proteins represents a weakness of the oncogenic network (FIG. 3). By inference, ABT-737 will synergize with approaches that target oncogenic pathways to which cancer cells are addicted (a phenomenon known as oncogene addiction that is understood to result from the induction, by multifarious oncogenic pathways, of both death and survival signals)[52] and will have little effect on cells that do not have these dependencies. Consistent with this, the combined inhibition of BCL-X_L (by ABT-737) and MEK efficiently killed RAS-mutant cells that are addicted to RAS, but not RAS-independent cells[53].

Lessons from the clinic. Due to the poor pharmacological properties of ABT-737, it is the orally bioavailable ABT-263 that has been used in the clinic. Several clinical trials have been carried out, and the results from some of these trials are available. A Phase I dose-escalation study enrolled 55 patients with relapsed or refractory lymphoid malignancies[54]. Common toxicities included fatigue and grade 1/2 gastrointestinal effects that might have been due to the drug vehicle used (phosphatidylcholine solubilizer). Thrombocytopenia and neutropenia represented serious common toxicities: grade-3 and grade-4 toxicities were observed in 29 and 17 patients, respectively. These are probably due to an on-target effect because BCL-X_L inhibition induces apoptosis of ageing platelets in a BAK-dependent manner[55,56]. In order to limit treatment-induced thrombocytopenia, a lead-in dose of ABT-263 was tried as a stimulant for platelet formation in the bone marrow, followed by a continuous administration at a higher dose. Of 46 patients who were evaluable for response, ten achieved a partial response. Eight of these patients had CLL or small lymphocytic lymphomas, one patient had follicular lymphoma and one patient had a natural-killer T-cell lymphoma. The antitumor activity observed in CLL was confirmed in another trial that included 29 patients with CLL[57]. Nine of the patients with CLL (31%) achieved a partial response. Median progression-free survival (PFS) was 25 months. It should be noted that residual diffuse CLL involvement was observed in all patients in whom a bone marrow biopsy was carried out. This is consistent with preclinical data showing that the interaction of CLL cells with the bone marrow stroma enhances apoptosis resistance, induces a significant upregulation of BCL-X_L and BCL2-A1 and a global decrease of apoptotic priming[58–60].

To date, the results obtained from treating solid tumours with ABT-263 have been disappointing. A Phase II trial that was carried out in 39 patients with relapsed SCLC had only one partial response according to response evaluation criteria in solid tumours (RECIST) with a median PFS of 1.5 months[61]. These results contrast with the preclinical data obtained using SCLC cell lines, yet are consistent with the observations made in patient-derived tumour xenografts[62]. Lower expression of BCL-2, and of its pro-apoptotic partners BAX and BIM, in primary SCLCs compared with SCLC cell lines might, in part, explain these results.

The dose-limiting thrombocytopenia induced by ABT-263 as a result of BCL-X_L inhibition, led to the development of ABT-199, which is specific for BCL-2 (REF. 63). The affinity of the compound, especially with the BCL-2 P4 hydrophobic pocket, was increased by promoting an electrostatic bond between an indole group grafted on to the compound and Asp103, which is one of the few amino acids that is not found in the BCL-X_L P4 hydrophobic pocket. Preliminary results of a Phase I trial showed activity in patients with CLL. Of 21 evaluable patients, 14 (67%) achieved a partial response. Tumour lysis syndrome (reflecting a rapid induction of apoptosis) was a dose-limiting toxicity, justifying a treatment schedule consisting of an initial low dose with stepwise dose escalation. ABT-199 has also recently been reported to be active in non-Hodgkin lymphomas. Eight out of 15 evaluable patients (53%) achieved a partial response, including all six patients with mantle cell lymphoma.

These clinical results establish BCL-2 as a valid target in B cell lymphomas. The sensitivity of CLL cells to BCL-2 inhibition may be partly explained by the predominant role of BCL-2 in lymphomagenesis, the high levels of BCL-2 molecules (compared with BCL-X_L or BCL-W) in peripheral-blood CLL cells[57] and the abundance of BCL-2–BIM complexes in CLL[64]. Several of the studies with ABT-263 have also shown that inhibiting BCL-2 has potential efficacy in the difficult-to-treat subset of CLL patients that have deletions in the short arm of chromosome 17 (del(17p)). However, BCL-2 inhibitors might need to be combined with other drugs to substantially improve clinical outcome: tumour cells interacting with

Box 3 | **BH3 profiling**

BH3 profiling is a functional assay that determines the sensitivity versus resistance of cancer cells to various pro-apoptotic stimuli. It assesses the occupation (binding) state of pro-survival BCL-2 family members by pro-apoptotic counterparts at the mitochondrial membrane by measuring the induction of mitochondrial outer membrane permeabilization (MOMP) by an array of functionally distinct BH3 peptides. Either isolated mitochondria or suspensions of permeabilized cells obtained from solid or haematopoietic malignancies are used. After the addition of BH3 peptides, cytochrome *c* release is measured by enzyme-linked immunosorbent assay (ELISA) or flow cytometry as a measure of MOMP. Alternatively, loss of the mitochondrial inner membrane potential is evaluated using a fluorescent marker and fluorimetry or flow cytometry. Mitochondrial priming, as determined by BH3 profiling, was shown to correlate with clinical responses to cytotoxic chemotherapy in multiple myeloma, acute myeloid leukaemia, acute lymphocytic leukaemia and ovarian cancer samples[16,65]. Results obtained from this functional assay have indicated the therapeutic advantage of raising the mitochondrial priming state by using small molecules that mimic the inhibitory features of BH3-only proteins. They have also shown that most normal tissues have a relatively low dependence on BCL-2-like proteins for survival (BOX 1) compared with some cancer samples.

Table 1 | **BCL-2L-inhibitory compounds**

Molecule	Targets (IC_{50} (μM))	Design or screening method used
HA-14	BCL-2 (>100)	Computer-based
BH3Is	BCL-2 (1.14), BCL-W (2.33), BCL-B (1.08) and MCL1 (2.17)	Fluorescence polarization
Antimycin A3	BCL-2 (2.95), BCL-X_L (2.7), BCL-B (1.83) and MCL1 (2.51)	Computer-based
Chelerythrine	BCL-2, BCL-X_L and BCL2-A1 (all ~10)	Fluorescence polarization
ABT-737 and ABT-263	BCL-2 (0.12), BCL-X_L (0.064) and BCL-W (0.024)	Structure–function analysis and NMR
ABT-199	BCL-2 (<0.01)	X-ray structure-based reverse engineering
Obatoclax (GX015-070)	BCL-2 (1.11), BCL-B (2.15) and MCL1 (2.90)	Computer-based
Gossipol (AT-101)	BCL-2 (0.28), BCL-W (1.4), BCL-B (0.16) and MCL1 (1.75)	NMR and fluorescence polarization
Apogossypol (ApoG2)	BCL-2 (0.64), BCL-X_L (2.8), BCL-W (2.1) and BCL-B (0.37)	NMR and fluorescence polarization
Sabutoclax (BI-97C1)	BCL-2 (0.32), BCL-X_L (0.31), MCL1 (0.20) and BCL2-A1 (0.62)	Structure-based design using ApoG2
TW37 (Gossipol analogue)	BCL-2 (0.29), BCL-X_L (1.11) and MCL1 (0.26)	Structure-based design using Gossipol
YC137	BCL-2 (6.43), BCL-X_L (2.21), BCL-B (3.1) and MCL1 (2.47)	Computer-based
Terphenyl-14	BCL-X_L (0.114)	α-helix-mimicking
MIM1	MCL1	High-throughput competitive screen (fluorescence polarization and displacement of FITC–MCL1 $SAHB_A$ from MCL1ΔNΔC)
BIM $SAHB_A$	BCL-2 (0.0032), BCL-W (0.0027), MCL1 (0.0107) and BCL2-A1 (0.0014)	Stapled peptide derived from a BH3 domain

FITC, fluorescein isothiocyanate; IC_{50}, the concentration of a drug giving a 50% inhibition of the activity of a target enzyme; SAHB, stabilized α-helix of BCL-2 domains.

bone marrow stroma may constitute a reservoir of cells that are resistant to BCL-2 inhibition alone. Combining BCL-2 inhibitors with targeted therapies (provided that they hit pathways to which cancer cells are 'addicted', as discussed above) may soon represent a treatment of choice for lymphoid malignancies. We expect that combination with new drugs, such as PI3K δ-isoform or Bruton tyrosine kinase (BTK) inhibitors that target the B-cell-receptor signalling pathway, may open the door to a new era of treatment for CLL, which might eventually replace classical chemotherapy. In particular, it was shown recently that an inhibitor, CAL-101, of the PI3K δ-isoform antagonizes stroma-induced protection of CLL cells by displacing CLL cells into the blood[60]. BCL-2 might also be a valid target in AML, as BH3 profiling assays established that a subset of AMLs (including chemoresistant AMLs) rely on BCL-2 for survival[65].

Multilocking security

BCL-2 homologues display complementary anti-apoptotic functions. The distinctive phenotypes of mice that are genetically deficient for each of the BCL-2 homologues[13] may not solely reflect the preferred expression of each of these survival proteins in a particular tissue. Mouse lymphocytes require not only BCL-2 (REF. 66) but also MCL1 (REF. 67) for survival. Moreover, MCL1 destruction was shown to occur early during the course of apoptosis compared with BCL-X_L, indicating that MCL1 and BCL-X_L differentially regulate cell death[68]. There are major differences in the half-lives of BCL-2L anti-apoptotic proteins: MCL1 and BCL-2A1 are labile proteins, whereas BCL-2 and BCL-X_L are more stable. Moreover, anti-apoptotic proteins have distinct, partially overlapping BH3-binding profiles (FIG. 1); when multiple BCL-2L proteins are co-expressed, only their combined inhibition is expected to trigger efficient cell death[69]. Thus, there is a need to develop distinct compounds that can target each one of the anti-apoptotic BCL-2 family members, and to understand how and when selective compounds can be used.

Targeting MCL1. MCL1 is recognized as an important therapeutic target in cancer[70]. It also restrains the pro-apoptotic activity of ABT-737 (REF. 71). MCL1 expression is tightly regulated by multiple mechanisms, including transcriptional, post-transcriptional and post-translational mechanisms (FIG. 4). Although all these mechanisms represent potential drug targets, currently the best approach seems to be the development of a direct MCL1 inhibitor. Recently, a stapled BH3 peptide inhibitor[72] and a small-molecule antagonist[73] were reported. The small-molecule antagonist binds to MCL1 with poor affinity, but it can release BAX from MCL1, trigger the death of MCL1-dependent cells and synergize with ABT-737 to kill cells that express both MCL1 and BCL-X_L[73] and it might serve as a prototype for the development of next-generation small-molecule MCL1 inhibitors.

The issue of therapeutic window is of particular importance when targeting MCL1, as bone marrow toxicity is expected: *Mcl1* ablation in mice results in severe bone marrow defects after 21 days[74]. However, AML cells in mice are more sensitive to *Mcl1* ablation compared with normal haematopoietic stem and progenitor cells[14]. These different cell death kinetics indicate that there should be a therapeutic window for anti-MCL1 drugs, before bone marrow toxicity becomes a substantial issue. Another caveat is the short half-life of MCL1, which implies that the levels of intact MCL1 protein will be rapidly renewed once the inhibitor has disappeared from

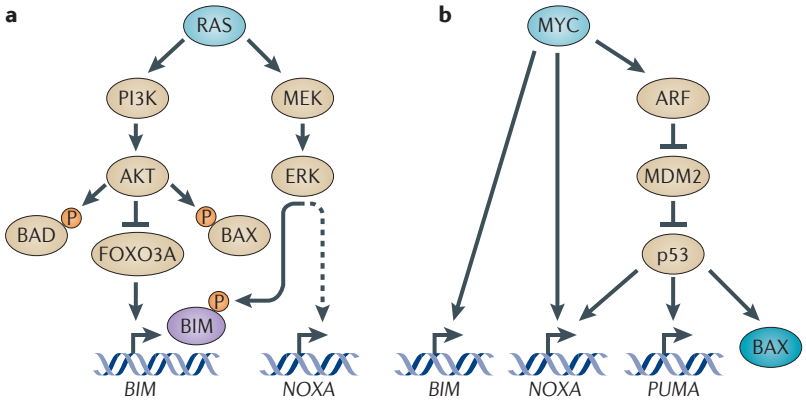

Figure 2 | **Regulation of BCL-2L proteins by oncogenic signalling.** RAS family members (part **a**) and MYC (part **b**) are shown as examples of upstream signalling pathways that have an impact on the BCL-2 family. **a** | RAS signalling can mitigate dependence on BCL-2-like (BCL-2L) proteins by inhibiting activator BH3-only proteins. For example, AKT-mediated inhibition of Forkhead box protein O3A (FOXO3A) can inhibit *BIM* transcription, and ERK1- or ERK2-induced phosphorylation (P) of BIM affects its stability, resulting in the inhibition of its function. AKT also regulates BAD[105] and BAX[106] activity by directly phosphorylating them. RAS has also been reported to promote ERK-dependent induction of *NOXA* transcription[107]. **b** | By contrast, MYC, which promotes apoptosis primarily by favouring mitochondrial outer membrane permeabilization[108], is thought to induce BCL-2L protein dependence. MYC directly regulates the transcription of *BIM*[17] and *NOXA*[109] and it triggers the p53 tumour suppressor pathway. p53 influences the BCL-2 network by inducing *PUMA* and *NOXA* transcription and by exerting a transcription-independent effect at the mitochondria that favours BAX activation[110].

tumour cells. Thus, the therapeutic window is likely to result from a 'fine tuning' between drug pharmacokinetics, schedule of administration and the kinetics of tumour cell death and MCL1 turnover, which could turn out to be challenging for drug discovery and clinical development. It is also possible that MCL1 might have functions other than its well-known anti-apoptotic role, which could explain why *Mcl1* deficiency in mice leads to peri-implantation embryonic lethality (a phenotype that is not clearly attributable to a defect in apoptosis regulation). If such functions are independent of the BH3-binding groove, *Mcl1* ablation experiments might have led to an underestimation of the potential therapeutic window of MCL1-specific BH3 mimetics.

Targeting BCL-X$_L$. Selective inhibition of BCL-2 seems to induce a similar antitumor activity in B cell malignancies compared with a dual BCL-2 and BCL-X$_L$ inhibitor. However, as BCL-X$_L$ binds a larger number of pro-apoptotic partners than other BCL-2L survival proteins (FIG. 1), the lack of BCL-X$_L$ inhibition could theoretically increase the risk of resistance owing to BCL-X$_L$ expression or restrict the spectrum of clinical activity to only BCL-2-dependent haematological malignancies. We have preliminary results in tumour cell lines *in vitro* that the sensitivity to a dual BCL-2 and BCL-X$_L$ inhibitor (such as ABT-737 or ABT-263), used as a single agent or in combination with cytotoxic drugs, is lost when BCL-X$_L$ is not inhibited (O.G. and S.D., unpublished observations). Thus, BCL-X$_L$ may have a major role in the control of cytotoxic-drug-induced apoptosis and it needs to be harnessed pharmacologically. A moderate peripheral thrombocytopenia of short duration could be clinically manageable, provided that novel BCL-X$_L$ inhibitors have optimized pharmacokinetic properties (with less inter-patient variation) compared with ABT-263; a more-predictable degree of treatment-induced thrombocytopenia will be easier to control. The schedule of administration should be finely adapted to allow platelet recovery in order to decrease the intensity of thrombocytopenia. Moreover, because the maintenance of cancer cell survival may rely on complexes — such as those containing BIM that might be structurally distinct (see below) from those containing BAK that maintain platelet survival — it is theoretically possible to dissociate antitumor activity from platelet toxicity with novel BCL-X$_L$ inhibitors. Designing selective BCL-X$_L$ inhibitors is achievable: recently, a high-throughput chemical screen, followed by a structure-guided medicinal chemistry campaign to improve affinity, led to the characterization of WEHI-539, which exclusively binds to, and functionally antagonizes, BCL-X$_L$[75]. The selectivity of this promising compound might stem from the nature of its binding mode, which relies on structural motifs that are unique to the BCL-X$_L$ P2 hydrophobic pocket.

Thinking about the combined use of selective BCL-2L inhibitors. As the synchronous inhibition of all anti-apoptotic BCL-2L proteins might have potent secondary effects, it is crucial to identify patients that will benefit from a selective inhibitor or from the combined use of distinct inhibitors. BH3 profiling (BOX 3) might be particularly useful as it can identify the specific dependency of tumour samples on selective subsets of anti-apoptotic proteins. It can predict an apoptotic response to ABT-263 (REF. 65). The triggering of MOMP by a NOXA BH3 peptide might also predict the likelihood of response to MCL1 inhibitors.

One might wonder why, given that BCL-2, BCL-X$_L$ and MCL1 add their effects to permit survival, inhibition of a single protein is sometimes sufficient to trigger cell death. Several mechanisms might account for the exclusive reliance on one BCL-2L protein. First, this could result from a default mechanism in which the overexpression of one BCL-2L protein during the early stages of oncogenesis decreases the selective pressure for additional anti-apoptotic mutations. This might explain the epistatic relationship that is found between high *MCL1* copy number and low BCL-X$_L$ expression[76]. Second, an apparent addiction to the survival activity of some BCL-2L proteins may come from their overexpression. Overexpression of BCL-2 was shown to increase the expression of pro-apoptotic proteins, such as BIM[77]. Likewise, BCL-X$_L$ actively contributes to the apoptotic response to its BH3-mimetic ligands, because it shifts the cellular equilibrium towards activated conformations of BAX by binding to BAX, and releases active BAX in response to BH3 mimetics[78]. Third, inhibition of one or two BCL-2L proteins may lead to inhibition of the third protein by dynamic responses: induction of NOXA (which inhibits MCL1) can be triggered by ABT-737 (REF. 79). This process involves the RB tumour suppressor protein and it might be lost in cancer cells if

RB expression is altered. Dynamic inhibitory responses might even be offset by ill-defined mechanisms whereby the inhibition of one BCL-2 homologue is compensated for by the upregulation of another. This compensation has been reported in lymphoma cells with acquired resistance to ABT-737, in which ABT-737 treatment leads to increased MCL1 expression[80]. BH3 profiling has shown that some human malignant myeloblasts acquire a dependence specifically on BCL-2, even though normal haematopoietic stem cells are (moderately) MCL1 dependent[65]. Thus, specific BCL-2L dependences might influence each other by many interconnected pathways. If specific BCL-2L dependencies interact dynamically, they might evolve during treatment, and selective inhibitors might need to be used one after the other.

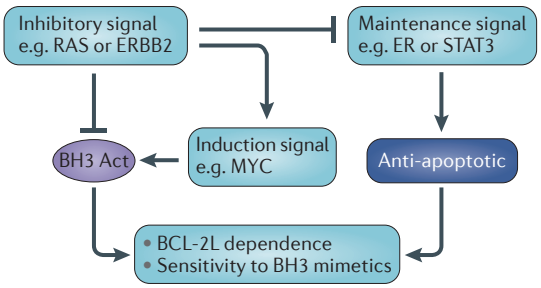

Figure 3 | **BCL-2L protein dependence as an output of complex, interconnected oncogenic signalling networks.** Sensitivity to BH3 mimetics is determined by the balance between signalling pathways that induce dependence on BCL-2-like (BCL-2L) proteins, those that maintain it and those that inhibit it. Activation of MYC can provide an induction signal by favouring the expression of BH3 activators. By contrast, oncogenic signals, such as the activation of RAS proteins or ERBB2 (also known as HER2), can provide an inhibitory signal by downregulating BH3-only activator proteins through the MAPK–ERK and AKT pathways. Oncogenic signals that favour the expression of individual anti-apoptotic proteins — for example, signal transducer and activator of transcription 3 (STAT3) leading to MCL1 expression as shown in FIG. 3, or oestrogen signalling leading to BCL-2 expression[111] — provide a maintenance signal. The net outcome is difficult to predict as these functionally distinct oncogene-induced signals regulate each other dynamically. Two types of pathway interactions are illustrated in the figure. In the first type of interaction the inhibitory signal activates the induction signal; that is, one oncogenic signal that promotes apoptotic resistance triggers a second oncogenic signal that promotes sensitivity. MYC, which functions downstream of RAS during oncogenesis[112] may contribute to render cancer cells with active RAS sensitive to BH3 mimetics. MYC was also shown to promote BIM expression downstream of ERBB2 (REF. 17). In the second type of interaction, the inhibitory signal counteracts a maintenance signal; that is, one oncogenic signal that promotes apoptotic resistance represses signals that are involved in maintaining the expression of anti-apoptotic proteins. As an example, the repression of oestrogen receptor (ER) signalling by the ERBB2 pathway was shown to limit BCL-2 expression in breast cancer cells[111]. The ERBB2 pathway may thus limit the usefulness of selective BCL-2 inhibitors as single agents and it might be appropriate to combine them with ERBB2 inhibitors.

Lock jamming: limitations and challenges

Terra incognita at subcellular membranes. Subcellular membranes contribute to BCL-2L protein-mediated cell death. Indeed, membrane insertion and oligomerization of BAX are rate-limiting steps in MOMP[81]. Activation of BAX by the cleaved BH3-only protein truncated BID (tBID) is particularly efficient when membranes are present. Moreover, survival proteins interfere with this process at subcellular membranes: BCL-X_L is recruited by tBID to membranes, where it prevents BAX-mediated membrane permeabilization[82]. From these data, an 'embedded together' model was proposed, which emphasizes that binding to protein partners and to membranes causes dynamic conformational and functional changes in the BCL-2 network (FIG. 1). This model depicts BCL-X_L as a dominant-negative protein that inhibits the activation and oligomerization of BAX: this is mostly consistent with the structural similarities of these two functionally opposed proteins. BAD prevents BCL-X_L-mediated inhibition of BAX in the membranes, but it is not yet clear whether BH3 mimetics equally suppress all the effects of BCL-X_L on BAX, as biophysical and structural studies of BH3-domain interactions with anti-apoptotic BCL-2 proteins were carried out in solution using pure BH3 peptides and recombinant BCL-2 homologues for which the hydrophobic carboxyl terminus was deleted to permit purification.

Several studies have now examined the interactions between full-length proteins in the presence of membranes and/or in intact cells[83]. One such study revealed an enhanced resistance of membrane-inserted BAX–BCL-X_L (and BAK–BCL-X_L) complexes to ABT-737, compared with tBID–BCL-X_L complexes[84]. This might be due to additional regions of interaction within each membrane-inserted partner. Understanding the mechanism involved in this resistance is important because inhibition of BAX oligomerization by BCL-2 proteins in membranes may represent a robust (and sufficient) way to permit cell survival despite multiple signals leading to BAX activation. As ABT-737 can inhibit BAX–BCL-X_L interactions in certain circumstances[78], BCL-X_L may in fact interact with BAX in multiple ways, and not necessarily in membranes. Consistent with this, BCL-X_L promotes the retrotranslocation of BAX from the mitochondrial outer membrane back to the cytosol. This mechanism, which may prevent the accidental insertion of BAX into proximate membranes, or its activation by nearby factors, is not fully characterized but it seems to rely on interactions that are ABT-737 sensitive[85,86].

The ability of BAX to interact with BCL-X_L in distinct complexes that differ in their sensitivities to ABT-737 extends to BIM. Indeed, BIM interactions with BCL-X_L (and possibly with BCL-2) at the mitochondria in live cells are refractory to ABT-737 treatment[87,88]. One plausible explanation is that additional uncharacterized domains are involved in these interactions. The resistance of mitochondrial BIM complexes to ABT-737 might also ensue from the fact that the conformations of the components of these complexes are changed in membranes: ABT-737 can efficiently compete with BIM for binding to BCL-X_L in the cytosol[87]. The types of changes

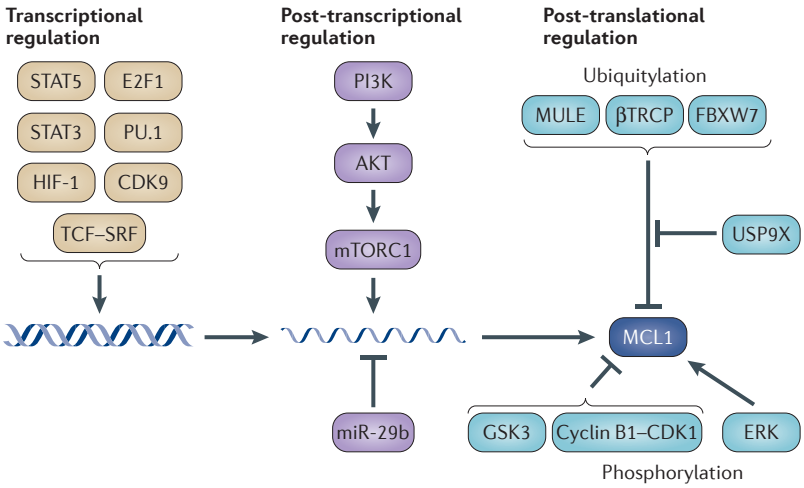

Figure 4 | **Transcriptional, post-transcriptional and post-translational regulation of MCL1 in cancer cells.** *MCL1* transcription is regulated by numerous transcription factors and their regulators, including signal transducer and activator of transcription 5 (STAT5) and hypoxia-inducible factor 1 (HIF1), that are downstream targets of key oncogenic pathways[113,114]. *MCL1* mRNA is subject to regulation by microRNAs (miRNAs), most notably by miR-29b[8], and is inhibited by the endoplasmic-stress-associated phosphorylation of translation initiation factor 2α (eIF2α; not shown)[115]. The PI3K–AKT–mammalian target of rapamycin complex 1 (mTORC1) pathway stimulates *MCL1* translation[116]. MCL1 is a short-lived protein[68,117], the stability of which is controlled by the ubiquitin–proteasome pathway. Three distinct E3 ubiquitin ligases (MULE, βTRCP and FBXW7) have been shown to contribute to MCL1 ubiquitylation leading to its degradation[118–121]. This is counterbalanced by the ubiquitin-specific protease, USP9X, which catalyses the removal of ubiquitin chains from MCL1 (REF. 122). Alterations of FBXW7 and USP9X expression have been reported in various tumours[121,122]. Phosphorylation of MCL1 on Thr163 by ERK increases the half-life of MCL1 (REF. 123), whereas the phosphorylation of Ser159 by glycogen synthase kinase 3 (GSK3) promotes MCL1 ubiquitylation and degradation[124]. Proteasomal degradation of MCL1 has also been shown to be increased by the phosphorylation of Thr92 by the cyclin B1–cyclin-dependent kinase 1 (CDK1) complex during mitosis[125]. SRF, serum response factor; TCF, ternary complex factor.

in BIM binding to BCL-X_L that are induced by membrane interaction are unknown and seem to be specific to BIM binding to BCL-X_L, as the interactions of tBID and BAD with BCL-2 or BCL-X_L at the mitochondria of live cells remain sensitive to ABT-737.

Novel biomarkers are needed. Arguably, picking the appropriate selective BH3 mimetic and choosing the efficient combination of BH3 mimetics with other drugs will only be possible if predictive biomarkers are available. Detecting a lack of expression of BAX or BAK might be useful to diagnose resistance. BAX and BAK loss-of-function has been implicated in the resistance to MCL1 inhibition in leukaemic cells from patients with AML. Notably, *BAX* frameshift mutations are present in ~50% of colon carcinomas in patients with a DNA mismatch repair defect[89], and epigenetic silencing of *BAX* was reported in 6% of human glioblastoma samples[90].

Predictors of response could also be derived from computational models of apoptotic sensitivity[91]. The data described above indicate that the most appropriate models should integrate the relative sensitivities of each complex to a given BH3 mimetic (which will depend on the existence of differing conformations of a single protein–protein interaction and/or on subcellular localization). It is also important to note that there are pools of BCL-2 family proteins in the endoplasmic reticulum and the Golgi[92] and that their contribution to MOMP in response to BH3 mimetics is not yet understood. Considerations that need to be taken into account include the stability of free and complexed proteins of differing conformations, the distinct synthesis rates of each protein (as the inhibition of *de novo* complex formation may occur differently from the displacement of preformed complexes) and the pathways that regulate BAX and BAK activation and activity[93,94].

BH3 profiling can provide a quick and reliable companion test if it can be carried out on available clinical samples (which might be technically challenging for solid tumour biopsy samples). The established predictability of this test advocates that the multiple signalling networks that determine BCL-2L dependence converge on the formation of BH3-peptide-sensitive mitochondrial complexes (peripherally bound or membrane-inserted). We think that computational models will be particularly useful if they can: confirm the results of BH3 profiling; define the number of mitochondrial complexes that need to be disrupted in BH3 profiling assays to predict a clinical response; and further refine the predictability of BH3 profiling.

Looking beyond apoptosis. The global impact of BCL-2L proteins on the cancer cell phenotype need to be better understood: anti-apoptotic BCL-2L proteins interact with, and regulate, proteins that are involved in biological functions beyond apoptosis. Interactions of BCL-2 with beclin[95] or RAD9 (REF. 96) rely on the BH3-binding site and thus directly plug the whole BH3-connected BCL-2 network into autophagy or DNA damage response regulation. The induction of autophagy[95] (and potentially of DNA repair) by BH3 mimetics may have consequences on their clinical efficacy that need to be fully understood. Other interactions involving BCL-2 homologues, but not their BH3-binding site, also require investigation[97]. For example, BCL-2 proteins regulate calcium homeostasis, at least in part, by interacting through their BH4 domain with the inositol triphosphate receptor (IP3R)[98]. These interactions are not inhibited by ABT-737, and might even be enhanced by it because the absence of BAX and BAK increases the interactions of BCL-2 proteins with IP3R[99]. BH3-mimetic-resistant regulation of calcium homeostasis, and the involvement of BCL-2 proteins in cell cycle control or cytoskeletal dynamics[97,100], may influence the biology of treated cells and account for unwanted side effects. Thus, a global description of the biological effects of BH3 mimetics needs to be carried out.

Conclusions

Genetic, biochemical and functional data have provided a sufficiently solid rationale to target BCL-2L proteins in cancer therapy, and to invest in the development of small-molecule inhibitors. The use of some of these molecules in preclinical studies and clinical trials confirm their potential as cancer therapeutics and as a tool to investigate the mechanisms that determine

BCL-2L dependence. Filling in the gaps in our structural understanding of BCL-2 family members, improving our comprehension of the BCL-2 network connectivity and describing the biological effects of inhibitors beyond cell death induction will be necessary to fully exploit BCL-2L dependence. This will allow the design of predictive biomarkers and companion tests that are necessary for the combined, or sequential, use of highly selective inhibitors of anti-apoptotic BCL-2 proteins in a fully implemented approach to personalized medicine.

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Acknowledgements
We apologize for our inability to cite all the contributing primary literature owing to space constraints. We wish to thank D. Andrews, J. Hickman, D. Huang, A. Letai, J.-C. Martinou, and A. Strasser for fruitful discussion, and all members of our laboratories for their constant support and seemingly unbreakable enthusiasm. P.J. thanks his friends from Bénodet (Finistère, France) for their support and warm welcome, especially during the initial stages of writing this manuscript.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
BCL-2 family database: http://bcl2db.ibcp.fr/site

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