From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors

Avi Ashkenazi[1], Wayne J. Fairbrother[1], Joel D. Leverson[2] and Andrew J. Souers[2]

Abstract | Members of the B cell lymphoma 2 (BCL-2) gene family have a central role in regulating programmed cell death by controlling pro-apoptotic and anti-apoptotic intracellular signals. In cancer, apoptosis evasion through dysregulation of specific BCL-2 family genes is a recurring event; accordingly, selective inhibition of specific anti-apoptotic BCL-2 family proteins represents an exciting therapeutic opportunity. A combination of nuclear magnetic resonance (NMR)-based screening and structure-based drug design has yielded the first bona fide BCL-2 homology 3 (BH3) mimetics, including the BCL-2 and BCL-X_L dual antagonist navitoclax, which is the first BCL-2 family inhibitor to show efficacy in patients with cancer. Clinical experience with navitoclax prompted the generation of the highly selective BCL-2 inhibitor venetoclax, which is now approved in the United States for the treatment of patients with chronic lymphocytic leukaemia with 17p deletion who have received at least one prior therapy. Recent advances have also been made in the development of potent and selective inhibitors of BCL-X_L and myeloid cell leukaemia 1 (MCL1), which are additional BCL-2 family members with established anti-apoptotic roles in cancer. Here we review the latest progress in direct and selective targeting of BCL-2 family proteins for cancer therapy.

Type I programmed cell death, also known as apoptosis, was first defined in the early 1970s[1] and is a tightly controlled process that is crucial for tissue homeostasis. The ability to avert apoptosis is a key characteristic of cancer cells[2]. Apoptosis can be triggered by extracellular or intracellular stimuli; in recent decades, the molecular mechanisms underlying the intrinsic[3] and extrinsic[4] apoptotic pathways have been elucidated[5]. The extrinsic pathway is activated in response to the binding of death-inducing ligands to cell-surface death receptors. Cell-intrinsic apoptotic stimuli include DNA damage, growth factor deprivation and oxidative stress. Initiation of the intrinsic pathway (FIG. 1) leads to mitochondrial depolarization, which allows the release of cytochrome *c*. In turn, cytochrome *c* binds to apoptosis protease-activating factor 1 (APAF 1) and procaspase 9, generating an intracellular 'apoptosome' that activates caspase 9. Disrupted mitochondria also produce second mitochondria-derived activator of caspase (SMAC; also known as DIABLO), which releases caspase 3 from X-linked inhibitor of apoptosis (XIAP)-mediated inhibition. Both the extrinsic and intrinsic apoptosis pathways converge on caspase 3 and caspase 7, which drive the terminal events of apoptosis[5].

The B cell lymphoma 2 (BCL-2) gene family encodes more than 20 proteins that regulate the intrinsic apoptosis pathway, and are fundamental to the balance between cell survival and death[6–8]. *BCL2* was initially discovered as part of the t(14;18) chromosomal translocation, which occurs in patients with follicular lymphoma and diffuse large B cell lymphoma (DLBCL), and leads to elevated *BCL2* transcription[9,10]. Although originally believed to act as a classical growth-driving oncogene, it was later shown that *BCL2* instead promotes malignant cell survival by attenuating apoptosis[11,12]. Members of the BCL-2 family are grouped together, as they contain up to four conserved BCL-2 homology (BH) regions. The multiregion (BH1–4) anti-apoptotic proteins BCL-2, BCL-X_L (also known as BCL-2L1), BCL-W (also known as BCL-2L2), myeloid cell leukaemia 1 (MCL1) and A1 (also known as BCL-2A1) antagonize pro-apoptotic BH3-only proteins, and they inhibit the essential apoptosis effectors BCL-2 antagonist killer 1 (BAK) and BCL-2-associated X protein (BAX)[13]. *BCL2* dysregulation results in the overexpression of the anti-apoptotic protein BCL-2, which alters the balance between pro-apoptotic and anti-apoptotic members of the BCL-2 family[14]. The resulting inhibition of apoptosis is thought to lead to chemoresistance[15] and

[1]*Genentech, 1 DNA Way, South San Francisco, California 94080, USA.*
[2]*AbbVie, 1 North Waukegan Road, North Chicago, Illinois 60064, USA.*

Correspondence to A.A.
ashkenazi.avi@gene.com

doi:10.1038/nrd.2016.253
Published online 17 Feb 2017

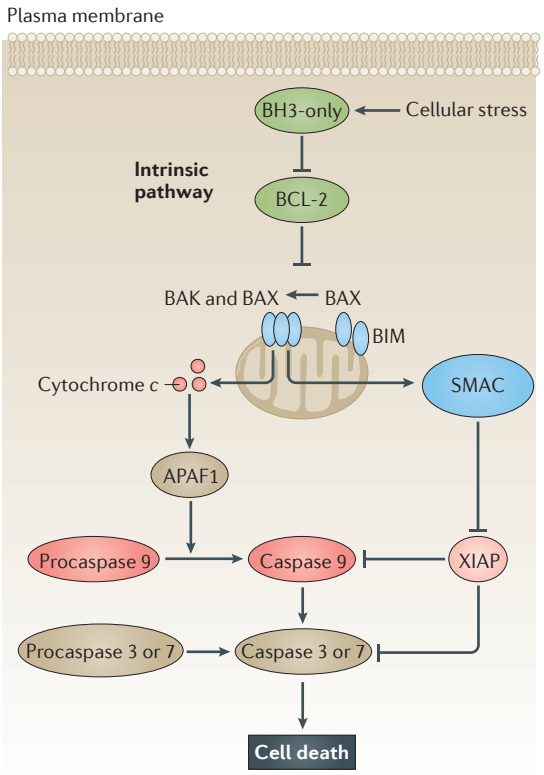

Figure 1 | **The intrinsic apoptosis pathway.** Following activation of the intrinsic pathway by cellular stress, pro-apoptotic BCL-2 homology 3 (BH3)-only proteins inhibit the anti-apoptotic proteins B cell lymphoma 2 (BCL-2), BCL-X_L, BCL-W and myeloid cell leukaemia 1 (MCL1). The subsequent activation and oligomerization of the pro-apoptotic proteins BCL-2 antagonist killer 1 (BAK) and BCL-2-associated X protein (BAX) results in mitochondrial outer membrane permeabilization (MOMP). This results in the release of cytochrome *c* and second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) from the mitochondria. Cytochrome *c* forms a complex with procaspase 9 and apoptosis protease-activating factor 1 (APAF1), which leads to the activation of caspase 9. Caspase 9 then activates procaspase 3 and procaspase 7, resulting in cell death. Inhibition of this process by anti-apoptotic BCL-2 proteins occurs via sequestration of pro-apoptotic proteins through binding to their BH3 motifs. BIM, BCL-2-interacting mediator of cell death; XIAP, X-linked inhibitor of apoptosis.

BH3 mimetics
Small molecules or modified peptides that mimic the action of B cell lymphoma 2 homology 3 (BH3)-only proteins, which insert their BH3 motifs into the hydrophobic groove of the anti-apoptotic B cell lymphoma 2 proteins and inhibit their functional activity, thereby inducing apoptosis.

Alanine scanning mutagenesis
The individual and systematic replacement of amino acids with alanine as a means of assessing the impact of each amino acid on the binding energy of a protein–protein interaction.

Hotspots
Groups of amino acids that make a major contribution to the binding energy of a protein–protein interaction.

has been identified in many cancers, including haematological malignancies[16] (such as multiple myeloma, chronic lymphocytic leukaemia (CLL), acute lymphocytic leukaemia (ALL), acute myeloid leukaemia (AML), myelodysplastic syndrome and myeloproliferative neoplasms) and solid tumours (such as breast cancer[17], lung cancer[18], melanoma[19] and mesothelioma[20]).

In contrast to anti-apoptotic proteins, BH3-only proteins such as BCL-2-interacting mediator of cell death (BIM) and BH3-interacting domain death agonist (BID) propagate intrinsic cell-death signals by suppressing anti-apoptotic proteins at the mitochondria and endoplasmic reticulum[13], and potentially also through direct stimulation of BAX and BAK[6]. Oligomerization of the pro-apoptotic proteins BAX and BAK results in mitochondrial outer membrane permeabilization (MOMP) and the aforementioned events, which culminate in apoptosis (FIG. 1). Anti-apoptotic BCL-2 proteins inhibit this process by sequestering pro-apoptotic proteins through binding to their BH3 motifs. As BCL-2-mediated resistance to intrinsic apoptosis is a hallmark of malignancy, targeting the anti-apoptotic BCL-2 proteins is an attractive therapeutic strategy in cancer.

Early efforts to target these proteins were based primarily on screening libraries of natural products and yielded several compound classes, as reviewed previously[21]. Members of these classes have shown activity against multiple BCL-2 family proteins. Although some of these compounds have progressed into cancer clinical trials, none has shown sufficient efficacy to warrant approval. More recently, combinations of nuclear magnetic resonance (NMR)-based screening, fragment chemistry and structure-based drug design have been implemented to more selectively target the anti-apoptotic proteins within the BCL-2 family. These efforts have produced the first bona fide BH3 mimetics; these compounds are capable of potently and specifically disrupting interactions between anti-apoptotic and pro-apoptotic BCL-2 family proteins.

Since the seminal discovery of the BCL-2 and BCL-X_L inhibitor ABT-737 and the initial clinical evaluation of the orally bioavailable compound navitoclax (also known as ABT-263), highly selective inhibitors of BCL-2, BCL-X_L and MCL1 have been generated. To date, there have been hundreds of peer-reviewed publications that have used these invaluable research tools. Moreover, the first BCL-2-selective inhibitor venetoclax (also known as ABT-199) has delivered highly promising clinical activity that could alter the course of treatment for specific haematological diseases. This Review considers recent advances in the rational design of small molecules that target the BCL-2 family to activate the intrinsic apoptotic pathway, with a focus on agents that directly and selectively inhibit anti-apoptotic proteins.

Rationally designed, selective BH3 mimetics

The 3D structures of anti-apoptotic proteins in the BCL-2 family share a common motif that comprises two hydrophobic α-helices surrounded by six or seven amphipathic α-helices. Four of the latter form a hydrophobic groove that serves as the binding site for pro-apoptotic proteins. Alanine scanning mutagenesis has shown that high-affinity binding of pro-apoptotic proteins to BCL-2 and BCL-X_L is largely mediated by protein–protein interactions in the P2 and P4 hydrophobic pockets[22]. These pockets become occupied by hydrophobic residues of the BH3 motifs of pro-apoptotic proteins[22,23] (FIG. 2). The binding hotspots within BCL-2 and BCL-X_L provide a structural framework for the rational design of selective BH3 mimetics. In addition, a conserved electrostatic interaction between an Arg in the anti-apoptotic proteins and an Asp in the pro-apoptotic proteins (such as BAX and BAK) is crucial for protein–protein interactions between BCL-2 family

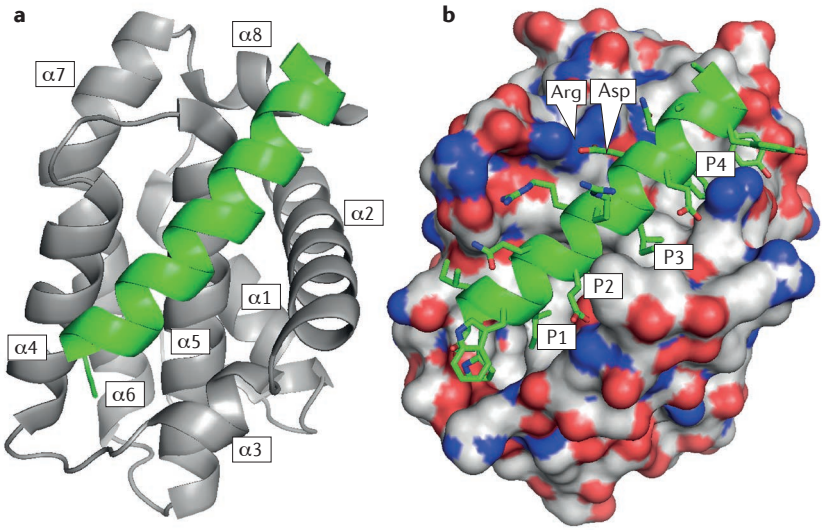

Figure 2 | **X-ray co-crystal structure of a B cell lymphoma X_L molecule complexed with a BCL-2-interacting mediator of cell death peptide. a** | α-helices of BCL-X_L (grey) form a hydrophobic groove that binds the B cell lymphoma 2 (BCL-2) homology 3 (BH3)-only protein BCL-2-interacting mediator of cell death (BIM) (green) **b** | Ile, Leu, Ile and Phe residues of the BH3-only protein BIM (green) bind within the P1, P2, P3 and P4 hotspots of BCL-XL , respectively. An Asp residue of BIM and other BH3-only pro-apoptotic proteins binds to a conserved Arg residue in BCL-X_L and other BCL-2 family anti-apoptotic proteins. This structure is described in REF. 106 and can be found in the Research Collaboratory for Structural Bioinformatics Protein Data Bank: entry 4QVF.

members (FIG. 2). Although not universally targeted by BH3 mimetics, this interaction has a key role in the high affinity of several selective BCL-2 family inhibitors.

ABT-737 and navitoclax: the first BH3 mimetics

In 1996, a paradigm-shifting technique known as structure–activity relationships (SAR) by NMR was developed to facilitate the identification of ligands for difficult protein targets[24]. This technique was essential for the generation of BH3 mimetics such as ABT-737 (TABLE 1), as it used high-throughput NMR to identify weakly yet cooperatively binding ligands of the BCL-X_L protein. Specifically, the first-site and second-site ligands bound respectively with affinities of approximately 0.3 mM and 4.0 mM to the P2 and P4 pockets of BCL-X_L. The successful chemical linking of these fragments resulted in a molecule with a binding affinity in the micromolar range and thus considerably increased activity compared with each fragment alone. Successive rounds of structure-based drug design were subsequently carried out to optimally occupy the key P2 and P4 hotspots of BCL-2 and BCL-X_L (FIG. 3) while simultaneously decreasing unwanted binding to albumin[25]. These efforts culminated in the discovery of ABT-737, which exhibited single-agent antitumour activity in preclinical models of lymphoma and small-cell lung carcinoma (SCLC), as well as in primary patient-derived CLL samples[26].

ABT-737 has been used extensively for studying and defining apoptosis biology, and its 2005 disclosure in *Nature*[26] has been cited more than 2,000 times. Ultimately, the fact that ABT-737 had poor oral absorption prompted the creation of its orally available second-generation analogue, navitoclax (TABLE 1). Navitoclax binds with high affinity to BCL-2 and BCL-X_L, with lower affinity to BCL-W and with very low affinity to MCL1 (REFS 26,27). In preclinical studies, navitoclax inhibited tumour growth when used as a monotherapy in SCLC xenograft models[27,28] and when used in combination with standard-of-care agents in both solid and haematological tumour cell lines[27,29,30], which provided a rationale for its clinical investigation. In phase I studies, navitoclax proved to be effective in B cell malignancies[31], including relapsed or refractory CLL, both as monotherapy[32] and in combination with fludarabine, cyclophosphamide and rituximab, or with bendamustine and rituximab[33]. Results of a phase II study showed that navitoclax was also effective as a first-line treatment in CLL when combined with rituximab[34]. During the course of preclinical development, navitoclax was found to cause temporary yet substantial decreases in platelet counts in animal studies[35,36]. This was subsequently determined to result from on-target inhibition of a critical function of BCL-X_L in maintaining the lifespan of circulating platelets[35,36]. In the clinic, thrombocytopenia was found to be a major dose-limiting toxicity for navitoclax, particularly in the single-agent setting. Although thrombocytopenia limited the use of navitoclax in patients, the observed efficacy provided seminal proof-of-concept for the basic strategy and underscored the therapeutic potential of selective BCL-2 family inhibitors.

Since the disclosures of ABT-737 and navitoclax, several inhibitors of BCL-2 and BCL-X_L that contain the acylsulfonamide pharmacophore or isosteres thereof have been reported[37]. BM-1197 consists of a *N*-phenylbenzenesulfonamide-based scaffold that is linked to a P4-binding moiety (FIG. 3), which is similar to the structure of navitoclax (TABLE 1). It binds to BCL-2 and BCL-X_L with subnanomolar affinity, and disrupts interactions between pro-apoptotic and anti-apoptotic BCL-2 family proteins to induce BAX–BAK-dependent apoptosis in SCLC cell lines. Long-lasting tumour regression was achieved in SCLC mouse xenograft models following intravenous administration on a daily or weekly schedule[38]. S44563 is another dual inhibitor of BCL-2 and BCL-X_L that has shown promising antitumour activity in patient-derived uveal melanoma xenografts (TABLE 1). This compound is effective as a single agent in xenograft models and shows robust combination activity when administered with fotemustine[39]. BCL2-32 was also recently described as a nanomolar-range binder of both BCL-2 and BCL-X_L (REF. 40). This compound has shown promising antitumour activity in ALL models as a single agent and in DLBCL models in combination with standard chemotherapy agents (TABLE 1). In these models, BCL2-32 was administered intermittently to allow for full platelet recovery between doses. AZD4320 is another dual BCL-2–BCL-X_L inhibitor that was recently disclosed as a clinical candidate (TABLE 1). AZD4320 was reported to induce complete and sustained tumour regression (lasting longer than 24 days) in the RS4;11 ALL model following a single intravenous dose, and there was a recovery of platelets within 72 hours[41]. Intravenous dosing and scheduling are reported strategies to manage thrombocytopenia

Pharmacophore
The collective molecular features of a molecule that define the requirements for binding to a biological target and triggering a biological response.

Table 1 | **Characteristics of small molecules that target B cell lymphoma 2 family proteins**

Compound	Targets	Affinity	Refs
BCL-2 inhibitors			
ABT-737	BCL-2, BCL-X_L and BCL-W	Subnanomolar to nanomolar	26
Navitoclax (also known as ABT-263)	BCL-2, BCL-X_L and BCL-W	Subnanomolar to nanomolar	27
BM-1197	BCL-2 and BCL-X_L	Subnanomolar	38
S44563	BCL-2 and BCL-X_L	Nanomolar	39
BCL2-32	BCL-2 and BCL-X_L	Nanomolar	40
AZD4320	BCL-2 and BCL-X_L	≤1 nM	41
Venetoclax (also known as ABT-199)	BCL-2	Subnanomolar	43
S55746 (also known as BCL201)	BCL-2	Nanomolar	60
BCL-X_L inhibitors			
WEHI-539	BCL-X_L	Subnanomolar	65
A-1155463	BCL-X_L	Subnanomolar	66
A-1331852	BCL-X_L	Subnanomolar	64
MCL1 inhibitors			
UMI-77	MCL1	Nanomolar	94
Compound 9	MCL1	Nanomolar	95
A-1210477	MCL1	Subnanomolar	97
Compound 34	MCL1	Nanomolar	99
S63845	MCL1	Subnanomolar	103
AMG176	MCL1	Nanomolar	104

BCL, B cell lymphoma; MCL1, myeloid cell leukaemia 1. Selected structures are shown in FIG. 4.

at the preclinical level for some of these compounds. Nevertheless, differentiation between these dual inhibitors may require clinical evaluation.

Monoselective BCL-2 inhibitors

Venetoclax. The protein BCL-2 has been shown to have a dominant role in promoting cell survival in multiple lymphoid malignancies[11,42]. In the context of clinical experience with navitoclax, this gave rise to the hypothesis that a BCL-2-selective inhibitor could mitigate the dose-limiting thrombocytopenia observed with dual BCL-2–BCL-X_L inhibition while maintaining efficacy against lymphoid malignancies. Subsequent research efforts led to the development of the first highly selective inhibitor of BCL-2, venetoclax[43] (TABLE 1). Venetoclax was shown to bind to its target BCL-2 with high affinity and selectivity, and to induce BAX–BAK-dependent apoptosis. This potency and selectivity was achieved by careful examination of an X-ray crystal structure of BCL-2 in complex with a smaller acylsulfonamide compound that lacks the P4 pocket-binding thiophenyl moiety of navitoclax[43]. Within the crystal lattice, a Trp side chain from a neighbouring BCL-2 protein was found to be inserted into the P4 pocket of the small molecule-bound BCL-2 protein; the indole side chain of this intercalating amino acid thus captured hydrophobic contacts in P4 while also forming an electrostatic interaction with a BCL-2-specific Asp residue. This observation provided the rationale for chemical modifications of the small-molecule core to allow for efficient binding in the P4 hotspot along with the formation of a BCL-2-selective hydrogen bond similar to that observed with the intercalating Trp residue[43]. Ultimately, this design hypothesis led to the development of the highly potent and selective compound that is now known as venetoclax. The functional potency of venetoclax was demonstrated using patient-derived primary CLL cells, and was validated further with human DLBCL, follicular lymphoma, mantle cell lymphoma, AML and ALL cell lines. Administration of venetoclax to the BCL-2-dependent ALL cell line RS4;11 resulted in multiple apoptotic hallmarks[43]. Furthermore, *in vivo* assessments of single-agent venetoclax in human xenograft models of B cell lymphoma with the t(14;18) translocation and models of ALL showed dose-dependent tumour inhibition and growth delay[43]. In xenograft models of non-Hodgkin lymphoma (NHL) and mantle cell lymphoma, venetoclax increased the efficacy of immunotherapy and chemotherapy agents, including the combination of rituximab and bendamustine, and the combination of rituximab, cyclophosphamide, hydroxydaunomycin, vincristine and prednisolone (R-CHOP)[43]. Notably, venetoclax was effective at clearing systemic haematological tumours *in vivo* while inducing an apoptotic response[44]. Compared with navitoclax, venetoclax had a markedly diminished effect on platelets *ex vivo* and *in vivo*, which is consistent with its substantially reduced inhibition of BCL-X_L (REF. 43).

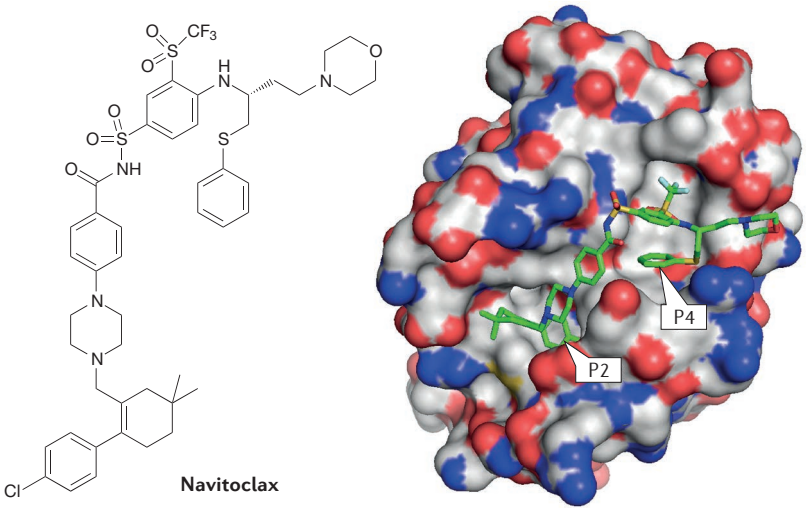

Figure 3 | **X-ray co-crystal structure of navitoclax bound to B cell lymphoma X_L.** Pharmacophore binding in the P2 and P4 hotspots is indicated[43].

Following the observation of venetoclax cytotoxicity in primary samples of human CLL, a recently completed first-in-human clinical study of venetoclax in patients with relapsed or refractory CLL (ClinicalTrials.gov identifier: NCT01328626) has provided encouraging data that support the concept of venetoclax monotherapy[45] (TABLE 2). Among the first three patients treated with a single dose of venetoclax, a rapid reduction in palpable lymphadenopathy was observed within 24 hours, and of two patients who had pretreatment lymphocytosis, both had a greater than 95% reduction in peripheral blood lymphocytosis. These antileukaemic effects were much greater and occurred more quickly than results reported previously for a single dose of navitoclax. Among a larger patient cohort, an objective response rate (ORR) of 79% was achieved, including response rates of 71–79% in poor-prognosis groups. Complete responses occurred in 20% of patients, 5% of whom were negative for minimal residual disease (MRD)[45]. These data prompted a pivotal phase II study of high-risk patients with relapsed or refractory CLL who had a deletion of the 17p chromosomal region (del(17p); NCT01889186)[46]. Achievement of the primary end point in the latter study has led to the first successful US Food and Drug Administration (FDA) registration for venetoclax. Venetoclax was also the first agent to demonstrate activity in patients previously treated with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib or the phosphoinositide 3-kinase-δ (PI3Kδ) inhibitor idelalisib[47] (NCT02141282; TABLE 2).

Studies of venetoclax in combination with rituximab[48], obinutuzumab[49] and rituximab plus bendamustine[50] in patients with relapsed or refractory CLL have also shown significant antitumour activity (TABLE 2). In a phase Ib study (NCT01682616) of venetoclax in combination with rituximab, an ORR of 86% was achieved, and 47% of patients showed a complete response or complete response with incomplete bone marrow recovery (CRi). MRD negativity was achieved in 74% of patients who showed a complete response or CRi, and in 55% of patients overall. Although the data for combinations of venetoclax with obinutuzumab[49] and with rituximab plus bendamustine[50] are less mature, the ORRs in patients with refractory or first-line CLL are reported to be 100%. The deep and durable responses, and the high rates of MRD negativity, for various venetoclax combination regimens are very promising and may offer new treatment options that achieve better disease control in patients with CLL than do current therapies.

Venetoclax has also shown favourable antitumour activity and an acceptable safety profile in early trials in patients with other haematological malignancies, including NHL[51,52], AML[53,54] and multiple myeloma[55,56] (TABLE 2). Objective responses and complete responses following single-agent administration have been observed across these indications. In addition, the combination of venetoclax with standard-of-care agents such as rituximab plus bendamustine, R-CHOP, CHOP plus obinutuzumab (G-CHOP), hypomethylating agents, low-dose cytarabine and bortezomib plus dexamethasone have demonstrated encouraging levels of antitumour activity in their respective disease settings.

The extensive thrombocytopenia associated with navitoclax was circumvented by the selectivity of venetoclax. In addition, the substantially higher potency of the latter molecule conferred a rapid reduction in tumour burden in patients with CLL; this observation was linked to the risk of developing tumour lysis syndrome (TLS) during the initial weeks of treatment, a risk that was associated with multiple factors, including tumour burden and comorbidities. Among patients treated in the early CLL trials of venetoclax, two deaths and an episode of acute renal failure attributable to TLS occurred. Subsequently, an analysis was performed on 135 patients with CLL who were treated with venetoclax in four clinical studies to identify pretreatment risk factors for TLS, and to establish and evaluate a new dosing schedule and patient monitoring[57]. The dosing regimen was then modified to include a gradual 5-week ramp-up, and prophylactic and monitoring measures were implemented on the basis of tumour burden. Since these amendments, no clinical TLS has been reported, and the overall risk of TLS has been effectively mitigated[57].

S55746

The second BCL-2-selective inhibitor to enter clinical development is an orally administered compound called S55746 (also known as BCL201 and Servier-1). Although the structure of S55746 has not yet been publicly disclosed, a compound with the representative structure shown in FIG. 4 has been reported to demonstrate efficient killing of BCL-2-dependent tumour cells *in vitro* and has shown *in vivo* efficacy in mouse models following oral dosing[58]. This class of BCL-2-selective inhibitors was derived from a series of tetrahydroisoquinoline amide-substituted phenyl pyrazoles that, in turn, originated from a modestly potent and selective high-throughput screening (HTS) hit[59]. A phase I dose-escalation study is currently assessing the safety and tolerability of S55746 in patients with refractory or relapsed CLL and those with B cell NHL (ISRCTN Registry, ISRCTN04804337)[60]. Results are pending, and

Table 2 | **Clinical trials of venetoclax monotherapy or combination therapy in certain lymphomas, leukaemia or multiple myeloma**

Clinical trial	Number of patients	Indication	Regimen	Response	Comments	Refs
Phase I (NCT01328626)	56 (60*)	Relapsed or refractory CLL	Venetoclax 150–1,200 mg per day (up to 400 mg per day in a modified dose ramp-up schedule*)	• ORR: 79% • CR: 20% • MRD negative: 5% • PFS at 15 months†: 69%	Clinical TLS in 3 of 56 patients; 1 death (*TLS in 0 of 60 patients; no deaths)	45
Phase II (NCT01889186)	107	Relapsed or refractory CLL with del(17p)	Venetoclax 20–400 mg per day in a dose ramp-up schedule	• ORR: 79% • CR or CRi: 8% • MRD negative: 17%	11 deaths: 7 due to progressive disease and 4 due to adverse events	46
Phase II (NCT02141282)	54	Relapsed or refractory CLL§	Venetoclax 20–400 mg per day in dose ramp-up schedule; patients in arm A had previously received ibrutinib; patients in arm B had previously received idelalisib	Ibrutinib arm‖ (*n* = 38) • ORR: 61% • CR: 8% • PR or nPR: 53% Idelalisib arm‖ (*n* = 10) • ORR: 50% • PR: 50%	Laboratory TLS in 2 patients	47¶
Phase Ib (NCT01682616)	49	Relapsed or refractory CLL	Venetoclax 20 or 50 mg per day ramped up to 200–600 mg per day then rituximab every 4 weeks for 6 doses	• ORR: 86% • CR or CRi: 47% • PR or nPR: 39% • MRD negative: 55% overall, and 74% with CR or CRi • PFS 24-month estimate: 83%	TLS in 1 patient led to death	48¶
Phase Ib (NCT01685892)	35	Relapsed or refractory or untreated CLL	Venetoclax 100–400 mg per day plus obinutuzumab	Relapsed or refractory CLL‖ (*n* = 21): • ORR: 100% • CR or CRi: 19% • PR or nPR: 81% • MRD negative: 43% First-line‖ (*n* = 6): • ORR: 100% • CR or CRi: 50% • PR or nPR: 50% • MRD negative: 17%	Laboratory TLS in 4 patients, but all continued treatment	49¶
Phase Ib (NCT01671904)	38	Relapsed or refractory or untreated CLL	Venetoclax 400 mg per day (ramp-up schedule) with bendamustine plus rituximab	Relapsed or refractory CLL‖ (*n* = 22): • ORR: 100% • CR or CRi: 18% • PR or nPR: 82% • MRD negative: 76% (16 of 21) First-line‖ (*n* = 11): • ORR: 100% • CR or CRi: 27% • PR or nPR: 73% • MRD negative: 56% (5 of 9)	No DLTs or TLS observed for the maximum protocol dose of 400 mg	50¶
Phase I (NCT01328626)	70	Relapsed or refractory NHL#	Venetoclax 200–1,200 mg per day in a dose ramp-up schedule	DLBCL** (*n* = 34): • ORR: 18% • CR: 12% • PR: 6% Follicular lymphoma (*n* = 29): • ORR: 38% • CR: 14% • PR: 24%	Laboratory TLS in 2 patients	51¶
Phase I (NCT01594229)	48	Relapsed or refractory NHL	Venetoclax 50–800 mg per day with bendamustine plus rituximab	• ORR: 65% • CR: 27% • PR: 38%	DLTs in 4 patients during cycle 1	52¶
Phase II (NCT01994837)	32	Relapsed or refractory AML	Venetoclax 20–800 mg per day in a dose ramp-up schedule	• ORR (CR+CRi): 19% • CR 6%	No reported events of clinical or laboratory TLS	53¶
Phase Ib (NCT02203773)	34	Untreated AML	Venetoclax 20–800 mg per day in a dose ramp-up schedule plus decitabine or azacitidine	Decitabine arm‖ (*n* = 16): • ORR: 81% • CR or CRi: 69% • PR: 13% Azacitidine arm‖ (*n* = 15): • ORR: 87% • CR or CRi: 87%	No clinical or laboratory TLS or DLTs	54¶

Table 2 (cont.) | **Clinical trials of venetoclax monotherapy or combination therapy in certain lymphomas, leukaemia or multiple myeloma**

Clinical trial	Number of patients	Indication	Regimen	Response	Comments	Refs
Phase I (NCT01794520)	48	Relapsed or refractory multiple myeloma	Venetoclax 300–1,200 mg per day after dose ramp-up	• ORR‡‡: 12%, including 24% of patients with t(11;14) and 4% of patients without t(11;14) • CR‡‡: 5%	DLTs in 2 patients in the 600 mg dose cohort; no clinical or laboratory TLS	55¶
Phase Ib (NCT01794507)	45	Relapsed or refractory multiple myeloma	Venetoclax 50–1,200 mg per day with bortezomib plus dexamethasone	All evaluable ($n = 41$): • ORR: 51% • ≥VGPR: 27% • sCR: 5% • CR: 5% Bortezomib-naive cohort ($n = 6$): • ORR: 100% • ≥VGPR: 83% • sCR: 17% • CR: 17% Bortezomib-sensitive cohort ($n = 17$): • ORR: 71% • ≥VGPR: 35% • sCR: 6% • CR: 6%	3 deaths occurred and were due to progressive disease; no TLS	56¶

AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CR, complete response; CRi, complete response with incomplete bone marrow recovery; del(17p), deletion of the 17p chromosomal region; DLBCL, diffuse large B cell lymphoma; DLT, dose-limiting toxicity; MRD, minimal residual disease; NHL, non-Hodgkin lymphoma; nPR, nodular partial response; ORR, objective response rate; PFS, progression-free survival; PR, partial response; sCR, stringent complete response; TLS, tumour lysis syndrome; ≥VGPR, at least a very good partial response. *Expansion cohort. ‡Estimate for the 400 mg dose group. §Relapsed after or refractory to ibrutinib or idelalisib. ‖Number of evaluable patients. ¶Based on data presented at congress. #DLBCL and follicular lymphoma. **Did not include patients with DLBCL-RT (Richter's transformation). ‡‡Based on evaluable patients.

a recent dose expansion into patients with multiple myeloma has been reported[61]. An ongoing phase Ib open-label study (NCT02603445) aims to examine the safety and efficacy of S55746 in combination with the PI3Kδ inhibitor idelalisib in patients with relapsed or refractory follicular lymphoma and those with mantle cell lymphoma[62], thus constituting the first clinical combination of S55746 with another targeted agent.

Selective BCL-X_L inhibitors

Whereas BCL-2 overexpression and cancer cell dependence on BCL-2 are associated mainly with haematological malignancies, BCL-X_L is known to have an anti-apoptotic role in solid tumours as well as in some haematological cancers[15,63]. Based on robust preclinical data, early clinical development was initiated in the solid-tumour setting, and navitoclax was combined with various chemotherapy agents. However, the doses of navitoclax that could be used were limited by neutropenia that exceeded that typically induced by chemotherapy alone. More recently, preclinical experiments with navitoclax, venetoclax and BCL-X_L-selective inhibitors have suggested that inhibition of BCL-X_L is responsible for the observed activity when it is used in combination with chemotherapy for the treatment of solid tumours, whereas BCL-2 inhibition probably accounts for the neutropenia observed in this context; thus, selective inhibitors of BCL-X_L may maintain efficacy in solid tumours while avoiding dose-limiting neutropenia[64].

The first reported BCL-X_L inhibitor with high affinity and high selectivity, WEHI-539, was developed using HTS and SAR[65] (TABLE 1). Further structure-based medicinal chemistry efforts led to development of the BCL-X_L-selective inhibitors A-1155463 and A-1331852, the latter of which is the first truly potent, selective and orally bioavailable inhibitor of BCL-X_L. All three compounds have key interactions with the P2 and P4 hotspots of BCL-X_L and, similarly to BH3-only proteins, possess an acidic moiety that forms a hydrogen bond with Arg139 in BCL-X_L (REFS 66,67). A-1155463 and A-1331852 have been studied alongside navitoclax and venetoclax to further discern the roles of BCL-2 and BCL-X_L in various haematological and solid tumour cell lines[64,66,67] (TABLE 1). The on-target activity of BCL-X_L-selective inhibitors has also been confirmed in distinct engineered cell lines and *in vivo*[68,69]. Their relative contributions to both the inhibition of tumour growth and mechanism-based toxicity (that is, neutropenia) observed previously with a navitoclax–docetaxel combination[70] were also examined. Selective BCL-2 inhibition with venetoclax, but not selective BCL-X_L inhibition with A-1155463, suppressed granulopoiesis in colony-forming assays[64], which suggests that the dose-limiting neutropenia observed in the clinical setting with the navitoclax–docetaxel combination was attributable to inhibition of BCL-2. In addition, there were no notable increases in thrombocytopenia with the navitoclax–docetaxel combination compared with navitoclax alone[64]. These data suggest the potential for BCL-X_L-selective inhibitors to enhance the efficacy of docetaxel in solid tumours while limiting the occurrence of neutropenia that is observed upon treatment with navitoclax. Moreover, several tumour cell lines — such as colorectal cancer cell lines[71] — are dependent

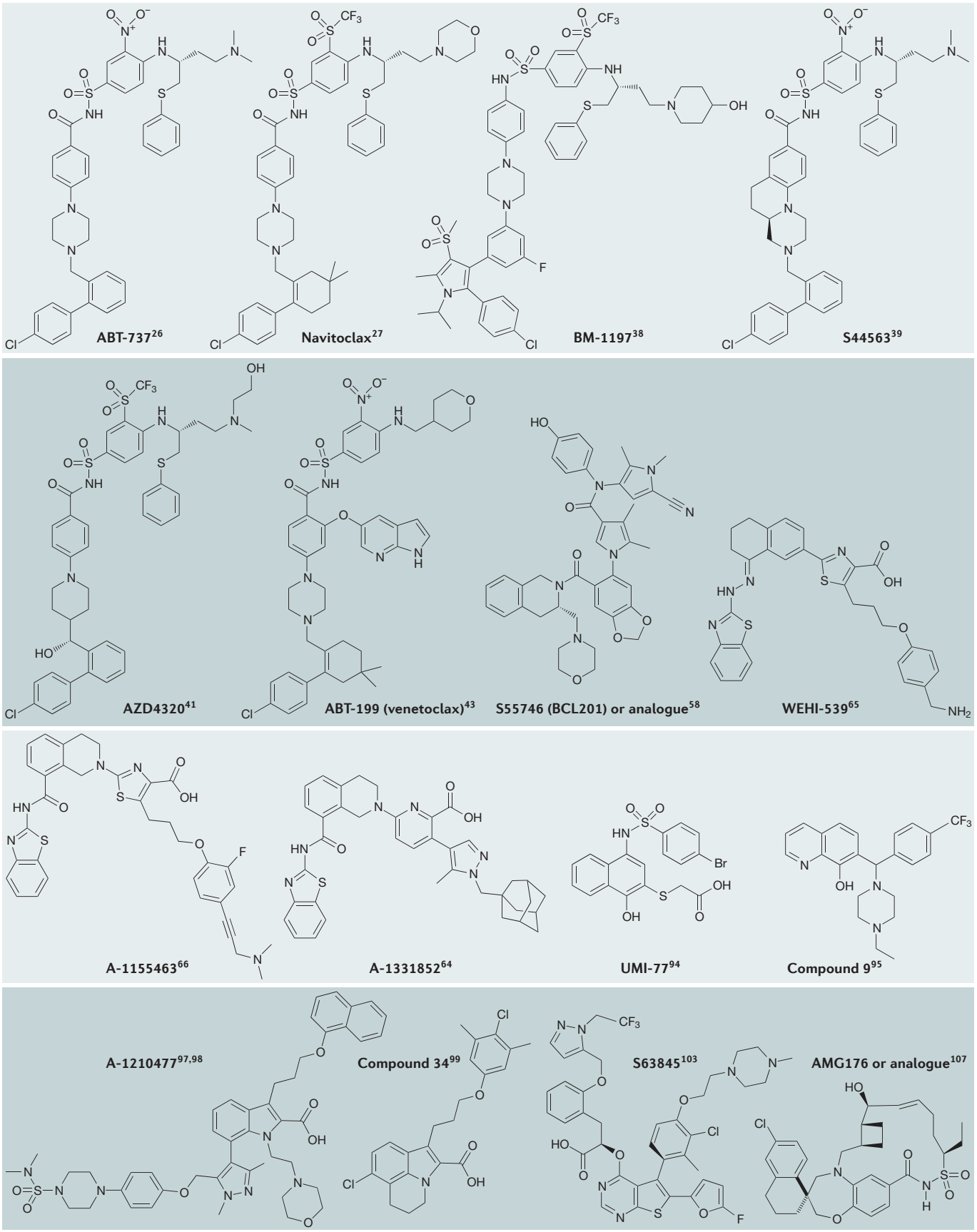

Figure 4 | **Structures of compounds listed in** TABLE 1**.** The structures of S55746 and AMG176 have not yet been publicly disclosed, and so representative structures are shown.

on BCL-X_L, which suggests that there is therapeutic potential for single-agent use of BCL-X_L inhibitors in cancer.

Selective MCL1 inhibitors

The anti-apoptotic protein MCL1 is implicated in mediating malignant cell survival in several primary tumour types[72], including breast cancer and non-SCLC (NSCLC)[73], and in models of multiple myeloma[74,75], AML[76], NSCLC[77,78] and MYC-driven lymphomas[79]. MCL1 is not inhibited by ABT-737, navitoclax or venetoclax[80], and has been identified as a potential resistance factor for these inhibitors[81–83]. MCL1 also seems to contribute to resistance to various chemotherapeutic agents, including gemcitabine, vincristine and taxol[84–86]. The P4 hotspot is one point of difference among MCL1, BCL-2 and BCL-X_L, with alanine scanning results suggesting that P4 binding is not crucial for MCL1 activity[87]. The conformational rigidity of the hydrophobic binding groove of MCL1 (REF. 88), together with the very high affinity of its endogenous ligands, has made it challenging to design small molecules that directly inhibit MCL1 potently enough to induce on-target, mechanism-based cell death.

Multiple approaches have been taken to directly target MCL1, including stapled peptides (stabilized α-helix of BH3-only proteins)[89,90], and α-peptide or β-peptide foldamers[91], which have been reviewed elsewhere. Several compounds with reported affinity for MCL1 have also been identified via screening of natural products and small-molecule library collections, as well as structure-based drug design. As some of these compounds have reported off-target activity[21,92,93] or lack reported cellular effects[90], we focus here on rationally designed small molecules that have characterized cellular effects and have demonstrated activity against MCL1-dependent cells (TABLE 1). Comparisons of activity across biochemical and cellular assays are confounded by differing conditions and formats; it should be noted, however, that some of the compounds described here have reported affinities for MCL1 that may not be expected to cause on-target cellular activity. In addition, selectivity over other BCL-2 family members is not reported for all compounds, although published structural comparisons of MCL1 alongside other family members[88] support the expectation that high-affinity binders to MCL1 would not bind tightly to the diverging proteins BCL-X_L and BCL-2. Further evaluation of the various inhibitors using common techniques should provide more insights for comparison.

A series of 3-substituted-*N*-(4-hydroxynaphthalen-1-yl) arylsulfonamides were identified by HTS against MCL1 (REF. 94). *In silico* docking and 2D $^{1}H-^{15}N$ heteronuclear single quantum coherence spectroscopy NMR were then used to develop SAR and optimization strategies that were based on the HTS hit, which ultimately led to the discovery of the compound UMI-77 (REF. 94) (TABLE 1). Although its binding affinity for MCL1 is modest (concentration required to produce half maximum inhibition (K_i) = 500 nM), UMI-77 has been reported to inhibit cell growth and induce hallmarks of apoptosis at micromolar concentrations in BxPC-3 pancreatic cancer cells. Co-immunoprecipitation experiments demonstrated that UMI-77 blocks the heterodimerization of MCL1 with BAX and BAK in cells, thus supporting its direct engagement with cellular MCL1. In a BxPC-3 xenograft model, two cycles of intravenous dosing with UMI-77 afforded modest yet statistically significant tumour growth inhibition.

The MCL1 inhibitor compound 9 was identified through HTS coupled with directed hit optimization[95]. *In silico* docking was used to select compounds that were predicted to form a hydrogen bond with the side chain amide of Asn260 in MCL1. Interestingly, although the HTS hit contained a carboxylic acid that is essential for other reported MCL1 inhibitors via the Arg263 electrostatic interaction, this chemical moiety was removed during the optimization process to generate compound 9. The reported binding affinity of compound 9 is 390 nM (according to the concentration of the inhibitor that reduces the response by half (IC_{50})) with selectivity over BCL-X_L. The administration of this compound to a panel of cell lines showed differential activity and, notably, compound 9 showed low micromolar activity against the MCL1-dependent H929 cell line. Furthermore, the antiproliferative response to compound 9 in a panel of cell lines was reported to correlate with the extent of mitochondrial priming via the BH3-profiling technique.

The generation of a previously reported series of indole-2-carboxylic acids[96] has recently been described in greater detail[97,98]. This compound series originated from a 2-carboxy-indole HTS hit that bound weakly to MCL1 yet showed selectivity for MCL1 over BCL-2 and BCL-X_L. Co-crystal structures of early compounds complexed with MCL1 indicated that the 2-carboxy-indole core bound deeply within the P2 hotspot of the target protein and, similarly to the endogenous BH3-only protein–protein interaction, was anchored by a hydrogen bond between the carboxylic acid and Arg263. Structure-based design was used to promote further interactions with the P3 and P4 hotspots of MCL1, yielding compounds such as A-1210477. This inhibitor demonstrated subnanomolar binding affinity to MCL1 (K_i = 0.45 nM) while showing high selectivity for MCL1 over other BCL-2 family proteins, as well as panels of kinases and G protein-coupled receptors (GPCRs). A-1210477 disrupts endogenous MCL1–BIM complexes in cells via the formation of small-molecule–MCL1 complexes that effectively increase cellular MCL1 levels by protecting the protein from ubiquitylation and degradation. This compound also induces the hallmarks of intrinsic apoptosis and demonstrates single-agent killing of specific cancer cell lines that are demonstrated to be MCL1 dependent by BH3 profiling or short interfering RNA (siRNA) rescue experiments. Finally, this compound synergized with navitoclax to kill several additional cell lines[97,98].

The 2-carboxy-indole core has been used to generate additional and similar chemical series of inhibitors. An NMR-based screen of a fragment library of approximately 15,000 compounds[99] identified a starting core that was elaborated into compounds such as the tricyclic structure 34 (REF. 99) and acylsulfonamide 71 (REF. 100) using structure-based design techniques. Both compounds demonstrate binding K_i values of less than 10 nM to MCL1 and

more than 1,000-fold selectivity for MCL1 over BCL-X_L. Characterization of cellular activity was not reported in these publications, although a subsequent abstract describing the ability of compounds within this general series to induce apoptosis in specific MCL1-dependent cell lines and to exert antiproliferative activity in a broad panel of tumour cell lines has been disclosed[101].

A series of thienopyrimidine-based MCL1 inhibitors (TABLE 1) has been reported[102]; these are structurally distinct from the indole acids and contain a preferred atropisomeric chirality that partially contributes to their high level of activity in MCL1 binding assays (a dissociation constant (K_d) of 0.19 nM has been reported for compound S63845)[103]. S63845 demonstrates low nanomolar activity against H929 cells (IC_{50} = 4 nM) and induces various hallmarks of apoptosis *in vitro*. In addition, 5 days of consecutive dosing conferred substantial tumour regression in a xenograft model of human AML, and complete remission was observed in six of eight mice after 80 days. Substantial *in vivo* activity was also reported in a model of human multiple myeloma, as well as a MYC-driven murine lymphoma. Representatives of the thienopyrimidine pharmacophore are the first structures to be associated with potent *in vivo* activity in MCL1-dependent tumour models, which is consistent with their biochemical and cellular activity.

AMG176 is the first putative MCL1 inhibitor to reach clinical evaluation (NCT02675452)[104], although no data in humans have been reported yet. This compound will be assessed for tolerability, pharmacokinetics and antitumour response in patients with refractory multiple myeloma. A secondary measurement also includes the pharmacodynamic assessment of MCL1 inhibition. Although the exact structure of AMG176 has not been disclosed, a class of complex macrocyclic MCL1 inhibitors with as many as six chiral centres was recently disclosed by the same group (a representative example is shown in FIG. 4). These macrocyclic structures also contain an acylsulfonamide group, which is a chemical moiety that has similar acidity to the carboxylic acid group found in several distinct MCL1 inhibitors and is capable of forming an electrostatic interaction with Arg263 of the MCL1 protein. Several compounds are reported to have submicromolar activity *in vitro* against the OPM-2 multiple myeloma cell line, and some compounds induce robust tumour growth inhibition in an OPM-2 mouse xenograft model following several days of dosing. This exciting clinical entry represents a considerable advance for the fields of BCL-2 family inhibitors and oncology drug discovery.

Discussion

In the past few years, substantial progress has been made in the development of selective BCL-2 family inhibitors. Structure-based design and translational medicine have been paramount in fuelling this progress, as preclinical and clinical observations from studies of the first dual-targeting BH3 mimetics have driven the development of highly selective agents that target single anti-apoptotic proteins. Specifically, the BCL-2–BCL-X_L inhibitor navitoclax was clinically active when used as a single agent or in combination with rituximab, but it was also associated with toxicity to platelets. Although the clinical activity validated the relevance of these protein targets for anticancer therapy, the dose-limiting thrombocytopenia caused by BCL-X_L inhibition led to the hypothesis that selective BCL-2 inhibition could provide a wider therapeutic index and greater inhibition of this protein in the clinic. Subsequent studies with the first BCL-2-selective compound venetoclax showed high and durable response rates in patients with refractory CLL[45]. Additional studies in this patient population led to the recent FDA approval of this agent in the United States for patients with relapsed or refractory CLL who have del(17p), thus providing a seminal drug registration for compounds that target the intrinsic apoptosis pathway and the BCL-2 family of proteins in cancer therapy. Venetoclax has been studied in other haematological malignancies, and objective data have led to a total of three FDA breakthrough therapy designations. The first was in April 2015 for single-agent venetoclax for the treatment of relapsed or refractory CLL in patients with del(17p); the second was in January 2016 for venetoclax in combination with rituximab in patients with relapsed or refractory CLL; and the third was in January 2016 for venetoclax in combination with hypomethylating agents for treatment-naive patients with AML who are unable to receive standard induction therapy[105]. Further encouraging data are anticipated for other tumour types and drug combinations. Finally, the discovery of a second compound in this class (S55746) should offer additional exciting opportunities to explore BCL-2 inhibition in the clinic.

Venetoclax is also being studied in patients with metastatic oestrogen receptor-positive breast cancer[17], thereby marking the first instance of this compound being tested in the setting of solid tumours. Although this clinical study is based on highly promising preclinical data, apoptosis evasion by malignant cells in solid tumours has been more closely associated with overexpression of BCL-X_L than of BCL-2 (REF. 15). Navitoclax inhibits both BCL-2 and BCL-X_L; however, the ability to target the latter in solid tumours was hampered by dose-limiting neutropenia when it was used in combination with chemotherapy. These findings led to the hypothesis that neutropenia was driven by inhibition of BCL-2 and that a BCL-X_L-selective inhibitor could maintain efficacy in solid tumours while averting neutropenia. Highly potent and selective BCL-X_L inhibitors have since been identified. Preclinical studies with agents such as A-1331852 have demonstrated that selective inhibition of BCL-X_L can indeed drive antitumour efficacy in preclinical models — when a BCL-X_L inhibitor is used either as a single agent or in combination with chemotherapy — while avoiding the suppression of neutrophil precursors[64].

In the process of studying navitoclax, venetoclax and other inhibitors of the BCL-2 family, MCL1 has emerged as an important driver of innate and acquired resistance. MCL1 is a highly compelling target in its own right, given its reported levels of amplification in solid tumours and its role in resistance to several anticancer agents. For these reasons, efforts to inhibit MCL1 via small molecules have greatly intensified over the past several years.The resulting molecules seem to have greater chemical diversity and

complexity than do those targeting BCL-2 and BCL-X_L. Recent disclosures indicate that highly potent inhibitors capable of inducing tumour regression in preclinical models have been realized, and in the case of AMG176, we may see the first patient to be treated with an MCL1 inhibitor in the near future.

In conclusion, unravelling the complexities of the BCL-2 family has opened the door to the development of selective pro-apoptotic cancer therapeutics that may redefine cancer treatment in specific malignancies. The BCL-2 drug-discovery process has also helped to advance new methods such as chemical parsing, which have enabled more precise functional categorization of individual BCL-2 family members in malignant and normal cells. These advances will no doubt accelerate the development of cancer therapeutics targeting the BCL-2 family in particular and apoptosis pathways in general.

1. Kerr, J. F., Wyllie, A. H. & Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26**, 239–257 (1972).
2. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
3. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116**, 205–219 (2004).
4. Ashkenazi, A. & Dixit, V. M. Death receptors: signaling and modulation. *Science* **281**, 1305–1308 (1998).
5. Jin, Z. & El-Deiry, W. S. Overview of cell death signaling pathways. *Cancer Biol. Ther.* **4**, 139–163 (2005).
6. Shamas-Din, A., Brahmbhatt, H., Leber, B. & Andrews, D. W. BH3-only proteins: orchestrators of apoptosis. *Biochim. Biophys. Acta* **1813**, 508–520 (2011).
7. Chipuk, J. E., Moldoveanu, T., Llambi, F., Parsons, M. J. & Green, D. R. The BCL-2 family reunion. *Mol. Cell* **37**, 299–310 (2010).
8. Czabotar, P. E., Lessene, G., Strasser, A. & Adams, J. M. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* **15**, 49–63 (2014).
This is a comprehensive Review on extrinsic (death receptor) and intrinsic (mitochondrial) pathways of apoptosis and their regulation. It focuses on the BCL-2 family, the pathological consequences of deregulated apoptosis, and how structural and functional analysis of the BCL-2 family is guiding the development of anticancer drugs.
9. Tsujimoto, Y., Finger, L. R., Yunis, J., Nowell, P. C. & Croce, C. M. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* **226**, 1097–1099 (1984).
10. Tsujimoto, Y. *et al.* Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. *Science* **224**, 1403–1406 (1984).
11. Vaux, D. L., Cory, S. & Adams, J. M. *Bcl-2* gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**, 440–442 (1988).
12. McDonnell, T. J. *et al. bcl*-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell* **57**, 79–88 (1989).
13. O'Neill, K. L., Huang, K., Zhang, J., Chen, Y. & Luo, X. Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane. *Genes Dev.* **30**, 973–988 (2016).
14. Kang, M. H. & Reynolds, C. P. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. *Clin. Cancer Res.* **15**, 1126–1132 (2009).
15. Amundson, S. A. *et al.* An informatics approach identifying markers of chemosensitivity in human cancer cell lines. *Cancer Res.* **60**, 6101–6110 (2000).
16. Kitada, S., Pedersen, I. M., Schimmer, A. D. & Reed, J. C. Dysregulation of apoptosis genes in hematopoietic malignancies. *Oncogene* **21**, 3459–3474 (2002).
17. Vaillant, F. *et al.* Targeting BCL-2 with the BH3 mimetic ABT-199 in estrogen receptor-positive breast cancer. *Cancer Cell* **24**, 120–129 (2013).
18. Gandhi, L. *et al.* 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.* **29**, 909–916 (2011).
19. Bedikian, A. Y. *et al.* Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. *J. Clin. Oncol.* **24**, 4738–4745 (2006).
20. Cao, X. X., Mohuiddin, I., Ece, F., McConkey, D. J. & Smythe, W. R. Histone deacetylase inhibitor downregulation of *bcl-xl* gene expression leads to apoptotic cell death in mesothelioma. *Am. J. Respir. Cell Mol. Biol.* **25**, 562–568 (2001).
21. Lessene, G., Czabotar, P. E. & Colman, P. M. BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug Discov.* **7**, 989–1000 (2008).
This Review focuses on preclinical and early clinical data on cytotoxic compounds that inhibit the interactions between BCL-2 family proteins and their natural ligands, BH3 motif-containing pro-apoptotic proteins, as well as their potential to be used as anticancer drugs.
22. Sattler, M. *et al.* Structure of Bcl-x_L–Bak peptide complex: recognition between regulators of apoptosis. *Science* **275**, 983–986 (1997).
23. Bharatham, N., Chi, S. W. & Yoon, H. S. Molecular basis of Bcl-X_L–p53 interaction: insights from molecular dynamics simulations. *PLoS ONE* **6**, e26014 (2011).
24. Shuker, S. B., Hajduk, P. J., Meadows, R. P. & Fesik, S. W. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **274**, 1531–1534 (1996).
This study was the first to describe the SAR-by-NMR technique that is used to develop ligands for proteins by identifying molecules that bind to proximal sites of a protein, optimizing their binding and linking these fragments to obtain a high-affinity ligand. This approach is extremely useful in target-based drug discovery.
25. Bruncko, M. *et al.* Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-x_L. *J. Med. Chem.* **50**, 641–662 (2007).
This paper describes the generation of multiple selective MCL1 inhibitors through structure-guided design, which advanced our understanding of MCL1 biology and aided drug discovery.
26. Oltersdorf, T. *et al.* An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**, 677–681 (2005).
This study was the first to report the discovery of the BH3 mimetic ABT-737 using NMR-based screening (described in reference 3) and to demonstrate preclinically that BCL-2 family inhibitors may be useful for the treatment of lymphoma and SCLC.
27. Tse, C. *et al.* ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* **68**, 3421–3428 (2008).
28. Shoemaker, A. R. *et al.* Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. *Clin. Cancer Res.* **14**, 3268–3277 (2008).
29. Ackler, S. *et al.* The Bcl-2 inhibitor ABT-263 enhances the response of multiple chemotherapeutic regimens in hematologic tumors *in vivo*. *Cancer Chemother. Pharmacol.* **66**, 869–880 (2010).
30. Chen, J. *et al.* The Bcl-2/Bcl-X_L/Bcl-w inhibitor, navitoclax, enhances the activity of chemotherapeutic agents *in vitro* and *in vivo*. *Mol. Cancer Ther.* **10**, 2340–2349 (2011).
31. Wilson, W. H. *et al.* 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**, 1149–1159 (2010).
This phase I dose-escalation study evaluated the pharmacokinetics and pharmacodynamics of navitoclax, an inhibitor of the anti-apoptotic proteins BCL-2 and BCL-X_L, as well as the pharmacodynamic effects of the drug on T cells and platelets. It was the first study to establish navitoclax as a drug with a high therapeutic index and a low incidence of indirect toxic effects.
32. Roberts, A. W. *et al.* Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J. Clin. Oncol.* **30**, 488–496 (2012).
33. Kipps, T. J. *et al.* Navitoclax (ABT-263) plus fludarabine/cyclophosphamide/rituximab (FCR) or bendamustine/rituximab (BR): a phase 1 study in patients with relapsed/refractory chronic lymphocytic leukemia (CLL). *Blood* **116**, 2455 (2010).
34. Kipps, T. J. *et al.* A phase 2 study of the BH3 mimetic BCL2 inhibitor navitoclax (ABT-263) with or without rituximab, in previously untreated B-cell chronic lymphocytic leukemia. *Leuk. Lymphoma* **56**, 2826–2833 (2015).
35. Zhang, H. *et al.* Bcl-2 family proteins are essential for platelet survival. *Cell Death Differ.* **14**, 943–951 (2007).
36. Mason, K. D. *et al.* Programmed anuclear cell death delimits platelet life span. *Cell* **128**, 1173–1186 (2007).
37. Sleebs, B. E. *et al.* Quinazoline sulfonamides as dual binders of the proteins B-cell lymphoma 2 and B-cell lymphoma extra long with potent proapoptotic cell-based activity. *J. Med. Chem.* **54**, 1914–1926 (2011).
38. Bai, L. *et al.* BM-1197: a novel and specific Bcl-2/Bcl-x_L inhibitor inducing complete and long-lasting tumor regression *in vivo*. *PLoS ONE* **9**, e99404 (2014).
39. Nemati, F. *et al.* Targeting Bcl-2/Bcl-X_L induces antitumor activity in uveal melanoma patient-derived xenografts. *PLoS ONE* **9**, e80836 (2014).
40. Adam, A. *et al.* A dual Bcl-2/x_L inhibitor induces tumor cell apoptosis in a hematopoietic xenograft model. *Blood* **124**, 5304 (2014).
41. Hennessy, E. J. *et al.* Discovery of potent inhibitors of the antiapoptotic proteins Bcl-2 and Bcl-xL, resulting in the identification of a clinical candidate for the treatment of cancer (AZD4320). *ACS Medicinal Chemistry annual meeting* https://www.acsmedchem.org/ama/orig/abstracts/mediabstractf2015.pdf abstr. 24 (2015).
42. Huang, J. Z. *et al.* The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. *Blood* **99**, 2285–2290 (2002).
43. Souers, A. J. *et al.* ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat. Med.* **19**, 202–208 (2013).
This study describes the use of structure-based design to generate a highly potent and first-in-class orally bioavailable selective BCL-2 inhibitor, ABT-199, which induced tumour regression *in vivo* without affecting platelets.
44. Ackler, S. *et al.* Clearance of systemic hematologic tumors by venetoclax (Abt-199) and navitoclax. *Pharmacol. Res. Perspect.* **3**, e00178 (2015).
45. Roberts, A. W. *et al.* Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N. Engl. J. Med.* **374**, 311–322 (2016).
This paper describes a phase I, first-in-human, dose-escalation study of venetoclax in patients with CLL. This study provided encouraging data on venetoclax monotherapy, reporting a manageable safety profile and favourable responses in patients with relapsed CLL who had a poor prognosis.
46. Stilgenbauer, S. *et al.* Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol.* **17**, 768–778 (2016).

47. Jones, J. A. *et al.* Preliminary results of a phase 2, open-label study of venetoclax (ABT-199/GDC-0199) monotherapy in patients with chronic lymphocytic leukemia relapsed after or refractory to ibrutinib or idelalisib therapy. *Blood* **126**, 715 (2015).
48. Ma, S. *et al.* Deep and durable responses following venetoclax (ABT-199 / GDC-0199) combined with rituximab in patients with relapsed/refractory chronic lymphocytic leukemia: results from a phase 1b study. *Blood* **126**, 830 (2015).
49. Flinn, I. W. *et al.* Safety and efficacy of a combination of venetoclax (GDC-0199/ABT-199) and obinutuzumab in patients with relapsed/refractory or previously untreated chronic lymphocytic leukemia: results from an ongoing phase 1b study (GP28331). *Blood* **126**, 494 (2015).
50. Salles, G. *et al.* Updated safety and preliminary efficacy data from a phase 1b study combining venetoclax (GDC0199, ABT199) with bendamustine/rituximab in patients with relapsed/refractory or previously untreated chronic lymphocytic leukemia. *Blood* **126**, 829 (2015).
51. Gerecitano, J. F. *et al.* A phase 1 study of venetoclax (ABT-199 / GDC-0199) monotherapy in patients with relapsed/refractory non-Hodgkin lymphoma. *Blood* **126**, 254 (2015).
52. de Vos, S. *et al.* A dose-escalation study of venetoclax (ABT-199/GDC-0199) in combination with bendamustine and rituximab in patients with relapsed or refractory non-Hodgkin's lymphoma. *Blood* **126**, 255 (2015).
53. Konopleva, M. *et al.* A phase 2 study of ABT-199 (GDC-0199) in patients with acute myelogenous leukemia (AML). *Blood* **124**, 118 (2014).
54. DiNardo, C. *et al.* A phase 1b study of venetoclax (ABT-199/GDC-0199) in combination with decitabine or azacitidine in treatment-naive patients with acute myelogenous leukemia who are ≥65 years and not eligible for standard induction therapy. *Blood* **126**, 327 (2015).
55. Kumar, S. *et al.* Safety and efficacy of venetoclax (ABT-199/GDC-0199) monotherapy for relapsed/refractory multiple myeloma: phase 1 preliminary results. *Blood* **126**, 4219 (2015).
56. Moreau, P. *et al.* Safety and efficacy of venetoclax (ABT-199/GDC-0199) in combination with bortezomib and dexamethasone in relapsed/refractory multiple myeloma: phase 1b results. *Blood* **126**, 3038 (2015).
57. Seymour, J. F. *et al.* Reduction of tumor lysis syndrome (TLS) risk in chronic lymphocytic leukemia (CLL) patients treated with ABT-199 (GDC-0199): results of modifications to dosing schedule and TLS prophylaxis [abstract P868]. *Haematologica* **99** (Suppl. 1), 321 (2014).
58. Casara, P. *et al.* Novel derivatives of indole and pyrrole, method for the production thereof and pharmaceutical compositions containing same. World Intellectual Property Organization patent *WO2015011396* (2015).
59. Porter, J. *et al.* Tetrahydroisoquinoline amide substituted phenyl pyrazoles as selective Bcl-2 inhibitors. *Bioorg. Med. Chem. Lett.* **19**, 230–233 (2009).
60. International Standard Randomised Controlled Trial Number Registry. *ISRCTN* http://www.isrctn.com/ISRCTN04804337 (2016).
61. Garcia, M. *et al.* Phase 1 trial with Bcl-2 inhibitor S55746 in t(11:14) multiple myeloma. *Health Research Authority* http://www.hra.nhs.uk/news/research-summaries/phase-1-trial-with-bcl-2-inhibitor-s55746-in-t1114-multiple-myeloma/ (2016)
62. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02603445 (2016).
63. Minn, A. J., Rudin, C. M., Boise, L. H. & Thompson, C. B. Expression of bcl-x_L can confer a multidrug resistance phenotype. *Blood* **86**, 1903–1910 (1995).
64. Leverson, J. D. *et al.* Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. *Sci. Transl Med.* **7**, 279ra240 (2015).
This study used selective BCL-2 family inhibitors to determine the relative contributions of BCL-2 and BCL-X_L inhibition to dose-limiting thrombocytopenia and neutropenia caused by combined treatment of solid tumours with navitoclax and docetaxel combination. Selective inhibition of BCL-2, but not of BCL-X_L, suppressed granulopoiesis, whereas BCL-X_L inhibition was sufficient to replicate the efficacy of navitoclax in combination with docetaxel.
65. Lessene, G. *et al.* Structure-guided design of a selective BCL-X_L inhibitor. *Nat. Chem. Biol.* **9**, 390–397 (2013).
66. Tao, Z. F. *et al.* Discovery of a potent and selective BCL-X_L inhibitor with *in vivo* activity. *ACS Med. Chem. Lett.* **5**, 1088–1093 (2014).
67. Koehler, M. F. *et al.* Structure-guided rescaffolding of selective antagonists of BCL-X_L. *ACS Med. Chem. Lett.* **5**, 662–667 (2014).
68. Koss, B. *et al.* Defining specificity and on-target activity of BH3-mimetics using engineered B-ALL cell lines. *Oncotarget* **7**, 11500–11511 (2016).
69. Lopez, J. *et al.* Mito-priming as a method to engineer Bcl-2 addiction. *Nat. Commun.* **7**, 10538 (2016).
70. Puglisi, M. *et al.* A phase I safety and pharmacokinetic (PK) study of navitoclax (N) in combination with docetaxel (D) in patients (pts) with solid tumors. *J. Clin. Oncol.* **29**, 2518 (2011).
71. Zhang, H. *et al.* Genomic analysis and selective small molecule inhibition identifies BCL-X_L as a critical survival factor in a subset of colorectal cancer. *Mol. Cancer* **14**, 126 (2015).
72. Perciavalle, R. M. & Opferman, J. T. Delving deeper: MCL-1's contributions to normal and cancer biology. *Trends Cell Biol.* **23**, 22–29 (2013).
73. Beroukhim, R. *et al.* The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899–905 (2010).
74. Derenne, S. *et al.* Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x_L is an essential survival protein of human myeloma cells. *Blood* **100**, 194–199 (2002).
75. Zhang, B., Gojo, I. & Fenton, R. G. Myeloid cell factor-1 is a critical survival factor for multiple myeloma. *Blood* **99**, 1885–1893 (2002).
76. Glaser, S. P. *et al.* Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia. *Genes Dev.* **26**, 120–125 (2012).
77. Song, L., Coppola, D., Livingston, S., Cress, D. & Haura, E. B. Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells. *Cancer Biol. Ther.* **4**, 267–276 (2005).
78. Zhang, H. *et al.* Mcl-1 is critical for survival in a subgroup of non-small-cell lung cancer cell lines. *Oncogene* **30**, 1963–1968 (2011).
79. Kelly, G. L. *et al.* Targeting of MCL-1 kills MYC-driven mouse and human lymphomas even when they bear mutations in *p53*. *Genes Dev.* **28**, 58–70 (2014).
80. Bose, P. & Grant, S. Mcl-1 as a therapeutic target in acute myelogenous leukemia (AML). *Leuk. Res. Rep.* **2**, 12–14 (2013).
81. Konopleva, M. *et al.* Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell* **10**, 375–388 (2006).
82. van Delft, M. F. *et al.* The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. *Cancer Cell* **10**, 389–399 (2006).
83. Tahir, S. K. *et al.* Influence of Bcl-2 family members on the cellular response of small-cell lung cancer cell lines to ABT-737. *Cancer Res.* **67**, 1176–1183 (2007).
84. Wei, S. H. *et al.* Inducing apoptosis and enhancing chemosensitivity to gemcitabine via RNA interference targeting *Mcl-1* gene in pancreatic carcinoma cell. *Cancer Chemother. Pharmacol.* **62**, 1055–1064 (2008).
85. Wertz, I. E. *et al.* Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. *Nature* **471**, 110–114 (2011).
86. Akagi, H. *et al.* Suppression of myeloid cell leukemia-1 (Mcl-1) enhances chemotherapy-associated apoptosis in gastric cancer cells. *Gastric Cancer* **16**, 100–110 (2013).
87. Lee, E. F. *et al.* A novel BH3 ligand that selectively targets Mcl-1 reveals that apoptosis can proceed without Mcl-1 degradation. *J. Cell Biol.* **180**, 341–355 (2008).
88. Lee, E. F. *et al.* Conformational changes in Bcl-2 pro-survival proteins determine their capacity to bind ligands. *J. Biol. Chem.* **284**, 30508–30517 (2009).
89. Stewart, M. L., Fire, E., Keating, A. E. & Walensky, L. D. The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. *Nat. Chem. Biol.* **6**, 595–601 (2010).
90. Muppidi, A. *et al.* Rational design of proteolytically stable, cell-permeable peptide-based selective Mcl-1 inhibitors. *J. Am. Chem. Soc.* **134**, 14734–14737 (2012).
91. Smith, B. J. *et al.* Structure-guided rational design of α/β-peptide foldamers with high affinity for BCL-2 family prosurvival proteins. *Chembiochem* **14**, 1564–1572 (2013).
92. Belmar, J. & Fesik, S. W. Small molecule Mcl-1 inhibitors for the treatment of cancer. *Pharmacol. Ther.* **145**, 76–84 (2015).
93. Varadarajan, S. *et al.* Evaluation and critical assessment of putative MCL-1 inhibitors. *Cell Death Differ.* **20**, 1475–1484 (2013).
94. Abulwerdi, F. *et al.* A novel small-molecule inhibitor of Mcl-1 blocks pancreatic cancer growth *in vitro* and *in vivo*. *Mol. Cancer Ther.* **13**, 565–575 (2014).
95. Richard, D. J. *et al.* Hydroxyquinoline-derived compounds and analoguing of selective Mcl-1 inhibitors using a functional biomarker. *Bioorg. Med. Chem.* **21**, 6642–6649 (2013).
96. Elmore, S. W. *et al.* 7-substituted indole MCL-1 inhibitors. World Intellectual Property Organization patent *WO2008131000 A2* (2008).
97. Leverson, J. D. *et al.* Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax). *Cell Death Dis.* **6**, e1590 (2015).
98. Bruncko, M. *et al.* Structure-guided design of a series of MCL-1 inhibitors with high affinity and selectivity. *J. Med. Chem.* **58**, 2180–2194 (2015).
99. Burke, J. P. *et al.* Discovery of tricyclic indoles that potently inhibit Mcl-1 using fragment-based methods and structure-based design. *J. Med. Chem.* **58**, 3794–3805 (2015).
100. Pelz, N. F. *et al.* Discovery of 2-indole-acylsulfonamide myeloid cell leukemia 1 (Mcl-1) inhibitors using fragment-based methods. *J. Med. Chem.* **59**, 2054–2066 (2016).
101. Sensintaffar, J. L. *et al.* Small molecule Mcl-1 inhibitors induce apoptosis and death in multiple cancer subtypes *in vitro*. *Cancer Res.* **76** (14 Suppl.), abstr. 3727 (2016).
102. Kotschy, A. New thienopyrimidine derivatives, a process for their preparation and pharmaceutical compositions containing them. European patent *EP2886545 B1* (2016).
103. Kotschy, A. *et al.* The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature* **538**, 477–482 (2016).
This manuscript describes S63845, a recently developed small molecule that specifically binds with high affinity to the BH3-binding groove of MCL1. *In vivo* studies indicate that S63845 has potent antitumour activity and an acceptable safety profile as a single agent in several cancers.
104. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02675452 (2016).
105. AbbVie. Venetoclax receives 3rd breakthrough therapy designation from the FDA for the combination treatment of patients with untreated acute myeloid leukemia not eligible for standard induction chemotherapy. *PR Newswire* http://www.prnewswire.com/news-releases/venetoclax-receives-3rd-breakthrough-therapy-designation-from-the-fda-for-the-combination-treatment-of-patients-with-untreated-acute-myeloid-leukemia-not-eligible-for-standard-induction-chemotherapy-300211208.html (2016).
106. Rajan, S., Choi, M., Baek, K. & Yoon, H. S. Bh3 induced conformational changes in Bcl-X_L revealed by crystal structure and comparative analysis. *Proteins* **83**, 1262–1272 (2015).
107. Brown, S. P. *et al.* Tetrahydronaphthalene derivatives that inhibit MCL-1 protein. World Intellectual Property Organization patent *WO2016033486* (2016).

Acknowledgements
Under the direction of the authors, editorial assistance was provided by T. N. Miller of Envision Scientific Solutions. T. N. Miller was funded by F. Hoffmann-La Roche.

Competing interests statement
The authors declare competing interests: see Web version for details.

DATABASES
ClinicalTrials.gov: https://clinicaltrials.gov/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF