HHS Public Access

Author manuscript

Cancer Discov. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

Cancer Discov. 2017 December ; 7(12): 1376–1393. doi:10.1158/2159-8290.CD-17-0797.

Found in translation: how preclinical research is guiding the clinical development of the BCL-2-selective inhibitor venetoclax

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Abstract

Since the discovery of apoptosis as a form of programmed cell death, targeting the apoptosis pathway to induce cancer cell death has been a high priority goal for cancer therapy. After decades of effort, drug discovery scientists have succeeded in generating small-molecule inhibitors of antiapoptotic BCL-2 family proteins. Innovative medicinal chemistry and structure-based drug design, coupled with a strong fundamental understanding of BCL-2 biology, were essential to the development of BH3 mimetics such as the BCL-2-selective inhibitor venetoclax. We review a number of preclinical studies that have deepened our understanding of BCL-2 biology and facilitated the clinical development of venetoclax.

Introduction

Many in the drug discovery and development community bemoan the inability of preclinical studies to validate therapeutic targets, identify sensitive patient populations, and predict clinical outcomes. Preclinical systems are felt to be poor substitutes for the diseases they are intended to model and inadequate for predicting how patients will respond. Although preclinical models are rarely perfect predictors of what will occur in the clinical setting, many have shown utility in generating hypotheses that can be tested in clinical studies and have contributed to some notable translational successes. Representing one such success is the B-cell lymphoma 2 (BCL-2)-selective inhibitor venetoclax (ABT-199/GDC-0199), which has emerged as a promising agent for a variety of hematologic malignancies. Conceived when the development of its less selective predecessor, navitoclax, was hindered

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Disclosure of Potential Conflicts of Interest

Joel D. Leverson, Andrew J. Souers, Saul H. Rosenberg are AbbVie employees and may own stock.
Deepak Sampath and Wayne J. Fairbrother are Genentech employees and may own stock.
Martine Amiot has no relevant conflicts to disclose.

by dose-limiting thrombocytopenia, venetoclax was designed to spare platelets and was recently approved by the Food and Drug Administration for the treatment of relapsed chronic lymphocytic leukemia with 17p deletion. The navitoclax-to-venetoclax story is an excellent example of translational medicine guided by iterative learning, with discoveries made in both the lab and the clinic guiding the development of an optimized drug target profile. In addition, the evolving story of venetoclax includes a number of other translational successes that may be less appreciated. This review will highlight some of these successes and discuss how preclinical findings are being translated into the clinical setting.

Know thy targets: the BCL-2 family of proteins

Apoptosis, a type of programmed cell death, is crucial to the development of multicellular organisms and for ensuring healthy tissue homeostasis. The intrinsic (mitochondrial) pathway of apoptosis is governed by the BCL-2 family of proteins, which function within a complex network of protein-protein and protein-membrane interactions. They are structurally and functionally related, containing up to four BCL-2 homology [BH] motifs (BH1–4), and can be divided into three groups: 1) antiapoptotic proteins containing all 4 BH regions; 2) membrane-permeabilizing proapoptotic effectors containing BH regions 1–3; and 3) BH3-only proteins that respond to cellular stresses and promote cell death indirectly by inhibiting antiapoptotic proteins or directly by activating proapoptotic effectors (Fig. 1A). A number of models have been proposed to describe how interactions between different family members regulate mitochondrial outer membrane permeabilization (MOMP) and the release of apoptogenic factors such as cytochrome *c* from the inter-membrane space into the cytosol (1–3). These factors promote activation of proteolytic caspases, which dismantle the cell and ultimately cause the phenotypic changes characteristic of apoptosis.

BCL2 was the first mammalian apoptotic regulator to be identified, discovered as part of the t(14;18) reciprocal chromosomal translocation commonly found in human B cell lymphomas, such as follicular lymphoma (FL) (4–8). Subsequently, other antiapoptotic members were identified, including BCL-X_L, MCL-1, BCL-W, and BFL-1, as well as proapoptotic members such as BAX, BAK and BOK, and the BH3-only proteins BIM, BAD, BID, BIK, BMF, HRK, NOXA and PUMA. Antiapoptotic proteins like BCL-2, BCL-X_L and MCL-1 are often expressed at high levels in cancer cells, where they maintain survival by sequestering high levels of their pro-death counterparts – a state referred to as “primed for death” (Fig. 1B). These antiapoptotic proteins are now well established as validated, high-value cancer targets, but it has also come to be appreciated how extraordinarily challenging it is to generate drugs capable of inhibiting them. Several approaches have been explored (9–11), including the use of synthetic antisense oligodeoxynucleotides to suppress *BCL2* expression, the use of natural products with proapoptotic activity, and the synthesis of small-molecule “BH3 mimetics” (Fig. 1B) or chemically constrained peptides designed to bind antiapoptotic proteins directly and competitively displace proapoptotic proteins (12). While several of these approaches have shown promise, small-molecule BH3 mimetics are currently the most advanced, with multiple examples currently under assessment in the clinic (13).

Designing inhibitors selective for specific antiapoptotic BCL-2 family members has been especially challenging due to the extended hydrophobic nature of the target BH3-binding sites, the structural similarities among BCL-2 family members, and the necessity to compete for binding with high-affinity endogenous ligands. As with any difficult targets, innovative structure-based drug design approaches and sophisticated medicinal chemistry efforts have been central to the development of BCL-2 family inhibitors. Equally crucial has been the elucidation of BCL-2 biology that has facilitated the discovery and development of BH3 mimetics. Pioneering work using gene transfer techniques and genetic models like *C. elegans* (14, 15) and transgenic mice (have elucidated the role of *BCL2* as a novel oncogene that, instead of driving cell growth and proliferation, maintains tumor cell survival in the presence of other cancer-driving mutations by inhibiting programmed cell death (6, 8, 16–18). Decades of intense research in academia and industry, often performed in close collaboration, have led to a deeper understanding of how programmed cell death is regulated in both normal and cancer cells, how to assess on-target activity of direct apoptosis inducers (19), and how to identify tumor types and patient populations most likely to respond to these agents. These studies were instrumental in providing a framework for drug discovery scientists to design small-molecule BH3 mimetics.

BH3 mimetics: selective inhibitors of prosurvival BCL-2 family proteins

In 2005, Oltersdorf et al. reported the discovery of the BH3 mimetic ABT-737 using a novel nuclear magnetic resonance (NMR)-based chemical screening approach termed ‘SAR by NMR’, followed by parallel synthesis and structure-based design (20). ABT-737 bound BCL-2 and BCL-X_L with high affinity and demonstrated potent (sub-μM) single-agent killing of primary cancer cells and cancer cell lines as well as anti-tumor activity in mouse models (20–22). Later, Tse and colleagues described an orally bioavailable analog, navitoclax (ABT-263), which also inhibits BCL-2 and BCL-X_L (23). Oral administration of navitoclax induced tumor regressions in xenograft models of small-cell lung cancer (SCLC) and acute lymphoblastic leukemia (ALL), and enhanced the efficacy of regimens used to treat aggressive B-cell lymphoma and multiple myeloma (MM) in xenograft models (23). However, BCL-X_L was found to be essential for the survival of mature platelets (24–27) and navitoclax induced rapid, concentration-dependent decreases in circulating platelets. This was distinct from most types of chemotherapy-induced thrombocytopenia as navitoclax did not kill megakaryocytes or inhibit their production of young platelets. In a phase 1 trial exploring navitoclax monotherapy in patients with CLL, significant reduction in lymphocytosis was observed in 19/21 patients with baseline lymphocytosis, consistent with data from *bcl-2* knockout mice that had shown significant reductions in lymphocytes (28–29). Nine of 26 patients receiving ≥110 mg/day navitoclax achieved a partial response and 7 maintained stable disease for more than 6 months (30); however, thrombocytopenia was dose-limiting and restricted the ability to achieve higher, more efficacious exposures (30–32).

Faced with this challenge, drug discovery scientists set out to generate BCL-2-selective BH3 mimetics, hypothesizing that such molecules would maintain anti-tumor activity while sparing platelets. Leveraging clues from a unique BCL-2–small molecule cocrystal structure to guide rational design, AbbVie discovery scientists synthesized venetoclax (ABT-199/

GDC-0199), a potent, selective and orally bioavailable inhibitor of BCL-2 (33). Venetoclax demonstrated substantially less platelet killing than navitoclax *ex vivo* and *in vivo*, while at the same time showing striking anti-leukemic activity in BCL-2–dependent cell lines and xenograft models. This activity translated into the clinical setting, where venetoclax was recently approved by the Food and Drug Administration and the European Medicines Agency for the treatment of relapsed chronic lymphocytic leukemia with 17p deletion. Clinical development of venetoclax has continued apace with encouraging signs of activity in a broader CLL population as well as a variety of other hematologic malignancies. Below, we describe the preclinical approaches and findings that have informed a number of the most promising venetoclax clinical studies.

The preclinical data package

While few drug developers would confess to a strong belief in the predictive power of preclinical models, most would agree that some level of preclinical evidence is preferred when deciding to initiate clinical studies. Ideally, preclinical data packages in oncology should include the following elements: 1) evidence of the target's expression in the tumor type of interest, including cancer cell lines, *in vivo* tumor models and primary tumor tissues (patient samples); 2) evidence of the tumor type's dependence on that target for survival, as well as a mechanistic explanation for that dependence and for a therapeutic index relative to essential normal tissues; 3) pharmacodynamic evidence that the agent being considered is able to engage and inhibit the target(s) of interest *in vitro* and *in vivo* at clinically achievable concentrations; 4) evidence of strong anti-tumor activity *in vitro* (ideally across a number of cell lines and/or primary patient samples) and *in vivo* (ideally durable tumor regressions in multiple models); and 5) strong hypotheses regarding predictive markers of sensitivity (ideally related to the target's biology and linked to the agent's mechanism of action). Most often, all of these elements emerge from a strong understanding of the target's biology and serve to enhance confidence when selecting the tumor types and biomarker strategies to explore clinically. The sections that follow describe some key considerations specific to BCL-2 family inhibitors, novel approaches that have been taken to define BCL-2 family sensitivity profiles, and some striking examples of preclinical data packages that have directly informed the clinical development of venetoclax.

Defining BCL-2 dependence

What makes a tumor cell BCL-2 dependent and how can one determine this, either through surrogate markers or empirically? A good starting point is target expression. Some cell line panels exhibit sensitivity to BCL-2 inhibitors that correlates directly with BCL-2 expression – the more BCL-2 protein the cell line expresses, the more sensitive it is (33). Often, the cause of this high expression can be attributed to known genetic lesions, including the t(14;18) *Ig-BCL2* translocation or amplification of the *BCL2* locus on chromosome 18. These lesions offer convenient surrogate markers of likely protein overexpression (the functional "business end" of the DNA-to-RNA-to-Protein central dogma); however, they may miss tumors that overexpress *BCL2* through other mechanisms, such as aberrantly active cell signaling pathways or defects in microRNA-mediated regulation.

Of course, simply looking at BCL-2 expression alone is unlikely to tell the whole story. Because other antiapoptotic proteins such as BCL-X_L and MCL-1 can sequester proapoptotic proteins liberated from BCL-2 by BH3 mimetics (Fig. 1B), their expression levels may also weigh significantly in the predictive equation. Indeed, BCL-2 family ratios (for example, *BCL2*/*MCL1* mRNA) are better predictors of venetoclax sensitivity in some settings (see section on multiple myeloma, below). Taking a step further, the most relevant information may be the relative amount of proapoptotic proteins being sequestered by each relevant antiapoptotic protein at any given time, as well as the capacity and competence of their "backup catchers" to sequester free proapoptotic proteins before they can induce apoptosis. The dynamic interactions between BCL-2 family members are complex and can be influenced by a number of factors, likely to include the expression of different isoforms (34), post-translational modifications such as phosphorylation and ubiquitylation (35), and subcellular localization (for example, cytosolic versus membrane-embedded proteins may behave quite differently) (2, 36). These factors complicate things even further and could limit the utility of expression-based markers as predictors of venetoclax sensitivity. One potential solution is to determine the BCL-2 family dependence profile of a given cell population empirically. Enter the concept of priming and the era of "BH3 profiling".

As described earlier, "priming" refers to a state in which BCL-2 or other antiapoptotic family members sequester high levels of their proapoptotic counterparts to ensure the survival of a given cell (21, 37, 38). In 2006, Certo et al. reported a novel approach for determining which BCL-2 family members a given cell population depends on for survival, a method termed "BH3 profiling". Based on the pattern of mitochondrial sensitivity (using mitochondrial depolarization as an indicator of MOMP) to a panel of BH3 peptides with known binding affinity profiles, the BCL-2 family dependence profile of any cell population can be determined (1). This approach has led to the identification of a number of BCL-2-dependent tumor types and has provided strong support for the mitochondrial-based, on-target mechanism of action of venetoclax, a key criterion for any true BH3 mimetic. For example, BH3 profiling identified BCL-2 dependence and predicted sensitivity to the BCL-2 antagonist ABT-737 in a panel of 18 lymphoma cell lines (37), primary CLL cells and two myeloma cell lines (21), and in ALL primary cells and cell lines (39). In some cases BCL-2 dependence has been correlated with sequestration of the BH3-only activator protein BIM. Displacement of BIM from the BH3-binding pocket of BCL-2 by BH3 peptides allowed BIM to bind and activate BAX (Fig. 1A), leading to permeabilization of the outer mitochondrial membrane and triggering cell death (1, 21). BH3 profiling indicated that occupation of BCL-2 by certain BH3-only proteins prevents BCL-2 from buffering death signals and primes cancer cells with high BCL-2 expression (e.g., CLL and FL) for apoptosis, making them sensitive to conventional chemotherapies (21, 37). Of equal importance, BH3 profiling across many tissues showed that, with the exception of certain blood cells, the mitochondria of normal adult cells are not generally primed for apoptosis, leaving an excess of free antiapoptotic proteins relative to their proapoptotic counterparts. This may explain, in part, the favorable therapeutic index exploited by venetoclax in the treatment of BCL-2-dependent cancers (40, 41). Recently, Montero et al. described "Dynamic BH3 Profiling" (42), a technique that can be used to predict the response of cancer cells to chemotherapeutic agents, as well as identify agents capable of inducing

BCL-2 priming (Fig. 2A–B). In this approach, BH3 profiling is performed before and after treatment with a given agent to determine how a cell population's BCL-2 family-dependence profile changes. Some agents leave cells in a state where BCL-2 is highly primed, for example with BIM or BAX (Fig. 2A), making them excellent candidates for combination with BCL-2 inhibitors like venetoclax. A number of preclinical studies have identified agents that combine effectively with venetoclax by inducing BCL-2 priming (see Fig. 2B for examples).

Another means of defining cellular BCL-2 family dependence profiles involves the use of selective small-molecule inhibitors, an approach that has been referred to as "chemical parsing" (43). Instead of BH3 peptides, cell-permeable BH3 mimetics can be used in cell killing assays to determine which antiapoptotic proteins a given population depends on for survival. For example, a recent study used the BCL-2-selective inhibitor venetoclax and the BCL-X_L-selective inhibitors A-1155463 and A-1331852 to define the BCL-2 family dependence profiles of AML and lung cancer cell lines, and to parse out the effects of navitoclax when combined with taxanes (44). Whereas BCL-X_L inhibition was sufficient to enhance the efficacy of docetaxel in a range of solid tumor types, BCL-2 inhibition accounted for inhibitory effects on granulopoiesis, which potentially explains the exacerbation of neutropenia that was observed when navitoclax was combined with docetaxel in the clinic. These data also suggest that adverse events of neutropenia, which are commonly observed in venetoclax clinical trials (see Table 1), reflect on-target toxicity. These results indicate that a BCL-X_L-selective profile may be superior for therapeutics aimed at solid tumors, and demonstrate the utility of 'chemical parsing' beyond the dissection of basic apoptosis biology. Indeed, more and more examples of chemical parsing are being reported as the uptake and use of these potent and selective probes increases (45–49).

Identifying sensitive tumor types and likely responders

Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) was among the first B-cell tumor types found to be highly sensitive to ABT-737 and navitoclax, based largely on pioneering preclinical work. Although CLL cells do not typically exhibit *BCL2* amplification or translocations, this tumor type is now appreciated to be amongst the most BCL-2 dependent. Using CLL patient samples and cell lines, Cimmino et al. reported decreased levels or deletion of two microRNAs, miR-15a and miR-16, that normally suppress the expression of BCL-2 (50). Oltersdorf et al. had reported that ABT-737 induced concentration-dependent apoptosis in patient-derived CLL B-cell samples (20), and similar activity was later observed with navitoclax and venetoclax, demonstrating that BCL-2 inhibition is sufficient to kill CLL cells (30, 31, 33, 51). It was not until 2007 that it was clearly shown, using BH3 profiling, that CLL cells are uniformly dependent on BCL-2, and hence a promising target for treatment with BH3 mimetics (21).

As noted earlier, navitoclax showed promising activity in CLL, inducing a number of objective responses, but its potential was hampered by dose-limiting thrombocytopenia driven by BCL-X_L inhibition. Venetoclax was specifically designed as a BCL-2-selective

inhibitor to remedy this problem and the initial proof of concept in CLL was striking. Two of the first three patients to receive a single dose of venetoclax exhibited extensive reductions in circulating tumor burden within 8 hours, reduced palpable lymphadenopathy within 24 hours, and signs of laboratory tumor lysis syndrome – a clear sign of biological activity (33). Despite the majority of patients on that study having received multiple previous lines of treatment, venetoclax proved to be active at all dose levels tested (150–1200 mg per day). Among 116 patients who received venetoclax, 79% had an objective response and 20% achieved complete remission (52). As an exploratory objective, minimal residual disease (MRD) was evaluated in 17 of the 23 patients who had a complete response and 6 (35%) had negative MRD results (less than 1 tumor cell detected in every 10,000 blood cells analyzed by flow cytometry). The 15-month progression-free survival (PFS) estimate was 69% at the 400-mg dose level. Tumor lysis syndrome (TLS) was the most significant safety concern, with clinical TLS occurring in 3 of 56 patients (including one TLS-related death) in the initial dose-escalation cohort. Other side effects reported included mild diarrhea, upper respiratory tract infection, nausea, and grade 3/4 neutropenia (52). In a phase 2 open label, multicenter study, venetoclax monotherapy demonstrated activity in patients with relapsed/refractory del(17p) CLL (53). Overall, objective response was achieved in 85 (79%) of the 107 patients enrolled in the trial. Most common grade 3/4 adverse events were neutropenia (40%), infection (20%), anemia (18%), and thrombocytopenia (15%). Encouraging results from these studies prompted additional investigations of venetoclax as monotherapy and in combination regimens in patients with CLL (see Table 1). Based on the safety and efficacy demonstrated in phase 1 and phase 2 trials, venetoclax was granted Breakthrough Therapy Designation by the U.S. Food and Drug Administration (FDA) in May 2015 and approved in April 2016 for use in patients with relapsed/refractory CLL with del(17p) who received at least one prior therapy. Conditional marketing approval was granted by the European Medical Agency in 2017, followed by approvals in Canada, Mexico, Puerto Rico, Uruguay, Argentina, Israel, Saudi Arabia, Turkey, Singapore and Australia.

When added to the CD20 antibody rituximab, either alone, or as part of multi-drug regimens like BR or R-CHOP, venetoclax enhanced efficacy in a number of preclinical models (33). The venetoclax-rituximab combination demonstrated an 86% overall response rate (ORR) in R/R CLL, with 51% complete responses (CR) (54), and was granted a Breakthrough Therapy Designation by the FDA for the treatment of relapsed/refractory CLL in January 2016. Additionally, the combination of venetoclax with the second generation anti-CD20 antibody obinutuzumab, which is also approved for the treatment of CLL, resulted in increased cell death in CLL patient samples treated *ex vivo* and durable tumor regressions in NHL xenograft models when compared to each agent alone (55). These promising preclinical results have translated well in the run-in safety portion of a randomized phase III clinical trial in which a small cohort of patients were treated with the combination of venetoclax and obinutuzumab (NCT02242942). Remarkably, an overall response rate of 100% and a 92% MRD-negativity rate in peripheral blood 3 months after treatment cessation were observed (56). While the clinical results are promising, it will be important to determine whether similar efficacy can be observed in the randomized phase of the trial.

A number of other promising agents have also been approved for CLL in recent years, and the next phase of venetoclax development has focused on rational drug combinations (Fig. 2, Table 1). Abnormal activation of the B-cell receptor (BCR) signaling pathway contributes to the pathogenesis of CLL, in part through driving cell autonomous proliferation and survival and in part by driving tumor cell homing to protective niches in the bone marrow and lymph nodes. This provides a rationale for testing venetoclax in combination with BTK, PI3Kδ, and SYK inhibitors, which are known to mobilize malignant cells into peripheral circulation (Fig. 2A, right panel). From BH3 profiling studies, Davids et al. observed that treatment with the phosphatidylinositide 3-kinase (PI3K) delta-isoform inhibitor idelalisib caused CLL cell de-adhesion from supporting stromal cells, leading to increased apoptotic priming (57). These findings suggest that the addition of idelalisib could be used to deepen responses to BCL-2 inhibitors by freeing relatively resistant CLL cells in lymph nodes and the bone marrow into the blood, where they can be more effectively killed. Similarly, Patel et al. reported that BCL-2 was upregulated in peripheral blood mononuclear cells from patients treated with the PI3K delta/gamma inhibitor duvelisib (58). Additionally, *ex vivo* incubations of pre- and post-duvelisib patient samples with venetoclax induced significantly greater apoptosis in post-therapy samples as compared to pre-treatment samples. These data provide further support for the combination of venetoclax with PI3K inhibitors as a rational approach to treating CLL patients. Pharmacological profiling and BH3 profiling of residual CLL cells from patients treated with the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, revealed BCL-2 dependence and sensitivity to venetoclax *ex vivo* (59). Following ibrutinib treatment, there is a transient increase in circulating leukemia cells due to mobilization of lymphocytes from lymph nodes, and levels of MCL-1 and BCL-X_L in these cells were decreased. A number of clinical studies are now assessing regimens including ibrutinib and venetoclax (see Table 1 for one example).

Non-Hodgkin Lymphoma

Because *BCL2* was first discovered as part of the t(14;18) translocation that defines follicular lymphoma (FL) and other non-Hodgkin lymphomas, additional clinical efforts with venetoclax were focused on these diseases. Even before BH3 mimetics were available for *in vivo* use, proof of principle was provided genetically that loss of BCL-2 alone in a BCL-2-overexpressing lymphoid malignancy was sufficient to cause rapid tumor remission (60). Early preclinical work with venetoclax had demonstrated potent cell killing activity in a subset of NHL cell lines, including diffuse large B-cell lymphoma (DLBCL), FL and mantle cell lymphoma (MCL) (33). Not surprisingly, venetoclax was especially active against NHL cell lines bearing the t(14;18) translocation or amplification of the *BCL2* locus, and its cell killing potency correlated directly with BCL-2 protein expression, which may thus serve as a predictive marker of tumor response. Venetoclax was also highly active *in vivo*, demonstrating single agent efficacy in xenograft models of DLBCL (33) and aggressive progenitor cell lymphomas derived from bitransgenic *MYC/BCL2* mice (61), as well as in combination with commonly used regimens (R, BR, R-CHOP) (33). Spurred by these data, a phase 1 monotherapy study and a number of combination studies were initiated to explore venetoclax in patients with relapsed or treatment refractory NHL (Table 1). Results from the phase 1 monotherapy study demonstrated a 44% ORR with an estimated median progression-free survival of 6 months in patients with NHL (62). Although these

data were encouraging, they suggest that the t(14;18) translocation is not wholly sufficient to predict deep responses to a BCL-2 inhibitor, at least not to the same degree that a lesion like *BCR-ABL* predicts response to ABL tyrosine kinase inhibitors like imatinib.

One key culprit may be the pro-survival protein MCL-1 (Fig. 1B), which is frequently coexpressed with BCL-2 in DLBCL patient samples and cell lines (63). BCL-2 and MCL-1 dependent subgroups can be identified by pharmacological targeting of the two proteins (64). Single-agent treatment with venetoclax was found to induce only modest antitumor activity against certain DLBCL cell lines and resulted in a compensatory increase in expression of MCL-1 (63). The combination of venetoclax with MCL-1 modulators such as dinaciclib (a potent cyclin-dependent kinase [CDK] 9 inhibitor that induces loss of MCL-1) or other standard DLBCL chemotherapy agents affecting MCL-1 levels resulted in potent antitumor activity in xenograft models and a murine model of *MYC-BCL2* double-hit lymphoma (63). Certain t(14;18)-positive cell lines such as SU-DHL-4, although high BCL-2 expressers, have been demonstrated to be resistant to venetoclax due to MCL-1 expression. In these cases, adding a CDK9 inhibitor (alvocidib/flavopiridol) or a direct inhibitor of MCL-1 (A-1210477) to venetoclax leads to synergistic cell killing (65). Additional synergies may also exist with other pathways known to promote tumor cell survival, as has been reported using the dual PI3K/mTOR (mammalian target of rapamycin) inhibitor BEZ235 and venetoclax (66).

MYC, BCL-2 and/or BCL-6 overexpression due to genetic rearrangements are key features of double-hit or triple-hit lymphoma (DHL/THL), aggressive forms of B cell lymphoma with poor survival rates and no truly effective treatment options (67, 68). Venetoclax has been shown to induce cell death in DHL cell lines Sc-1 and Ocl-LY18, the RL cell line and primary human DHL cells that overexpress BCL-2. Synergistic killing of DHL cell lines was observed when venetoclax was combined with doxorubicin, cytarabine, bortezomib or JQ-1 (a BET inhibitor which down-regulates c-MYC expression), providing a rationale for combination therapies for patients with DHL (68).

Mantle cell lymphoma (MCL) is an aggressive lymphoma with limited treatment options, and is characterized by the t(11;14) translocation that drives Cyclin D1 overexpression. Ackler et al. described a novel systemic tumor model that uses a bioluminescent Granta-519 reporter cell line to recapitulate MCL disease progression, including bone marrow engraftment and central nervous system penetrance (69). Treatment with venetoclax or navitoclax, alone or in combination with standard of care agents bendamustine and rituximab (BR), resulted in extensive tumor cell apoptosis in this model. Synergistic inhibition of MCL proliferation has also been observed when the BTK inhibitor ibrutinib was combined with venetoclax *in vitro* (70). The combination also displayed significant induction of apoptosis in primary cells from two recurrent MCL patients. Recently, the first data from a Phase 2 clinical study combining venetoclax and ibrutinib for MCL patients revealed a higher response rate than historically observed with either agent alone (71). Moreover, a greater portion of complete responses was observed, with many of those patients exhibiting MRD negativity (Table 1). Sequential treatment with venetoclax and BTK inhibitors may also overcome impaired sensitivity to venetoclax resulting from CD40-mediated increase in BCL-X_L expression, as demonstrated using MCL cell lines and

peripheral MCL cells acquired from a patient undergoing ibrutinib treatment (72). Combination of the BET inhibitor JQ-1 with venetoclax synergistically induced apoptosis of ibrutinib-resistant MCL cell lines (73), perhaps suggesting another strategy to improve treatment options and outcomes for patients with MCL.

Multiple myeloma

Although numerous effective treatment options have emerged for multiple myeloma in recent years (for example proteasome inhibitors, antibodies, and IMiDs), the disease remains lethal and better therapies are still needed. Early studies of the proteasome inhibitor bortezomib linked its activity to modulation of the intrinsic apoptosis pathway. By stabilizing the proteasome substrate NOXA, a BH3-only protein that binds and neutralizes MCL-1 (Fig. 1D), bortezomib was shown to liberate the prodeath proteins BIM and BAK (23, 74–76). This led to the hypothesis that bortezomib could be combined with BCL-2 inhibitors to enhance killing of MM cells that co-express MCL-1 and BCL-2. Indeed, treatment of MM xenograft models with bortezomib increased NOXA and enhanced anti-tumor activity when combined with venetoclax (77). Based on this strong mechanistic rationale and a strong preclinical data package, a phase 1 study investigating the combination of venetoclax with bortezomib and dexamethasone was initiated. Preliminary results have shown an acceptable safety profile and promising responses, particularly in patients naïve or sensitive to prior bortezomib therapy (78). Synergistic effects with BCL-2 antagonists have also been explored with histone deacetylase and insulin-like growth factor-1 inhibitors as potential therapeutic options for patients with MM (79–81).

Survival dependencies in MM cells have been shown to be governed by the distribution of BIM between BCL-2/BCL-X_L and MCL-1 (38). Various molecular subtypes of MM have been identified and the heterogeneity extends to differential expression of antiapoptotic BCL-2 members in each molecular subgroup (82), potentially influencing response to BCL-2 inhibitors. Bodet, Amiot and colleagues demonstrated that ABT-737 is particularly active against t(11;14)-translocated MM cells, which express high levels of *BCL2* relative to *MCL1* (83). More recently, Touzeau et al. reported that venetoclax behaves similarly, exhibiting the highest activity against cell lines or patient samples with a t(11;14) translocation and high *BCL2*/*MCL1* mRNA ratios (84). At about the same time, objective responses were being reported in a phase 1 study exploring venetoclax monotherapy in heavily pretreated patients with relapsed/refractory MM (85). Whereas the overall response rate was 21%, the subset of patients with t(11;14) MM demonstrated a 40% response rate, with a high proportion showing complete (10%) or very good partial (17%) responses. These data indicate that the t(11;14) translocation may enable an enrichment for patients more likely to respond to venetoclax-based therapy and could set the stage for the first biomarker-directed therapy in multiple myeloma.

More recent data indicate that BCL-X_L may also play a significant role in multiple myeloma. BH3 profiling identified subsets of MM cell lines and primary MM cells dependent on either BCL-2 alone or on BCL-2 and BCL-X_L together (86). Gong et al. also identified a sub-set of MM cell lines that were highly dependent on BCL-X_L (87). Similar observations were made by Punnoose and colleagues, whose chemical parsing experiments

demonstrated that MM cells co-expressing BCL-2 and BCL-X_L were resistant to venetoclax but sensitive to navitoclax or the BCL-X_L-selective inhibitor A-1155463 (77). MM xenograft models that co-expressed BCL-X_L or MCL-1 with BCL-2 were resistant to venetoclax. Immunohistochemistry of MM patient bone marrow biopsies and aspirates (n=95) revealed high levels of BCL-2 and BCL-X_L in 62% and 43% of evaluable samples, respectively, whereas 34% were characterized as BCL-2^{High}/BCL-$X_L{}^{Low}$ (77). Whereas BCL-2 dependence is quite homogeneous in CLL, MM provides an example of a disease where dependence on individual anti-apoptotic proteins varies greatly from case to case and a broader evaluation of predictive biomarkers in the clinical setting will be informative.

Acute myelogenous leukemia

AML is a particularly aggressive leukemia, with dismal outcome. Despite high rates of complete remissions with standard induction chemotherapy, only 30% will achieve long-term disease-free survival (88, 89). Because it is largely a disease of older adults, many of whom are ineligible for intensive chemotherapy-based induction, better-tolerated treatments are clearly needed. ABT-737 has been shown to effectively kill AML blast, progenitor and stem cells, while sparing normal hematopoietic cells (90). BH3 profiling identified differences in mitochondrial priming between myeloblasts and normal hematopoietic stem cells which suggested that BCL-2 inhibition would be selectively toxic to myeloblasts (91). In 2014, two studies reported that AML cell lines, primary patient samples and murine primary xenografts are sensitive to venetoclax, as are AML cells with the acute promyelocytic leukemia phenotype or harboring mixed-lineage leukemia (MLL) fusion genes (92, 93). BH3 profiling studies indicated an on-target mitochondrial mechanism of action with venetoclax that correlated with cytotoxicity (93, 94). Of interest, AML cells with mutations in the *isocitrate dehydrogenase (IDH) 1* and *2* genes have been found to be particularly sensitive to venetoclax (95). Mutant IDH1/2 proteins are known to catalyze production of the oncometabolite (R)-2-hydroxyglutarate, which can elicit epigenetic changes, dysregulate mitochondrial function, and induce BCL-2 dependence in AML cells (95–97). Together, these preclinical findings provided the impetus for a clinical evaluation of venetoclax monotherapy in AML patients. Results from a phase 2 study of venetoclax in relapsed/refractory AML patients demonstrated a modest response rate of 19% CR/CRi (complete response/complete response with incomplete blood count recovery) in heavily pretreated AML patients but higher response rates were observed in the subset of patients with IDH1/2 mutations (33% CR/CRi) (98). Although these data were a clear indicator of biological activity in AML, the effects of venetoclax monotherapy were not durable (median time on study ranging 13 – 246 days), and the next logical step was to explore combinations with currently approved standards of care.

ABT-737 and 5-Aza have exhibited strong synergy in short-term *ex vivo* cultures of myeloid malignancies, including *de novo* and secondary AML, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (99). Subsequent *ex vivo* studies using primary AML samples demonstrated ABT-737 and venetoclax single agent activity as well as synergy with 5-Aza (100). In an earlier study, Tsao et al. demonstrated synergistic AML cell killing by 5-Aza and ABT-737, in part through inhibition of MCL-1 by 5-Aza (22). In a phase 1b study, venetoclax in combination with the hypomethylating agents decitabine or azacitidine had a

tolerable safety profile in treatment-naïve AML patients not eligible for standard induction therapy, and achieved a 66% overall response rate (64% RR in patients treated with venetoclax and azacitidine) (101). In January 2016, the FDA granted venetoclax Breakthrough Therapy Designation in combination with hypomethylating agents for the treatment of patients with untreated (treatment-naïve) AML who are ineligible to receive standard induction therapy (high-dose chemotherapy). A separate phase 1/2 study assessed the combination of venetoclax with low-dose cytarabine for elderly, treatment naïve AML patients not eligible for standard induction chemotherapy (102). The combination of venetoclax with low-dose cytarabine was well tolerated, demonstrating a 61% ORR with 54% of patients achieving durable CR/CRi. In July 2017, the FDA granted venetoclax its fourth Breakthrough Therapy Designation for use in combination with low-dose cytarabine in treatment-naïve elderly patients with AML who are ineligible for intensive chemotherapy. Combinations with higher doses of induction chemotherapy in younger patients are also ongoing (Table 1).

Combinations with investigational targeted agents have also been explored preclinically, including pairings of ABT-737 or venetoclax with the MDM2 inhibitor Nutlin-3a (103), mitogen-activated protein kinase kinase (MEK) inhibitors PD0325901, GDC-0973 (cobimetinib) (104) and CI-1040 (105), the PI3K inhibitor GDC-0941 (106), and the dual mTORC1/2 inhibitor INK128 (107), all of which have shown strong synergistic anti-leukemic activity *in vitro* and robust combination responses in xenograft models. A synergistic increase in apoptosis has also been observed in AML cell lines and in primary cells, and confirmed in tumor xenograft models, with the combination of the MEK1/2 inhibitor pimasertib with navitoclax (108). Zhang et al. evaluated concomitant blockade of BCL-2, MEK and mTOR signaling pathways with ABT-737, selumetinib and AZD8055, respectively, and the triple combination produced marked cytotoxic effects in CD33+/CD34+ AML progenitor cells from primary AML samples with *NRAS* mutations (109). Combined inhibition of BCL-2 and the NEDD8 activating enzyme (NAE), which triggers increased expression of NOXA and MCL-1 antagonism, has also demonstrated synergistic activity in AML models (110). Recently, the combination of the selective MDM2 antagonist idasanutlin and venetoclax demonstrated synergistic anti-tumor activity in p53 wild-type AML cell lines, and led to superior efficacy and survival relative to either agent on its own in subcutaneous and systemic AML models (111, 112). These findings prompted the initiation of a Phase 1b study (Table 1).

Future considerations and conclusions

Venetoclax has demonstrated promising preclinical activity in a variety of hematologic tumor models, both as a single agent and in combination with other drugs. Table 1 summarizes these findings alongside the corresponding results from the clinical studies that they informed. In addition, preclinical data sets are now maturing regarding the use of venetoclax in numerous other tumor types, including MDS, acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML) and blastic plasmacytoid dendritic cell neoplasm (BPDCN). Based on the successful translation of venetoclax preclinical studies in CLL, NHL, MM and AML, there is reason for optimism that additional uses for BCL-2 inhibitors will be found and that additional patients will benefit.

Based on early signals of activity in AML, venetoclax activity is also being explored in MDS, which can transform into secondary AML. In a transgenic model of MDS progressing to AML, treatment with ABT-737 reduced bone marrow blasts and progenitor cells by increasing apoptosis and significantly extended lifespan (113). Jilg et al. demonstrated that inhibition of BCL-2 and BCL-X_L with ABT-737 or specific inhibition of BCL-2 with venetoclax was toxic to bone marrow cells from high-risk MDS and secondary AML patients, whereas cells from low-risk MDS and normal, age-matched bone marrow remained largely unaffected (114). Sensitivity to ABT-737 and venetoclax was observed in samples from treatment-naïve and treatment failure patients, suggesting that the effects of BH3 mimetics depend primarily on the level of mitochondrial priming irrespective of prior treatment. Additionally, both ABT-737 and venetoclax decreased CD34+ cells and colony-forming capacity, indicating that they may be able to target the MDS stem/progenitor cell population. Collectively, these results suggest that treatment with BH3 mimetics may delay disease progression in high-risk MDS patients, and studies evaluating venetoclax, alone or in combination with azacitidine, have been initiated (see Table 1).

Using BH3 profiling, Del Gaizo Moore et al. demonstrated BCL-2 dependence in both ALL cell lines and primary patient samples (39). Recently, Suryani et al. reported strong activity with navitoclax in pediatric ALL xenografts with low levels of *MCL1* mRNA (115). Primary B-cell lineage ALL cell cultures have been shown to be highly sensitive to navitoclax and venetoclax (116). T-ALL is a high-risk ALL subtype exhibiting therapy resistance or relapse despite intensified chemotherapy, and an early T-cell progenitor (ETP) subgroup of T-ALL has a very high-risk of relapse. BH3 profiling and studies using the LOUCY cell line indicate that venetoclax, alone or in combination with chemotherapeutic agents, may represent a powerful new therapeutic strategy for ETP T-ALL (117–119). Benito and colleagues found that blasts from mixed lineage leukemia-rearranged (MLLr) ALL express high levels of BCL-2, and ChIP sequencing analysis demonstrated that the *BCL2* gene is a direct MLL-AF4 target (120). MLL-AF4 maintains *BCL2* gene expression through H3K79 methylation, which translates into high sensitivity of MLLr cell lines and primary cells to venetoclax, alone or in combination with standard induction chemotherapeutics or H3K79 methylation inhibitors. Khaw and colleagues confirmed that PDX models of MLL-r infant ALL are sensitive to venetoclax; however, they did not observe venetoclax sensitivity in two PDX models of pediatric ETP-ALL (121), indicating that other factors may influence survival in these tumors.

In CML, tumorigenicity is driven by the BCR-ABL tyrosine kinase, which can regulate the expression of BCL-X_L and MCL-1. Venetoclax has shown synergistic effects against CML progenitor cells when combined with the tyrosine kinase inhibitor (TKI) imatinib and high expression levels of BCL-2 in CML and normal cord blood progenitors were found to predict sensitivity to venetoclax (122). More recently, Carter, Andreeff and colleagues demonstrated that TKIs can downregulate MCL-1, leading to synergy with venetoclax in killing CML cells, including quiescent cells and blast crisis cells – populations notoriously difficult to eradicate with TKIs alone (123). Moreover, a venetoclax-nilotinib combination was highly effective in a genetically engineered mouse model of chronic phase CML, reducing tumor burden in multiple compartments and prolonging survival. Most notably, the combination also reduced the levels of leukemic stem cells as assessed by flow cytometry

and gold standard serial transplantation experiments. These data suggest that venetoclax-TKI combinations could be employed with the aim of eradicating disease and discontinuing treatment, a major goal of cancer therapy. Similarly, the CLL field is also exploring whether finite treatments with venetoclax-containing regimens might enable treatment cessation and extended treatment-free intervals, based on encouraging rates of MRD-negativity in early venetoclax studies.

BPDCN is a rare but clinically aggressive hematologic malignancy that commonly manifests as cutaneous lesions with or without bone marrow involvement and leukemic dissemination. A study evaluating the dependence of plasmacytoid dendritic cells (pDCs) and conventional DCs (cDCs) on antiapoptotic proteins revealed that pDCs had higher levels of BCL-2 compared with cDCs, and were selectively killed when cultured *in vitro* with venetoclax, highlighting their dependence on BCL-2 for survival (124). Using a similar chemical parsing approach, the BCL-2 inhibitors venetoclax and ABT-737 were found to selectively kill mouse and human pDCs but not cDCs, and synergized with glucocorticoids to kill activated pDCs (125). These findings indicated that BCL-2 antagonists may be attractive candidates for treating pDC-associated diseases such as BPDCN. Indeed, a recent study from Montero, Lane, Pemmaraju and colleagues showed that BPDCN cells are exquisitely BCL-2 dependent, exhibiting *ex vivo* sensitivity to venetoclax on par with CLL cells (126). Venetoclax exhibited clear efficacy in patient-derived xenograft models of BPDCN, improving survival, and notable signs of activity were also observed in two BPDCN patients treated with venetoclax off-label, providing the first evidence that these preclinical data may translate well in the clinical setting.

Although the bulk of the peer-reviewed literature focuses on the role of BCL-2 in hematologic malignancies, BCL-2 inhibitors may also find utility in solid tumors and even in non-cancerous diseases. For example, Cittelly et al. described a mechanism in estrogen receptor (ER)-positive breast cancers that overexpress human epidermal growth factor receptor (HER) 2, which involves both BCL-2 overexpression and suppression of miR-15a and miR-16 (127), the same *BCL2*-targeting microRNAs that are commonly lost or downregulated in CLL (50). Both ABT-737 and venetoclax have been shown to enhance sensitivity to tamoxifen in ER-positive breast cancer PDX models, further supporting the hypothesis that BCL-2 is an important target in these tumors (128). A clinical study is now underway in Australia to explore the venetoclax-tamoxifen combination in patients with metastatic ER-positive HER2-non-amplified breast cancer (see Table 1). Treatment with ABT-737 has also been shown to restore sensitivity to paclitaxel in endocrine-resistant breast cancer (129). Additional approaches combining BH3 mimetics with investigational agents such as PI3K/mTOR inhibitors (130, 131) and gamma secretase inhibitors (132) are also being explored to bypass resistance mechanisms and enhance efficacy. In small cell lung cancer (SCLC), ABT-737, navitoclax and venetoclax have shown antitumor activity in cell lines and xenograft models (44, 133–136) and limited single-agent activity has been observed with navitoclax in patients with SCLC (137, 138). Combination with other chemotherapeutic agents including rapamycin and vorinostat, or inhibition of other signaling pathways such as PI3K/BMX, appears to be a promising approach to overcoming resistance and enhancing sensitivity to BH3 mimetics in SCLC (139–142).

Recently, Ko et al. reported that BCL-2 expression is elevated in infiltrating T- and B-lymphocytes found in the renal tubulointerstitium of lupus nephritis patient biopsies, indicating that BCL-2 may be an attractive therapeutic target in systemic lupus erythematosus (SLE) (143). Indeed, venetoclax was able to prevent proteinuria and tubulointerstitial inflammation in the New Zealand hybrid mouse model NZB/NZW, which manifests disease features consistent with human SLE. These findings suggest that targeting BCL-2 may be a viable treatment strategy in other inflammatory diseases where dysregulation of apoptosis occurs.

In summary, the history of venetoclax to this point includes a number of notable translational successes. Guided by many of the preclinical studies described here, venetoclax has demonstrated clear signs of antitumor activity both as monotherapy and in combination with standard-of-care regimens or novel targeted agents (see Fig. 2). Nevertheless, a number of challenges remain, including the identification of robust clinical biomarkers which can identify patients most likely to benefit from venetoclax. Smith et al. have described one approach which employs a fluorescence-based flow cytometry method for quantifying BCL-2 family members in cell lines and clinical samples from patients with CLL (144). BH3 profiling and chemical parsing may provide an alternative, functional means of assessing tumor sensitivities to BH3 mimetics. Another challenge is to better understand potential mechanisms of resistance to BCL-2-selective inhibitors, which include upregulation of BCL-X_L, MCL-1 or other antiapoptotic BCL-2 relatives, downregulation or mutation of proapoptotic proteins (BIM, BAX), and post-transcriptional modification or mutation of the BCL-2 protein (145–147). Understanding toxicities and identifying potential mitigation strategies will also be crucial. For example, some CLL patients receiving venetoclax have undergone Richter's transformation (52), which typically involves conversion into DLBCL. The mechanisms behind this are currently not understood and, thus far, no factors have been identified that might predict a patient's likelihood of experiencing Richter's transformation while on venetoclax.

Although it is well accepted that preclinical models may not always predict success in the clinic, the story of venetoclax provides a striking example of how sound preclinical work and a deep understanding of target biology can facilitate drug discovery and guide clinical development. An important lesson we have learned is that the job of assigning novel, biologically active drugs for use in different types of cancer cannot be left to genetics alone. In several of the tumor types discussed here - *e.g.*, AML, ALL and BPDCN, there are no obvious *BCL2* lesions that would indicate sensitivity to BCL-2 inhibition. Instead, these vulnerabilities were identified by careful *in vitro* and *in vivo* study based on the mechanism of BCL-2 family interactions and the mechanism of action of BH3 mimetics. There are still challenges ahead but the field's ever-increasing understanding of BCL-2 family biology will continue to guide the progression of venetoclax from bench to bedside as better therapeutic options are sought for patients with cancer.

Acknowledgments

Medical writing support was provided by Kakuri Omari, Ph.D., CMPP, of Evidence Scientific Solutions (Philadelphia, PA, USA), which was funded by AbbVie, Inc.

Marina Konopleva has been a consultant to and received research funding from AbbVie and Genentech.

Anthony Letai has been an advisor to and his laboratory has received research funds from AbbVie, AstraZeneca, Tetralogic, and XrX.

AbbVie: AbbVie Inc. funded medical writing for this manuscript, and participated in reviewing and approving of this publication. All authors participated in the development, review, and approval, and in the decision to submit this publication.

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Significance

Basic research into the pathways governing programmed cell death have paved the way for the discovery of novel apoptosis-inducing agents such as venetoclax, a BCL-2-selective inhibitor that was recently approved by the FDA and EMA. Preclinical studies aimed at identifying BCL-2-dependent tumor types have translated well into the clinic thus far, and will likely continue to inform the clinical development of venetoclax and other BCL-2 family inhibitors.

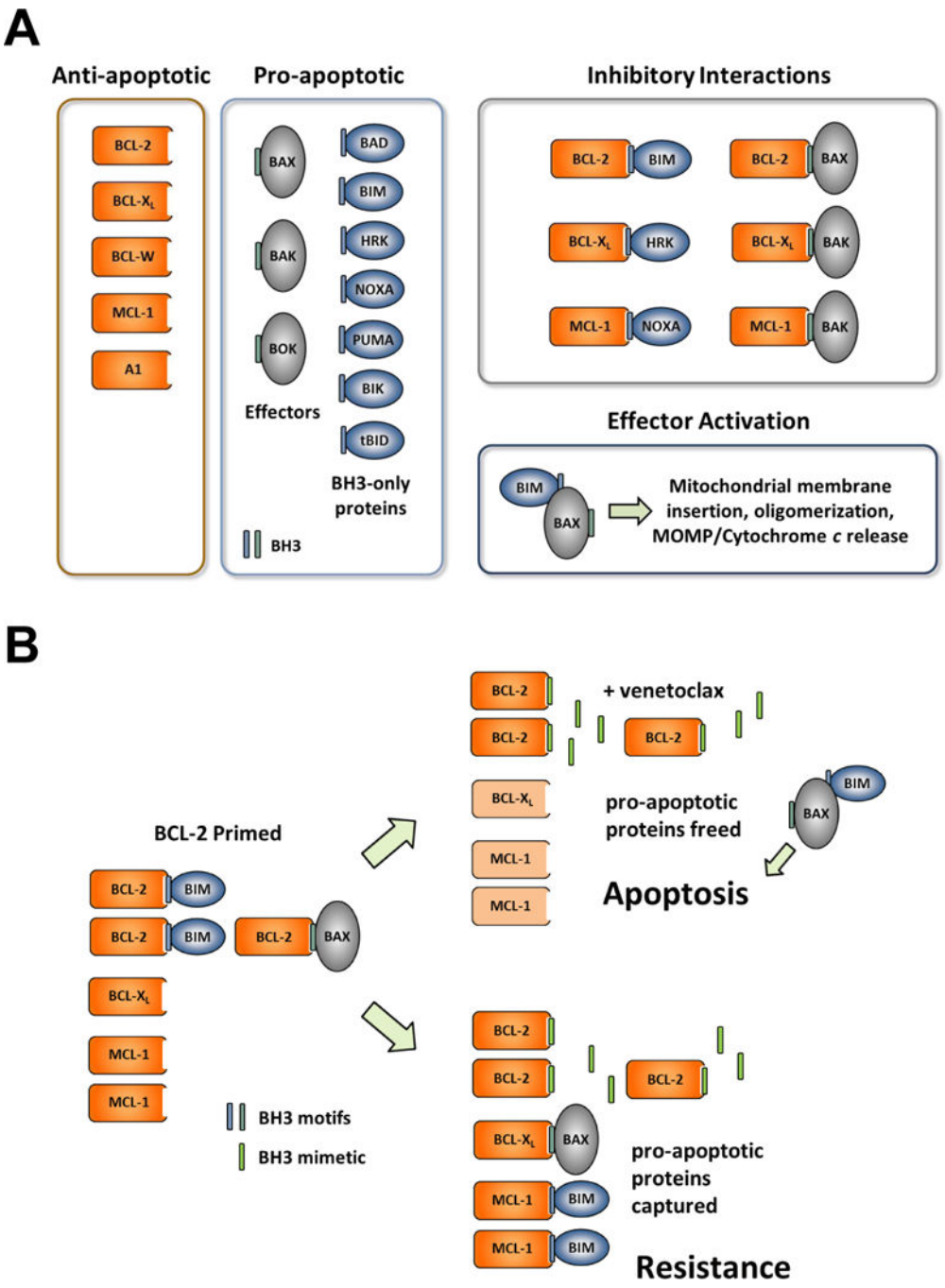
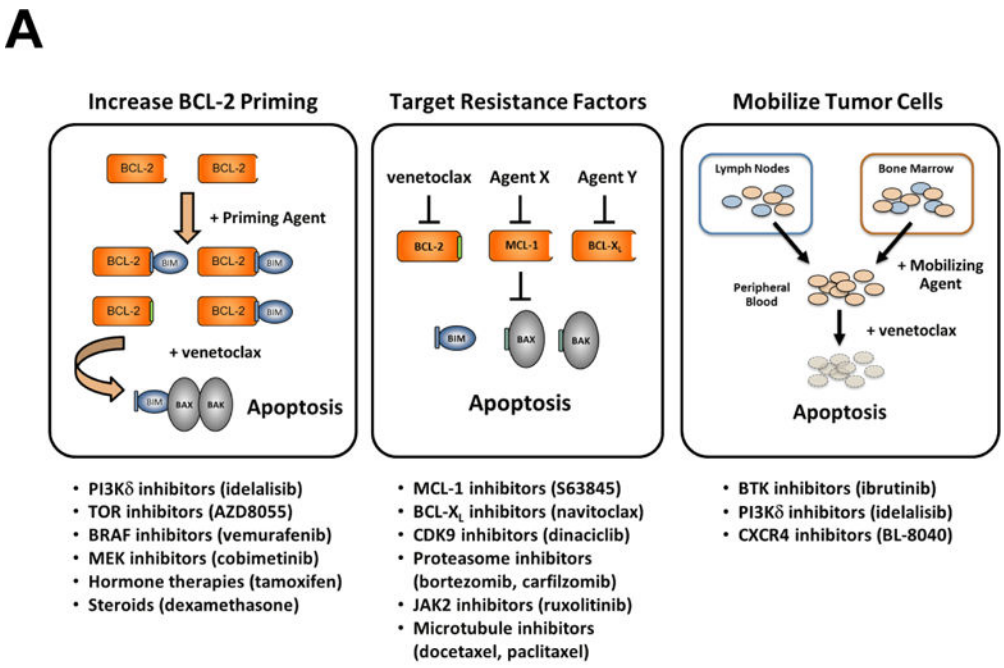


Figure 1. Apoptotic "priming" and BH3 mimetics

a) BCL-2 family proteins regulate the intrinsic pathway of apoptosis and can be divided into antiapoptotic and proapoptotic subgroups. Antiapoptotic proteins sequester proapoptotic proteins by binding to their BH3 motifs (blue rectangles) and often exhibit preferential binding to specific family members. Some BH3 proteins, such as BIM, can directly activate effector proteins, facilitating their insertion into the mitochondrial outer membrane, oligomerization, and subsequent mitochondrial outer membrane permeabilization (MOMP). **b)** Antiapoptotic proteins are often overexpressed in cancer cells, where they sequester high

levels of proapoptotic proteins to maintain survival. Such cells are poised to initiate apoptosis upon the release of sufficient quantities of proapoptotic proteins, a state referred to as “primed for death”. The figure at left represents a cell with primed BCL-2. Small-molecule BH3 mimetics such as venetoclax (green rectangles) can competitively displace proapoptotic proteins to trigger programmed cell death. However, other antiapoptotic proteins such as BCL-X_L and MCL-1 can capture proapoptotic proteins liberated by venetoclax, thereby acting as resistance factors.



B

	Mechanism	Agent(s)	Key Target	References
BCL-2	Direct inhibitor, BH3 mimetic	venetoclax S55746 (BCL201)	BCL-2	(33)
BCL-2 + BIM	Elevated BCL-2 priming – BIM stabilization/upregulation	UO126, selumetinib ibrutinib dexamethasone tamoxifen	MEK BTK HR ER	(162, 163) (149) (153) (128)
BCL-XL	Direct inhibitor, BH3 mimetic	navitoclax A-1331852 A-1155463 WEHI-539	BCL-XL	(23) (44) (164) (165)
MCL-1	Direct inhibitor, BH3 mimetic	S63845 A-1210477	MCL-1	(166) (43)
MCL-1 + NOXA	Reduced proteolysis of NOXA, a BH3-only protein which can bind/neutralize MCL-1	bortezomib	Proteasome	(75) (77)
MCL-1	Reduced RNA Pol2-mediated transcriptional elongation of *MCL1* mRNA	dinaciclib alvocidib	CDK9	(63) (65)
MCL-1	Inhibition of cap-dependent translation of *MCL1*	AZD8055	mTORC1/2	(167)
	Unknown	anthracyclines hypomethylators	DNA/RNA DNA/RNA	(157)

Figure 2. Rational combinations with the BCL-2-selective inhibitor venetoclax

a) A number of chemotherapeutics and targeted agents demonstrate synergistic cancer cell killing when combined with venetoclax, and their mechanisms of action fall into three general categories: 1) agents that trigger elevations in proapoptotic proteins and lead to BCL-2 priming in cancer cells, 2) direct or indirect inhibitors of BCL-X_L or MCL-1 (middle panel), and 3) agents that mobilize tumor cells away from protective niches in lymph nodes

and bone marrow (right panel). **b)** Additional examples of agents that have been shown to synergize with venetoclax and their respective mechanisms of action.

Table 1

Overview of preclinical and clinical studies with venetoclax

Tumor Type	Combination Agent(s)	Preclinical Reference(s)	Key Preclinical Findings	Key Clinical Findings	Clinical Study (NCT #) Clinical Reference(s)
CLL	monotherapy	(33, 44, 51)	Venetoclax exhibits potent killing of patient-derived CLL cells *ex vivo* while sparing platelets; BCL-2 inhibition sufficient to suppress neutrophil colony formation *ex vivo*	ORR: 79%, CR: 20%, MRD-negativity: 5% in relapsed/ refractory CLL (n=116); Grade 3/4 thrombocytopenia: 12%; Grade 3/4 neutropenia: 41%. Clinical TLS in 3/56 (1 lethal) in escalation and 0/60 in 400 mg expansion cohort.	M12-175 (NCT01328626) (52)
CLL	rituximab	(33)	Venetoclax-rituximab combination more efficacious than either agent alone in B-cell lymphoma xenograft models	ORR: 86%, CR: 51% in R/R CLL (n=49); MRD-negativity (bone marrow): 57%, 80% of CR Pts (20/25); Grade 3/4 neutropenia: 53% (26/49)	M13-365 (NCT01682616) (54)
CLL	obinutuzumab	(55)	Venetoclax-obinutuzumab combination demonstrates efficacy superior to either agent alone in three xenograft models	ORR: 100%, CR: 58%, in previously untreated CLL (n=12); MRD-negativity (peripheral blood): 92% (11/12); Grade 3/4 neutropenia: 58% (7/12)	CLL14 (NCT02242942) (56)
CLL	ibrutinib	(59, 148, 149)	CLL cells from Pts after ibrutinib dosing are highly sensitive to venetoclax-mediated killing *ex vivo*	Median CLL counts in blood went from 45×10^9/L to 60×10^9/L after 8 weeks ibrutinib, then to 0.042×10^9/L after 8 weeks ibrutinib + venetoclax in relapsed/ refractory CLL Pts (n=35)	CLARITY (ISCRTN13751862) (150)
NHL	monotherapy	(33, 61)	*BCL2* translocation t(14;18) or amplification predicts sensitivity of NHL cell lines to venetoclax *in vitro*; BCL-2 protein expression correlates with greater sensitivity	ORR: 44% (MCL, 75%; FL, 38%; DLBCL, 18%) in Pts with relapsed/refractory NHL (n=106); Grade 3/4 events: anemia (15%), neutropenia (11%), thrombocytopenia (9%). No clinical TLS.	M12-175 (NCT01328626) (62)
NHL	rituximab	(33)	Venetoclax enhances the efficacy of rituximab in NHL xenograft model	ORR: 30% (n=53) ven + R in relapsed/refractory FL; Grade 3/4 neutropenia: 25% (n=52)	BO29337/CONTRALTO (NCT02187861) (151)
NHL	bendamustine + rituximab	(33, 69)	Venetoclax enhances the efficacy of BR in xenograft and systemic models of NHL	ORR: 65% (n= 48) in relapsed/refractory NHL ORR: 75% (n=51) in relapsed/refractory FL; Grade 3/4 neutropenia: 61% (n=49)	M12-630 (NCT01594229) (152) BO29337/CONTRALTO (NCT02187861) (151)

Tumor Type	Combination Agent(s)	Preclinical Reference(s)	Key Preclinical Findings	Key Clinical Findings	Clinical Study (NCT #) Clinical Reference(s)
NHL	R-CHOP	(33)	Venetoclax enhances the efficacy of R-CHOP in xenograft model of NHL	ORR: 88% (n=24) venetoclax + R-CHOP in NHLs; Grade 3/4 neutropenia: 54%	GO27878/CAVALLI (NCT02055820) (153)
MCL	ibrutinib	(70)	Venetoclax and ibrutinib demonstrate synergistic killing of MCL cell lines and patient samples	ORR: 71%, CR: 63% in Pts w relapsed (n=23) or treatment naïve (n=1) MCL; MRD-negativity (bone marrow of CR patients): 80%; Grade 3/4 neutropenia: 25%; TLS in 2 Pts	AIM (NCT02471391) (71)
ALL (B-cell)	monotherapy	(120, 121)	B-cell precursor ALL cell lines and PDX models sensitive to venetoclax; MLL-AF4 drives BCL-2 expression and sensitivity to venetoclax	No data yet reported	M13-833 (NCT03236857) - pediatric
ALL (ETP)	monotherapy	(117–119)	ETP-ALL cell line (LOUCY) and patient samples highly sensitive to venetoclax-mediated killing	No data yet reported	M13-833 (NCT03236857) - pediatric
ALL	monotherapy	(121)	Pediatric ALL PDX models more sensitive to BCL-2/BCL-X_L inhibitor navitoclax than to venetoclax	No data yet reported	M16-106 (NCT03181126) venetoclax combination with navitoclax and chemotherapy
AML	monotherapy	(44, 93)	High proportion of AML cell lines and patient samples sensitive to venetoclax; efficacy observed in xenograft and PDX models	ORR: 19% (n=32) in relapsed/refractory AML; Grade 3/4 Febrile Neutropenia: 31%, No TLS	M14-212 (NCT01994837) (98)
AML (IDH1/2 mutated)	monotherapy	(95)	IDH1- and IDH2-mutated AML cells identified as highly sensitive to venetoclax.	RR: 33% (4/12) in IDH1/2 mutant tumors	M14-212 (NCT01994837) (98)
AML	cytarabine	(154)	Venetoclax and cytarabine demonstrate synergistic killing of AML patient samples *ex vivo*	ORR: 61%, CR/CRi: 54% (n=61) in treatment naïve AML Pts ≥65 years of age and unfit for standard induction therapy	M14-387 (NCT02287233) (102)
AML	idarubicin	(155)	Idarubicin reduces MCL-1 expression and synergizes with venetoclax to kill AML cell lines	No data yet reported	NCT03214562 NCT03194932 - pediatric
AML	idasanutlin	(111, 112)	Venetoclax significantly enhances the efficacy of idasanutlin and extends survival in subcutaneous and systemic xenograft models of AML	No data yet reported	GH29914 (NCT02670044)
AML	cobimetinib	(104)	Venetoclax and cobimetinib demonstrate synergistic killing of	No data yet reported	GH29914 (NCT02670044)

Tumor Type	Combination Agent(s)	Preclinical Reference(s)	Key Preclinical Findings	Key Clinical Findings	Clinical Study (NCT #) Clinical Reference(s)
			AML cell lines and patient samples *ex vivo*		
AML	azacitidine	(99, 100)	Venetoclax and azacitidine demonstrate synergistic killing of AML patient samples *ex vivo*	ORR: 64% (n=50) for venetoclax + azacitdine in treatment-naïve AML Pts ≥65 years of age and ineligible for standard induction therapy; No TLS	M14-358 (NCT02203773) (101)
MDS	monotherapy	(113, 114)	ABT-737 extends survival in *NRASD12/BCL2* transgenic model of MDS-AML transition; High-risk MDS and secondary AML patient samples sensitive to venetoclax-mediated killing *ex vivo*	No data yet reported	M15-522 (NCT02966782) +/− azacitidine in higher-risk MDS Pts after HMA failure M15-531 (NCT02942290) + azacitidine in treatment-naïve higher-risk MDS Pts
MM	monotherapy	(84, 86)	t(11;14)-positive cell lines and MM patient samples highly sensitive to venetoclax; high *BCL2*/*MCL1* mRNA ratio predicts sensitivity	ORR: 21%; (n= 66) RR for t(11;14)-positive: 40% (n=30); Grade 3/4 thrombocytopenia: 32%, Grade 3/4 neutropenia: 27%	M13-367 (NCT01794520) (85, 156)
MM	bortezomib	(77)	Venetoclax enhances the efficacy of bortezomib in multiple xenograft models of multiple myeloma	ORR: 67% (n=66) RR for Pts not refractory to bortezomib with 1-3 prior therapies: 97% (n=30); Grade 3/4 thrombocytopenia: 29%	M12-901 (NCT01794507) (78)
MM	dexamethasone	(157)	Dexamethasone induces increased BCL-2 priming with BIM and synergistic killing of MM cell lines	No data yet reported − venetoclax + dexamethasone expansion being performed in t(11;14) Pts	M13-367 (NCT01794520) (85)
WM	ibrutinib	(158)	Venetoclax synergizes with ibrutinib to kill WM cell lines and patient samples *ex vivo*	No data yet reported 4/4 Pts had PR (monotherapy)	A15-751 (NCT02677324) M12-175 (NCT01328626) (62)
CML	tyrosine kinase inhibitors (ABL)	(123)	Venetoclax + TKIs kills proliferating and quiescent CML cells, including blast crisis CML samples; kills LSCs	No data yet reported	No study
BPDCN	monotherapy	(126)	BH3 profiling identified BPDCN cell lines and patient samples as BCL-2-dependent; venetoclax efficacious in PDX models of BPDCN	Reduction in disease burden within 4 weeks	2 patients treated off label (126)

Tumor Type	Combination Agent(s)	Preclinical Reference(s)	Key Preclinical Findings	Key Clinical Findings	Clinical Study (NCT #) Clinical Reference(s)
ER+ breast cancer	tamoxifen	(128)	Venetoclax enhances the efficacy of tamoxifen in PDX models of ER+ breast cancer	ORR: 31% (n=13) in Pts with ER+ BCL-2+ HER2- metastatic breast cancer; Grade 1/2 lymphopenia: 67% (n=15)	m-BEP (ISRCTN98335443) (159)
NB	cyclophosphamide	(160, 161)	Venetoclax active as a single agent vs. some NB cell lines; enhances the efficacy of cyclophosphamide in PDX model	No data yet reported	M13-833 (NCT03236857) - pediatric

ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm; BR, bendamustine/rituximab; CLL, chronic lymphocytic leukemia; CR/CRi, complete response/complete response with incomplete recovery of blood count; DLBCL, diffuse large B-cell lymphoma; ER, estrogen receptor; ETP, early T-cell precursor; FL, follicular lymphoma; HMA, hypomethylating agent; LSC, leukemic stem cell; MRD, minimal residual disease; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MZL, marginal zone lymphoma; ORR, overall response rate; PB, peripheral blood; PDX, patient-derived xenograft; Pts, patients; R, rituximab; RR, response rate; TKI, tyrosine kinase inhibitor; WM, Waldenström's Macroglobulinemia