BCL-2 Antagonism to Target the Intrinsic Mitochondrial Pathway of Apoptosis
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Abstract
Despite significant improvements in treatment, cure rates for many cancers remain suboptimal. The rise of cytotoxic chemotherapy has led to curative therapy for a subset of cancers, though intrinsic treatment resistance is difficult to predict for individual patients. The recent wave of molecularly targeted therapies has focused on druggable-activating mutations, and is thus limited to specific subsets of patients. The lessons learned from these two disparate approaches suggest the need for therapies that borrow aspects of both, targeting biologic properties of cancer that are at once distinct from normal cells and yet common enough to make the drugs widely applicable across a range of cancer subtypes. The intrinsic mitochondrial pathway of apoptosis represents one such promising target for new therapies, and successfully targeting this pathway has the potential to alter the therapeutic landscape of therapy for a variety of cancers. Here, we discuss the biology of the intrinsic pathway of apoptosis, an assay known as BH3 profiling that can interrogate this pathway, early attempts to target BCL-2 clinically, and the recent promising results with the BCL-2 antagonist venetoclax (ABT-199) in clinical trials in hematologic malignancies.

Disclosure of Potential Conflicts of Interest
M.S. Davids is a consultant/advisory board member for AbbVie, Genentech, Gilead, Infinity Pharmaceuticals, and Janssen. No potential conflicts of interest were disclosed by the other author.

Introduction
Apoptosis
Most chemotherapeutic and targeted cancer therapies kill tumor cells through the generation of pro-death signaling that initiates the intrinsic apoptotic pathway of programmed cell death (the two other major operative mechanisms of tumor cell killing, the extrinsic cell death pathway and autophagy, are discussed in detail elsewhere in this CCR Focus section; refs. 1, 2). The point of no return in the apoptotic cascade is mitochondrial outer membrane permeabilization (MOMP); once it has occurred, mitochondrial permeabilization leads to the formation of an apoptosome, which facilitates caspase activation and subsequently triggers the other hallmarks of apoptotic cell death. The cellular decision to initiate MOMP is controlled by a delicate balance between the pro- and antiapoptotic molecules of the B-cell leukemia/lymphoma-2 (BCL-2) family. This review discusses the clinical use of agents designed to inhibit BCL-2 and related molecules; strategies for targeting other antiapoptotic mechanisms, in particular the IAP family of proteins that inhibit caspase activation, are discussed elsewhere in this CCR Focus section (3).

BCL-2
BCL-2, first identified as one of the genes involved in the t(14;18) found in follicular lymphoma (4), is one of the primary proapoptotic proteins (5), along with BCL-XL (6), BCL-w (7), MCL-1 (8), and BFL-1 (9). Antiapoptotic proteins act by binding proapoptotic activators such as BID and BIM (Fig. 1). When sequestered by antiapoptotic proteins, BID and BIM are unable to interact with the direct effector proapoptotic molecules BAX and...
BAK, preventing their oligomerization and therefore MOMP (10). Antiapoptotic proteins can also bind BAX and BAK directly, preventing their homo-oligomerization, which is required for MOMP. BCL-2 and its antiapoptotic cousins bind proapoptotic molecules at a shared domain known as BCL-2 Homology 3 (BH3).

Early studies of the relationships between these molecules suggested that many cancers have a dependence on BCL-2 and other antiapoptotic molecules for their survival (11). For example, relative to other peripheral blood mononuclear cells, chronic lymphocytic leukemia (CLL) cells express high levels of BCL-2 protein (12). Bcl-2 protein expression level alone, however, cannot account for the propensity of a cell to undergo apoptosis, as this intricate system also depends on the interactions of Bcl-2 with other anti- and proapoptotic proteins. A more physiologic assessment of these interactions can be obtained through BH3 profiling, a functional assay that measures the ability of a range of BH3-only domain-containing peptides to trigger cytochrome c release from mitochondria in a cell of interest (see Text Box 1). The pattern of response can then be compared with known molecular interactions between pro- and antiapoptotic BCL-2 family members to determine the specific antiapoptotic protein dependencies of the given cell (12, 13). BH3 profiling can also assess the proximity of a cell to the apoptotic threshold (a property known as “mitochondrial priming”; Fig. 2). The potential clinical relevance of BH3 profiling is discussed in additional detail below; a summary of the agents described in this article can be found in Table 1.

**Early Attempts to Target BCL-2 in the Clinic**

**Oblimersen**

Oblimersen is a single-stranded 18-mer DNA molecule complimentary to BCL-2 mRNA (14). In cell lines, oblimersen had been shown to inhibit BCL-2 protein expression, presumably by hybridizing with BCL-2 mRNA (15). Despite some evidence of benefit in phase I studies of CLL (16), myeloma (17), and melanoma (18), oblimersen was not effective in a phase III study in myeloma (19), and only modestly beneficial when added to fludarabine in a phase III study of CLL (20). Given these results, it
**Text Box 1. Mitochondrial priming and BH3 profiling**

The observation that different tumors have differing sensitivities to cytotoxic chemotherapy led to the concept of “mitochondrial priming,” which refers to a cell’s proximity to the apoptotic threshold. At a molecular level, primed cells have a high fraction of their antiapoptotic proteins such as BCL-2 bound to proapoptotic BH3-only proteins such as BIM and BID, whereas unprimed cells have a low fraction of antiapoptotic proteins bound to proapoptotic BH3-only proteins (see Fig. 2). Primed cells are more sensitive than unprimed cells to chemotherapy and to BH3-mimetic drugs such as venetoclax, which binds with high affinity to the antiapoptotic protein Bcl-2. BH3 profiling is a functional assay in which cells from a specific tumor sample are interrogated with a range of BH3-domain containing peptides that simulate the actions of their corresponding full-length BH3-only proapoptotic proteins. The degree of cytochrome c release (a proxy for mitochondrial outer membrane permeabilization) is then compared for different peptides at different concentrations to assess the degree of mitochondrial priming of the cell. BH3 profiling can also predict the specific antiapoptotic proteins on which a particular cell depends for survival.

**Obatoclax**

Another anti-BCL-2 agent tested in clinical trials was obatoclax (GX-15-070), a small molecule, which is thought to bind the BH3 domain of BCL-2 (as well as those of BCL-XL and MCL-1), thus preventing the antiapoptotic proteins from sequestering proapoptotic BH3-only proteins (22). Obatoclax was only modestly efficacious in the clinic. For example, a phase I trial adding it to fludarabine and rituximab in relapsed/refractory CLL showed a partial response (PR) rate of 54% with no complete responses (CR; ref. 23), and a phase II trial in small-cell lung cancer (SCLC) showed no benefit when it was added to the standard regimen of carboplatin and etoposide (24). As with oblimersen, subsequent analyses suggested that obatoclax may behave differently in vivo compared with the original in vitro studies (25). For instance, significant thrombocytopenia, a well-known on-target effect of BCL-XL inhibition, was never observed in patients treated with obatoclax. Because of its formulation, obatoclax also had neurologic side effects such as mental status changes, which further limited its clinical development (26). Furthermore, additional in vitro studies showed that obatoclax can trigger apoptosis in cells lacking BAX and BAK, suggesting an alternative mechanism of action (27). It is important to remember that, although the results of these early experiences with both oblimersen and obatoclax were disappointing, these results reflect the inadequacy of these individual molecules rather than that of the overall strategy of targeting BCL-2 in cancer.

**Navitoclax (ABT-263)**

The most potent and selective BCL-2 antagonists engineered to date are those developed by Abbott Laboratories (now AbbVie), beginning with ABT-737 (28) and its orally bioavailable counterpart navitoclax as well as venetoclax (ABT-263, ABT-199; refs. 29, 30). These “BH3-mimetic” molecules mimic the proapoptotic action of BH3-only proteins by binding directly to the BH3-binding domains of antiapoptotic molecules, thereby displacing native BH3-only proteins (e.g., BIM, BAD, Fig. 2). ABT-737 and navitoclax have binding affinities for BCL-2 family proteins on the order of 10 to 10,000 times greater than other molecules, including obatoclax (31). ABT-737, whose BH3-binding profile directly mirrors that of BAD BH3 protein, has poor oral bioavailability and has been limited to in vitro and animal studies.

Navitoclax (formerly ABT-263) is an orally bioavailable, relatively nonselective BCL-2 family inhibitor with high affinity for BCL-2, BCL-XL, and BCL-w, and substantially less affinity for MCL-1 (29). Early-phase clinical trials, particularly in hematologic malignancies, brought promising results. For example, in a phase I trial that included 29 patients with relapsed or refractory CLL, 9 (35%) had a PR with navitoclax alone and 7 others had stable disease for at least 6 months, with overall progression-free survival (PFS) in the cohort of 25 months (32). The activity of ABT-263 monotherapy in solid tumors was less promising. For example, in a phase II study in 39 patients with relapsed SCLC, only 1 patient (2.6%) had a PR, and 9 patients (23%) had stable disease, with a median PFS of only 1.5 months (33). Subsequent studies have shown that, at least in SCLC, high expression of BIM without concomitant MCL-1 expression predicts navitoclax efficacy, suggesting a potential opportunity to retarget the molecule in a more selected cohort (34).

The major limitation of navitoclax in clinical use, however, has been the frequent development of thrombocytopenia, which can be severe. This toxicity is a predicted consequence of the drug’s strong inhibition of BCL-XL, a primary barrier to apoptosis in aging platelets (35); platelet production, on the other hand, appears to be spared or even increased. Specific pharmacokinetic strategies, such as gradual dose increases and daily rather than pulsed administration, have been able to mitigate the thrombocytopenia to a certain extent (32), and navitoclax remains under clinical exploration in a number of cancers. Although clinically relevant bleeding has not been reported in the studies described above, this on-target toxicity of navitoclax has nevertheless limited its development, particularly in many hematologic malignancies, in which baseline thrombocytopenia is often prominent.

**Venetoclax (ABT-199)**

The elucidation of the mechanism by which navitoclax causes thrombocytopenia suggested that a more selective BCL-2 inhibitor might avoid this toxicity and allow for higher dosing to maximize clinical efficacy. This led to the rational reverse engineering of navitoclax to yield venetoclax, an orally bioavailable BCL-2–specific inhibitor originally known as ABT-199/GDC-0199 (also manufactured by AbbVie; ref. 30). Side-by-side pharmacodynamic comparison of venetoclax with navitoclax showed that venetoclax has a slightly higher avidity for BCL-2, and three orders of magnitude less avidity for BCL-XL. Initial in vitro studies confirmed that venetoclax rapidly kills malignant cells through the intrinsic mitochondrial apoptosis pathway and is selective for cells dependent on
BCL-2, but not those dependent on BCL-XL. In preclinical models, the drug exhibited efficacy against a wide variety of tumor types, including leukemias, non-Hodgkin lymphoma (NHL), and myeloma, with no significant thrombocytopenia observed in in vivo models.

**Clinical Uses of Venetoclax**

Chronic lymphocytic leukemia

Several results from preclinical studies suggested that CLL would be the logical disease in which to first study venetoclax in the clinic. CLL is known to express high levels of Bcl-2 and proapoptotic BH3-only proteins, and in vitro BH3 profiling of CLL patient samples has demonstrated on a functional level that CLL cells from most patients are dependent on Bcl-2 for survival (13), which may be due in part to interactions between CLL cells and the surrounding bone marrow stroma (37). Moreover, in a small cohort of CLL patients for whom baseline samples were collected prior to starting first-line therapy, the degree of mitochondrial priming appeared to correlate with treatment responsiveness (37). Primary CLL cells were among those shown to be most sensitive to ABT-199 ex vivo, with substantial induction of apoptosis observed within only an hour of treatment.
The first-in-human study of venetoclax (M12-175) is a large, ongoing multicenter dose-escalation study of venetoclax monotherapy in relapsed/refractory CLL (38) and NHL (39). An interim analysis of the CLL arm of this study found that the majority of the 105 patients had clinically high-risk disease [75% were immuno-globulin heavy chain variable region (IGHV) unmutated, and 22% harbored 17p deletions or TP53 mutations], and were heavily pretreated, with a median of four prior lines of therapy. Venetoclax was well tolerated by most patients. Mild gastrointestinal toxicity was seen, with diarrhea (40%) and nausea (35%) being most common, generally grade 1/2, and manageable with supportive care. One third of patients developed grade 3/4 neutropenia, but only 7% had febrile neutropenia. This neutropenia was not entirely unexpected given prior ex vivo studies showing that Bcl-2 blockade accelerates FasL-mediated apoptosis in neutrophils (40), and other work showing a specific sensitivity to BCL-2 inhibition in myeloid precursors that did not extend to inhibition of other proapoptotic molecules like BCL-XXL (34). Moreover, neutropenia was generally responsive to growth factor support. Consistent with predictions and preclinical models, grade 3/4 thrombocytopenia was uncommon (7% of patients). Other adverse effects included anemia (10%), hyperglycemia (7%), tumor lysis syndrome (TLS, 7%), and hypokalemia (5%). Only 7 serious adverse events were thought to be related to venetoclax (4 episodes of febrile neutropenia and 3 episodes of TLS). The most serious toxicity observed with venetoclax was tumor lysis syndrome, which resulted in acute kidney injury leading to the need for dialysis in 1 patient and presumed sudden cardiac death in a patient treated at a daily dose of 1,200 mg. On the basis of these events, the protocol was revised to include a lower initial dose, a slower stepwise intrapatient dose escalation, and intensive TLS prophylaxis and monitoring. Using this new strategy, no additional clinical TLS was observed, and the recommended phase II dose was determined to be 400 mg daily.

With regard to efficacy, deep responses were observed in the peripheral blood, lymph nodes, and bone marrow of the majority of patients. Of 78 evaluable subjects, 60 (77%) had an objective response by 2008 IW-CLL criteria (41), with 18 (23%) CRs and 42 (54%) PRs. Equivalent response rates were seen in all high-risk groups, including del(17p) [15/19 patients responded (79%), including 5 CRs], fludarabine-refractory patients [31/41 patients responded (76%), 9 CRs and IGHV unmutated patients [18/24 patients responded (75%), 7 CRs]. Six of the 18 patients with CRs were found to have no evidence of minimal residual disease (MRD) by four-color flow cytometry, although this MRD analysis was not preplanned and was assessed by heterogeneous local methodologies. At this interim analysis, 37 patients had discontinued treatment: 22 with progressive disease, 12 for adverse events, and 3 for other reasons (1 patient needed to start warfarin and 2 proceeded to allogeneic stem cell transplant in CR). The median PFS for the entire cohort was estimated at 18 months, but this included many patients treated at lower doses in the early dose-escalation phase of the trial. For those patients treated at or above a dose of 400 mg daily, median PFS had not been reached. Although these results were impressive for a single agent, preclinical studies showed that venetoclax sensitizes CLL cells to monoclonal antibodies and cytotoxic agents (30), suggesting that it might be even more effective as a component of a multidrug regimen. On the basis of these preclinical data, a phase Iib study of venetoclax plus rituximab (M13-982) was opened to assess the safety and efficacy of this combination (42). The most recent presentation of interim data reported results for 49 patients with relapsed/refractory CLL, 20% of whom had 17p deletions and 57% of whom had progressed after fludarabine. Compared with the monotherapy study, this cohort was somewhat less heavily pretreated, having received a median of only two prior therapies.

The initial results from the M13-982 study show that venetoclax plus rituximab has generally been a safe and tolerable regimen for most patients. Neutropenia was again the most frequent grade 3/4 adverse event (47% of patients), but febrile neutropenia remained rare (6%). Grade 3/4 thrombocytopenia and anemia were somewhat more frequent than in the monotherapy setting (16% and 14%, respectively). Serious adverse events attributed to the study drugs were rare, and included febrile neutropenia (4%), infusion reactions (4%), and tumor lysis syndrome (4%). During the initial venetoclax monotherapy lead-in period of this study, another case of fatal TLS occurred in a patient with extremely bulky lymphadenopathy. This death, in conjunction with the death on the M12-175 study, led to a revamping of the study design (as discussed above) after which no additional clinical TLS events were observed in the next 32 patients. The recommended phase II dose for venetoclax in combination with rituximab was the same as for monotherapy at 400 mg daily. At the time of interim analysis, 10 of 49 patients had discontinued treatment: 6 for progressive disease, 2 for adverse events, and 2 withdrew consent.

With regard to the preliminary efficacy the overall response rate (ORR) was 68% (43 of 49 patients), with 22 PRs (45%) and 15 CRs (31%). An additional 6 patients with PRs were not yet confirmed. MRD analysis by high-sensitivity flow cytometry showed that 9 of the 15 patients with CRs were MRD negative. Interestingly, 8 of 22 patients achieving PR were MRD negative in the marrow or blood; several of these patients had lymph nodes that just barely met criteria for enlargement, raising the question of whether this residual mass potentially represented scar tissue rather than residual CLL. Interestingly, 5 patients who achieved CR with MRD negativity have since opted to discontinue the venetoclax. Although 1 patient has since had slight progression and has technically moved back into the PR category, the other 4 continue to have no evidence of disease, with 3 now off venetoclax for longer than they were on it (median, 12 months) in continued MRD-negative CR, suggesting an impressive durability to these deep responses (42).

Non-Hodgkin lymphoma

Preclinical studies showed that venetoclax also has significant activity against a number of NHL cell lines, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and mantle cell lymphoma (MCL; ref. 30). Its in vitro activity is best in lymphomas with BCL-2 activation or translocations involving the BCL-2 locus, such as t(14;18), the hallmark of FL, and in double-hit lymphoma (DHL), which harbors translocations of both BCL-2 and MYC. As in CLL, venetoclax also appears to enhance the efficacy of chemotherapy in NHL xenograft models. At an interim analysis, the NHL arm of the phase I first-in-human M12-175 study included 62 patients with a range of NHL subtypes, including MCL (20 patients), DLBCL (19 patients), FL (14 patients), Waldenstrom macroglobulinemia (WM, 4 patients), marginal zone lymphoma (MZL, 3 patients), multiple myeloma (1 patient), and primary mediastinal B-cell lymphoma (PMBCL, 1 patient). Similar to the CLL cohort, the NHL cohort was heavily pretreated, having received a median of three prior lines of therapy.

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As in the CLL arm, grade 1/2 nausea, diarrhea, anemia, and fatigue were the most common adverse events observed. The most common grade 3 and 4 adverse event was anemia (12 patients, 19%). Neutropenia was somewhat less common than in the CLL cohorts (6 patients, 10%). Two dose-limiting toxicities were observed at the 600-mg range, including one episode of grade 4 neutropenia and one episode of grade 3 febrile neutropenia. Laboratory TLS was observed in some MCL patients, but was without clinical sequela.

Among 59 evaluable patients, the ORR was 48%, but as would be expected in such a heterogeneous cohort, the range was variable and depended on lymphoma subtype. The best activity was seen in MCL (13/19 patients [68%], including 1 CR). The ORR in DLBCL was 28% (5/18 patients, 1 CR) and in FL was 31% (4/13 patients, 1 CR). The relatively low response rate in FL is somewhat surprising, given the high levels of BCL-2 expression created by the 1CR). The relatively low response rate in FL is somewhat surprising, given the high levels of BCL-2 expression created by the

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acuity of single-agent venetoclax in patients with relapsed or refractory AML, or as first-line therapy in patients deemed unfit to receive intensive chemotherapy, was recently presented (46). At this interim analysis, 32 patients had enrolled on study (30 with relapsed/refractory disease). Most patients had high-risk features, including preexisting myelodysplastic syndrome (MDS, 37.5%), FLT3-ITD mutations (19%), and older age (median, 71 years). Venetoclax appeared to be safe and well tolerated, with a similar range of adverse events as those seen in other studies, though perhaps a slightly higher rate of grade 3/4 febrile neutropenia (25%). No clinically significant TLS was observed.

Only 1 of 28 evaluable patients in this study achieved a CR (3%), with 4 achieving CR with incomplete blood count recovery (CRi, 12%), for an ORR of 15%. Interestingly, 3 of these 5 patients had activating IDH1 or IDH2 mutations, and another 3 patients with IDH2 mutations showed antileukemic activity that did not correlate with a favorable prognosis and response to

Acute myeloid leukemia

Despite prior work showing high Bcl-2 expression in myeloblasts (43), it was somewhat surprising that in vitro treatment with venetoclax efficiently killed myeloblasts from a variety of sources, including cell lines, primary patient samples, and murine primary xenografts (44), as BCL-2 was previously thought to be more important as a survival factor in lymphoid, rather than myeloid lineages. BH3 profiling, however, showed that a significant proportion of myeloblasts are indeed BCL-2 dependent, and that differing degrees of apoptotic priming in patient-derived samples appear to correlate with the variance in chemoresponsiveness seen in the clinical setting (45). As referenced above, subsequent studies have gone on to show that this BCL-2 dependence may extend to normal myeloid precursors as well (34). These data provided a strong rationale for studying venetoclax as a treatment for patients with acute myelogenous leukemia (AML).

An interim analysis of a phase II, multicenter study to evaluate the efficacy of single-agent venetoclax in patients with relapsed or refractory AML, or as first-line therapy in patients deemed unfit to receive intensive chemotherapy, was recently presented (46). At this interim analysis, 32 patients had enrolled on study (30 with relapsed/refractory disease). Most patients had high-risk features, including preexisting myelodysplastic syndrome (MDS, 37.5%), FLT3-ITD mutations (19%), and older age (median, 71 years). Venetoclax appeared to be safe and well tolerated, with a similar range of adverse events as those seen in other studies, though perhaps a slightly higher rate of grade 3/4 febrile neutropenia (25%). No clinically significant TLS was observed.

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Other malignancies

Acute lymphoblastic leukemia

Early T-cell progenitor acute lymphoblastic leukemia (ETP-ALL) is a high-risk subgroup of T-cell ALL with a particularly poor prognosis. BH3 profiling of primary patient samples from patients with ETP-ALL revealed significant dependence on BCL-2 (47). This was distinct even when comparing these samples with primary samples derived from patients with other subtypes of T-ALL, some of which were dependent on BCL-XL. As might be expected, ETP-ALL samples displayed increased sensitivity to in vitro treatment with venetoclax. In a related study, venetoclax and cytarabine had a synergistic effect against the T-ALL cell line LOUCY, which approximates the ETP phenotype, but not against more differentiated T-ALL cell lines (48). These preclinical data provide a strong rationale for pursuing a clinical trial of venetoclax specifically in ETP-ALL.

Waldenstrom macroglobulinemia

The NHL arm of the M12-175 study discussed above had 4 patients with WM, all of whom responded to treatment with venetoclax. Preclinical studies give reason to hope that this efficacy will carry forward in larger cohorts of WM patients treated either with venetoclax alone or with combination therapy. In particular, recent studies in CXCR4WT WM cells, which carry a mutation conferring resistance to the BTK inhibitor ibrutinib, showed that treatment with venetoclax can restore ibrutinib sensitivity (49).

Venetoclax can also directly induce apoptosis in CXCR4WT WM cells and appears to sensitize WM cells to the PI3k-δ isoform inhibitor idelalisib.

Multiple myeloma

BCL-2 is expressed in many cases of myeloma, and in those cases appears to be important for survival (50). Preclinical studies using ABT-737 showed that the drug efficiently killed a number of myeloma cell lines, all of which were distinguished by the presence of translocations involving CCND1, the gene encoding cyclin D1 (51). These studies have since been replicated using venetoclax (52), and an interim analysis from a recent phase 1 trial of 24 patients with relapsed or refractory myeloma showed responses in 3 of 7 patients with CCND1/IGH translocation, with 2 of these patients achieving CR (53). Interestingly, this translocation is also the pathophysiologic hallmark of mantle cell lymphoma, an NHL subtype against which venetoclax appears to be particularly effective. Venetoclax is now in clinical trials in patients with relapsed or refractory myeloma as part of multidrug regimens that include bortezomib and dexamethasone (NCT01794507).

Breast cancer

BCL-2 expression appears to have variable prognostic significance in breast cancer. It has been best studied in luminal cancers, where it is expressed in 85% of cases and appears to correlate with a favorable prognosis and response to
Future Directions

A number of questions about the biology of BCL-2 in cancer and its therapeutic targeting remain unanswered or unexplored. First, is the optimal therapeutic role for venetoclax as monotherapy or in combination with other drugs? Although initially tested as monotherapy in early clinical studies, in vitro studies suggest that venetoclax may be most effective as a chemosensitizing agent, in effect removing cancer cells’ major lines of defense against the proapoptotic effects of chemotherapy. A number of ongoing or upcoming studies will investigate these possibilities, including in CLL, where trials have either recently opened or are in development to combine venetoclax with newer anti-CD20 monoclonal antibodies and kinase inhibitors (NCT02427451), both in the relapsed/refractory and eventually in the frontline setting, and in AML, where a recently opened trial combines venetoclax with the hypomethylating agents azacitidine or decitabine (NCT02203773) and future studies will combine venetoclax with chemotherapy.

Second, are there molecular tools that can predict clinical response to venetoclax? Thus far, BH3 profiling has been largely reserved for preclinical studies or descriptive studies incorporated into trials; however, the technique is also appealing as a clinically applicable assay, in which patient samples could be profiled in real time to assess potential sensitivity to small molecules such as venetoclax, and clinical decisions could be influenced by the results. Preliminary data with BH3 profiling suggest that the level of mitochondrial priming in pretreatment samples from patients on the M12-175 trial may be associated with the depth of response to venetoclax in CLL (57). BH3 profiling of pretreatment samples from 12 patients treated with venetoclax for relapsed/refractory AML showed similar utility in predicting treatment response (58). In addition, genetic profiling may uncover important mutations that predict either sensitivity or resistance to BCL-2 blockade. For example, IDH1 and IDH2 mutations have recently been shown to predict BCL-2 dependence in vitro (59), a fact that appears to be supported by the initial experience using venetoclax in patients with AML.

Third, what are the mechanisms that contribute to the development of venetoclax resistance? In some cancers, resistance may occur via upregulation of other antiapoptotic molecules, such as BCL-XL or MCL-1, though this has been difficult to conclusively show in the clinic. Recent preclinical studies have shown potent proapoptotic effects of specific MCL-1 inhibitors, both alone and in combination with ABT-263 (60). A number of other drugs, all thought to be acting via MCL-1 inhibition, are being explored for utility in overcoming venetoclax resistance, including CDK9 inhibitors (61), MEK inhibitors (62), sorafenib (63), and such novel agents as the pan-BCL2 family inhibitor (-)BI977D6 (64).

In other cancers, resistance may be due to acquired mutations in BCL-2 or other related proteins. For example, one recent study showed that prolonged exposure of lymphoma cell lines to venetoclax selected for missense mutations in BCL-2 that disrupt the drug’s binding to the BH3 domain, thereby inhibiting apoptosis, whereas other venetoclax-resistant lymphomas were found to harbor inactivating mutations in BAX that prevent the molecule from anchoring to the outer mitochondrial membrane (65). An improved understanding of these resistance mechanisms may ultimately allow the development of new strategies that subvert these resistance mechanisms; for example, if BCL-2 missense mutations were found to occur in patients on chronic dosing of venetoclax, trials of bolus, pulsatile dosing of venetoclax could explore whether abrogating the selective pressure for the development of resistance mutations would result in more durable responses. Given that the killing of malignant cells by venetoclax appears to depend more on achieving Cmax compared with AUC, it seems likely that a strategy of high doses of venetoclax given less frequently would be particularly effective, though this hypothesis will need to be explored in clinical trials.

Some of the observations made in studying the evolution of resistance in cancers previously sensitive to BCL-2 inhibition lead to a final question: Can strategies targeting addiction to antiapoptotic molecules be extended to other cancers? Despite promising results in the range of malignancies detailed above, the agents described in this review have shown little efficacy in many other cancers, including many solid tumors (66–68). Recent studies have shown at least two reasons for this. One is that some tumors are dependent on antiapoptotic molecules other than BCL-2 for survival; for example, a recent study showed that some non–small cell lung cancer cell lines appear to be more dependent on BCL-XL than on BCL-2, and selective BCL-XL inhibition significantly increased the anitumor effect of docetaxel in vitro (34). A second is that some cancers, although reliant to a certain extent on BCL-2, upregulate additional antiapoptotic molecules as well. Recently, for example, it was found that certain SCLC cell lines can be sensitized to BCL-2 inhibition with ABT-263 by inhibition of TORC1/2, which leads to reduced MCL-1 protein levels (69). These new insights suggest additional avenues of investigation that may significantly expand the role of venetoclax and other agents that similarly inhibit antiapoptotic proteins.

Conclusions

Our improving understanding of the fundamental protection afforded to cancer cells by the antiapoptotic protein BCL-2 has opened a new therapeutic avenue in cancer treatment. Although early efforts at therapeutically targeting BCL-2 were only modestly successful, the highly selective oral BCL-2 antagonist venetoclax has shown promise in a range of malignancies as both monotherapy and in combination with existing regimens. The FDA recently granted venetoclax a breakthrough designation for relapsed/refractory CLL with 17p deletion, and it appears likely that the drug will receive approval in the near future. Moving forward, biomarkers such as BH3 deletion will help us to further refine our understanding of BCL-2 biology and have the potential to become clinically relevant tools to predict sensitivity to venetoclax and other drugs targeting the BCL-2 family. Finally, a better understanding of the mechanisms by which resistance to BCL-2 inhibition develops will allow us to develop strategies to subvert these mechanisms, thereby optimizing the therapeutic potential of this powerful new approach. Although the early clinical studies have focused primarily on hematologic malignancies, the
fundamental role of the mitochondrial pathway of apoptosis in cancer more broadly suggests that the lessons learned from these initial studies have the potential to make a major impact on the broader world of cancer therapeutics.

Authors’ Contributions
Conception and design:


39. Bodet L, M… (R/R) non-Hodgkin lymphoma (NHL): responses observed in diffuse large B-cell (DLBCL) and follicular lymphoma (FL) at higher cohort doses. J Clin Oncol 32:5s, 2014 (suppl; abstr 8522).


49. BCL-2 Antagonism to Target Apoptosis


BCL-2 Antagonism to Target the Intrinsic Mitochondrial Pathway of Apoptosis

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