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

Christopher J. Gibson and Matthew S. Davids

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.

Editor’s Disclosures

The following editor(s) reported relevant financial relationships: S.E. Bates—None.

CME Staff Planners’ Disclosures

The members of the planning committee have no real or apparent conflicts of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the biology of the intrinsic pathway of mitochondrial apoptosis, including the BCL-2 family of proteins, an assay known as BH3 profiling that interrogates this pathway, and the limitations of early attempts to target BCL-2 therapeutically. The participant will also learn about the molecular mechanism of the BCL-2 antagonist venetoclax and why it is a promising new treatment for CLL, NHL, and other malignancies.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

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 antiapoptotic 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.

did not receive FDA approval and further development of the drug was not pursued. Subsequent studies in both primary tumor cells and mononuclear blood cells from patients treated with oblimersen suggested that its inhibition of BCL-2 protein expression was significantly less potent than had been predicted by the first in vivo studies in cell lines (21), highlighting the limitations of cell lines and the importance of assessing drug activity in primary tumor cells in preclinical development.

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 vivo 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 nonsclective 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 vivo 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.

**Table 1.** Agents targeting the BCL-2 family

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Diseases tested (phase of trial)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblimersen</td>
<td>Antisense DNA targeting BCL2</td>
<td>Myeloma (III)</td>
<td>Potency probably overestimated by preclinical studies</td>
</tr>
<tr>
<td>Obatoclax</td>
<td>Pan-inhibitor of BH3-domain containing proteins</td>
<td>CLL, MDS, AML, NSCLC, Hodgkin lymphoma (I)</td>
<td>Formulation and off-target effects led to poor tolerability</td>
</tr>
<tr>
<td>ABT-737</td>
<td>Similar to navitoclax</td>
<td>None</td>
<td>Not used clinically due to lack of oral bioavailability</td>
</tr>
<tr>
<td>Navitoclax (ABT-263)</td>
<td>BCL-2 and BCL-XL inhibitor</td>
<td>CLL, NHL (I), SCLC (I)</td>
<td>Use limited by significant thrombocytopenia due to concomitant BCL-XL inhibition</td>
</tr>
<tr>
<td>Venetoclax (ABT-199)</td>
<td>Specific BCL-2 inhibitor</td>
<td>CLL (I-III), NHL (I-II), AML (I-II), Myeloma (I)</td>
<td>Promising efficacy in hematologic malignancies, starting to be explored in solid tumors</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; NSCLC, non-small cell lung cancer.

**Figure 2.** Apoptotic priming and venetoclax (VCX) method of action. A, in an apoptotically primed cell, BCL-2 or other antiapoptotic molecules sequester BIM (or BID) and prevent interaction with effector molecules such as BAK or BAX. B, binding of VCX to BCL-2 displaces BIM, allowing it to interact with BAX (or BAK), which then oligomerizes and allows efflux of cytochrome C from the mitochondrion. C, a cell with a low degree of apoptotic priming has relatively little BIM or BID. In this case, treatment with VCX (D) has little effect in and of itself, though this might not preclude synergy with additional chemotherapy.
The first-in-human study of venetoclax (M12-175) is a large, ongoing multicenter dose-escalation study of venetoclax mono-
therapy in relapsed/refractory CLL (38) and NHL (39). An interim
analysis of the CLL arm of this study found that the majority of the
103 patients had clinically high-risk disease [75% were immu-
noglobulin 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 gastrointes-
tinal 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 neu-
tropenia, 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 neu-
trophils (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-XL (34).
Moreover, neutropenia was generally responsive to growth factor
support. Consistent with predictions and preclinical models,
grade 3/4 thombocytopenia 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 recom-
manded 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 discon-
tinued 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 Ib 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 resi-
dual 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.
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 sequelae.

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 1 CR). The relatively low response rate in FL is somewhat surprising, given the high levels of BCL-2 expression created by the hallmark t(14;18), but illustrates that BCL-2 expression alone is not sufficient to predict BCL-2 dependence. Notably, most of the responses in DLBCL and FL were observed in higher dosing cohorts, suggesting that a higher drug exposure may be required to achieve response in patients with these histologic subtypes (39).

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.

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 meet formal criteria for response due to a lack of hematologic recovery. Overall, 6 of 11 patients with IDH1 mutations had evidence of antitumor activity with venetoclax; of the 5 who did not, 2 had concomitant FLT3-ITD. Although 2 of the 5 patients with CRs became MRD negative by flow cytometry, the majority of responses were not durable. In particular, all 3 CR/CRi patients with IDH mutations had relapsed by 12 weeks on study, whereas the 2 patients with CR/CRi without IDH mutations remained in remission. The preliminary results of this study demonstrate antitumor activity of venetoclax as a single agent in AML with good tolerability. They support the development of additional clinical trials in AML looking at venetoclax in combination with chemotherapy and other targeted therapies; some of these, including a phase I and II study of venetoclax in combination with low-dose cytarabine (NCT02287233) and a phase Ib study combining venetoclax with one of the two hypomethylating agents decitabine or azacitidine (NCT02203773), are already enrolling patients.

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 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 isofrom 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...
chemotherapy (54). Although neither ABT-263 nor ABT-199 induce breast cancer xenograft regression when used as single agents, more recent studies have shown that the combinations of ABT-199 with tamoxifen (55) and ABT-737 with docetaxel (56) are more effective at inducing xenograft breast tumor regression than either tamoxifen or docetaxel alone. So far, these studies have only been performed in estrogen receptor–positive breast cancers, and responses appear to be limited to those with positive BCL-2 expression by immunohistochemistry. These intriguing findings are worthy of further exploration.

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 (-)-BIB197D6 (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 SCCL 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 in BCL-2-dependent CLL with 17p deletion, and it appears likely that 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

www.aacrjournals.org

Clin Cancer Res; 21(22) November 15, 2015 5027

Downloaded from clinicalcareres.aacrjournals.org on July 31, 2021. © 2015 American Association for Cancer Research.
fundamental role of the mitochondrial pathway of apoptosis in cancer more broadly suggests that the lessons learned from these initial clinical studies have the potential to make a major impact on the broader world of cancer therapeutics.

Authors’ Contributions
Conception and design: C.J. Gibson, M.S. Davids
Writing, review, and/or revision of the manuscript: C.J. Gibson, M.S. Davids
BCL-2 Antagonism to Target the Intrinsic Mitochondrial Pathway of Apoptosis

Christopher J. Gibson and Matthew S. Davids


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/21/22/5021

Cited articles
This article cites 63 articles, 29 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/22/5021.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/21/22/5021.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/21/22/5021.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.