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Rational combination strategies to enhance venetoclax activity and overcome resistance in hematologic malignancies

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Abstract

Venetoclax (ABT-199) is a Bcl-2-specific BH3-mimetic that has shown significant promise in certain subtypes of CLL as well as in several other hematologic malignancies. As in the case of essentially all targeted agents, intrinsic or acquired resistance to this agent generally occurs, prompting the search for new strategies capable of circumventing this problem. A logical approach to this challenge involves rational combination strategies designed to disable preexisting or induced compensatory survival pathways. Many of these strategies involve downregulation of Mcl-1, a pro-survival Bcl-2 family member that is not targeted by venetoclax, and which often confers resistance to this agent. Given encouraging clinical results involving venetoclax in both lymphoid and myeloid malignancies, it is likely that such combination approaches will be incorporated into the therapeutic armamentarium for multiple hematologic malignancies in the near future.

Keywords

Venetoclax; resistance; combination strategies

Introduction: hematologic malignancies and apoptosis

One of the hallmarks of hematologic malignancies, including acute (AML) and chronic myelogenous (CML) leukemia, multiple myeloma (MM) and non-Hodgkin lymphoma (NHL), is the failure of cells to undergo an organized program of cell death, for example, apoptosis, as occurs in diverse cancer cell types [1]. Apoptosis is a programmed form of cell suicide wherein activation of executioner caspases (e.g. caspases-3 and caspases-7) by noxious stimuli trigger degradation of cellular constituents, culminating in cellular demise

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[2]. It is strictly regulated by Bcl-2 family proteins, consisting of pro- and antiapoptotic proteins [3]. The former contain activator (e.g. Bim, Puma, Bid) or sensitizer (e.g. Bad, Noxa) BH3-only proteins as well as multidomain effectors (e.g. Bak and Bax) [4]. The latter trigger mitochondrial membrane permeabilization (MOMP), cytosolic release of cytochrome c and activation of initiator caspases (e.g. caspase-9) [5]. Multidomain antiapoptotic proteins (e.g. Bcl-2, Bcl-xL, Mcl-1, A1, etc.) act to neutralize sensitizer, activator and/or effector pro-apoptotic proteins [6]. Both relative levels and direct interactions between these pro- and antiapoptotic proteins determine cell fate [7]. Moreover, apoptotic-signaling pathways are divided into the intrinsic (mitochondrial) and extrinsic (death receptor) pathways [8]. The former is activated by mitochondrial injury (MOMP), whereas the latter, typically triggered by death receptors [9], involves caspase-8 activation through the formation of the DISC [10] or the recently defined ripoptosome (Complex II), composed of FADD, RIP1, cFLIP and caspase-8. The latter is a 2-MDa complex that activates either caspase-dependent apoptosis or RIP3-dependent necroptosis via the core-component RIP1, events tightly regulated by cIAP1/2 [11]. Of note, MM has been associated with deregulation of both the intrinsic and extrinsic pathways [12].

There is continuing debate regarding the mechanism(s) by which apoptosis is induced. According to one model (the indirect model), apoptosis is induced when pro-apoptotic proteins (e.g. Bim) act indirectly to inactivate antiapoptotic proteins, for example, Bcl-2 which restrain pro-apoptotic multi-domain proteins (e.g. Bax and Bak), leading to activation of the apoptotic cascade [13]. Alternatively, in the direct model, pro-apoptotic proteins like Bim activate multidomain proteins directly [14]. In many cases, antiapoptotic proteins cooperate to keep the apoptotic apparatus in check. For example, under normal circumstances, the pro-apoptotic protein Bak is tethered by both Bcl-xL and Mcl-1, preventing activation of the apoptotic cascade [15]. However, when physiologic death supervenes, Bcl-xL is inactivated by the pro-apoptotic protein Bad [16], and Mcl-1 is inactivated by the pro-apoptotic protein Noxa, allowing Bak conformational change and ultimately cell death [17]. The implications of these observations is that strategies designed to trigger cell death in neoplastic cells can involve both inactivation of antiapoptotic proteins as well as activation/upregulation of pro-apoptotic proteins [14]. In fact, a dual strategy involving simultaneous disabling of antiapoptotic proteins coupled with upregulation of pro-apoptotic proteins may be particularly effective.

Bcl-2 family proteins and malignant cell survival and resistance

Deregulation of Bcl-2 family proteins may both contribute to transformation and resistance to chemotherapeutic agents. For example, it has long been recognized that Mcl-1 (myeloid leukemia cell protein 1) plays a key role the survival of multiple myeloma cells and may also contribute to myeloma cell resistance to chemotherapeutic agents [18]. It also has been implicated in the survival of leukemia stem cells (LSC) [19]. In this context, downregulation of Mcl-1 by either genetic or pharmacologic means has been shown to be highly effective in diminishing multiple myeloma cell survival [20] as well as that of human myeloid leukemia cells [21]. However, Bcl-2, deregulation of which has been classically implicated in the development of B-cell malignancies (e.g. CLL, non-Hodgkin lymphoma), may also play a key role in multiple myeloma cell survival [22] as well as that of myeloid leukemia cells

[23]. For example, it has been shown that Bcl-2 represents a critical survival factor for at least a subset of multiple myeloma cells [24]. In addition to these antiapoptotic proteins, pro-apoptotic proteins may also represent key regulators of malignant hematopoietic cell survival and sensitivity to chemotherapeutic agents. For example, Bim, known to be a key determinant of neoplastic cell sensitivity to various targeted agents (e.g. kinase inhibitors), has also been shown to be a significant factor in resistance of multiple myeloma cells to proteasome inhibitors [25] and of myeloid leukemia cells to histone deacetylase inhibitors [26]. Furthermore, activation of the multidomain pro-apoptotic proteins Bak and Bax play key roles in cell death regulation. In this context, under physiologic conditions, Bak is held in check by binding to both Mcl-1 and Bcl-xL, whereas disruption of these associations leads to apoptosis [15]. Moreover, the relative distribution of pro-to antiapoptotic proteins may represent a major determinant of cell survival and response to various therapeutic agents [7]. Consistent with these findings, BH3 profiling, which assesses the sensitivity of tumor cells to BH3 peptides can accurately predict responses of these cells to agents that perturb members of the Bcl-2 family [27].

Finally, the mechanisms by which malignant cells, particularly malignant hematopoietic cells, become resistant to venetoclax represent a major area of investigation. However, to date, the most important mechanisms responsible for this phenomenon appear to stem from upregulation of antiapoptotic proteins not targeted by venetoclax, for example, Bcl-xL and Mcl-1, and/or downregulation of Bcl-2, presumably reflecting loss of addiction to this protein [28–31].

The goal of the following discussion is not to provide an overview of mechanisms of venetoclax resistance, as an excellent review of this topic has recently appeared [29]. Instead, the purpose of this review is to summarize molecularly rational combination strategies, focusing on other novel targeted agents, which are designed both to enhance venetoclax activity in various hematologic malignancies as well as to circumvent resistance to this agent.

Targeting bcl-2 with venetoclax in hematologic malignancies

B-cell malignancies

As B-cell malignancies are known to be dependent upon Bcl-2 for survival [32], it is not surprising that a specific Bcl-2 antagonist, such as venetoclax, would be effective in these malignancies. Indeed, venetoclax has shown marked activity in CLL [33] and has been formally approved for the treatment of relapsed/refractory CLL exhibiting the Del 17p in the United States [34]. It is also approved in Europe for patients without this translocation or TP53 mutations who have failed chemoimmunotherapy or BCR inhibitors. Surprisingly, relatively little is known about the mechanism(s) by which CLL cells become resistant to venetoclax in the clinical setting, although postulated mechanisms include upregulation of Mcl-1 and/or Bcl-xL, activation of intracellular signaling pathways (e.g. MEK/ERK), and microenvironmental forms of resistance [35,36], nor are extensive preclinical studies available to suggest strategies to overcome this phenomenon. In this regard, results of a recent study indicate that inhibition of the Bruton's tyrosine kinase (BTK) can lower the threshold for mitochondrial priming of CLL cells by venetoclax in association with Bim

upregulation and pronounced cell death [37]. These findings are in accord with those of Chiron et al., who presented a rationale for sequential administration of these agents in lymphoma cells [38]. Such findings raise the possibility of combining BTK antagonists and venetoclax in both frontline therapy of CLL and resistant disease [39]. Indeed, the combination of ibrutinib and venetoclax (with obinutuzumab) has been explored in patients with relapsed/refractory CLL [40], and trials of this combination in the frontline setting are currently being investigated (NCT02758665). Analogously, the dual delta- and gamma-PI3 kinase inhibitor duvelisib was shown to increase the sensitivity of CLL cells to venetoclax in association with upregulation of multiple pro-apoptotic Bcl-2 family members [41]. Notably, this strategy also circumvented stromal cell-mediated resistance mechanisms. A trial combining venetoclax with duvelisib for patients with relapsed/refractory CLL has been initiated.

Bojarczuk et al. examined the ability of various BCR inhibitors to potentiate venetoclax lethality in CLL cells. They discovered that SYK inhibitors were the most effective of the agents tested with respect to downregulation of Mcl-1 and enhancement of venetoclax lethality [42]. Analogously, a high-content microscopic assay identified the multikinase inhibitor sunitinib as superior to BCR antagonists in potentiating venetoclax lethality in activated B cells [43].

As in the case of CLL, relatively little information is available regarding strategies designed to overcome venetoclax resistance in non-Hodgkin lymphoma. However, Choudary et al. reported that NHL cell lines rendered resistant to venetoclax exhibited upregulation of the pro-apoptotic proteins Bcl-xL and Mcl-1 [44]. Notably, resistance of these cells could be reversed, at least in part, by interference with activation of the PI3K/AKT/mTOR pathway. Such findings are analogous to those reported in AML cells in which inhibition of the latter pathway led to dephosphorylation/activation of GSK and resulting degradation of Mcl-1, thus sensitizing cells to BH3-mimetics, for example navitoclax [45].

More recently, Li et al. demonstrated that the CDK inhibitor dinaciclib sharply increased the lethal effects of venetoclax in both *in vitro* and *in vivo* NHL (DLBCL) cell models [46]. The mechanism responsible for this phenomenon appeared to stem from dinaciclib-mediated inhibition of the pTEF-b transcription complex, culminating in downregulation of the short-lived protein Mcl-1 and subsequent cell death.

Very recently, BET inhibitors have been shown to enhance the activity of venetoclax in various tumor cell models including AML and NHL [47]. The mechanism(s) by which these agents interact remain to be fully elucidated. Interestingly, BET inhibitors have recently been shown to enhance venetoclax activity in T-cell acute lymphoblastic leukemia [48].

Chiron et al. showed that mitochondrial priming by anti-CD20-directed antibodies, for example, obinutuzumab could help to overcome microenvironment-mediated resistance in mantle cell lymphoma and potentially increase venetoclax sensitivity [49]. Similarly, Bodo et al. reported that t(14;18) lymphoma models with acquired resistance to venetoclax could be resensitized to this agent by anti-CD20 antibodies or MEK1/2 inhibitors [50]. Concordant results were obtained by Thijssen et al. [51]. Such findings provide a theoretical foundation

for combining venetoclax with such agents in NHL. In this context, the nucleoside analog acadesine downregulated Mcl-1 in mantle cell lymphoma cells and sensitized them to venetoclax [52].

In studies involving NHL systems, disabling of Mcl-1, for example, by either CDK inhibitors such as flavopiridol or specific Mcl-1 antagonists sharply increased the activity of venetoclax or navitoclax [53]. Such findings highlight the critical role of Mcl-1 in determining venetoclax sensitivity in NHL cells and emphasize the importance of targeting this molecule in circumventing venetoclax resistance. In accord with these findings, the protein translation inhibitor homoharringtonine downregulated Mcl-1 and increased the sensitivity of DLBCL cells to venetoclax [54].

Myeloid leukemia/AML

While the dependence of B-cell malignancies on Bcl-2 for survival has long been recognized, it was less obvious that AML cells would share such a dependence. However, initial preclinical studies revealed that AML cell lines, primary AML cells and murine AML xenograft models were highly susceptible to venetoclax [23]. Furthermore, BH3 mitochondrial profiling was able to predict the susceptibility of individual patient samples to this agent. Notably, this preclinical study provided a basis for launching a venetoclax trial in patients with AML, which revealed unexpected single-agent activity [55].

A subsequent study demonstrated that venetoclax sensitized relatively resistant AML cells to the hypomethylating agent 5-azacytidine, although navitoclax was more effective in this regard [56]. Levels of BCL-xL and MCL-1 were major determinants of venetoclax sensitivity, and silencing of these proteins increased venetoclax-mediated cell death. Notably, results of recent trials combining venetoclax with 5-azacytidine in patients with relapsed/refractory AML have yielded encouraging results [57]. However, such trials are currently on hold due to unanticipated toxicities (sepsis) and await amendments prior to reinitiation.

Chan et al. reported that mutations in IDH1/2 in human leukemia cells dramatically sensitized them to venetoclax [58]. This sensitization was mediated by 2-hydroxyglutarate-mediated disruption of the mitochondrial electron transport chain. Such findings raise the possibility that venetoclax may help to overcome resistance of IDH1/2-mutant AML cells to IDH1/2 antagonists.

Another metabolic strategy to enhance venetoclax activity was described by Jacque et al. who reported that glutaminase interruption, for example, by genetic knockdown of the upstream genes GLS1/2 or by the pharmacologic inhibition of these proteins by CB-839 in human myeloid leukemia cells disrupted oxidative phosphorylation [59]. This phenomenon was associated with mitochondrial priming and lowering the threshold for venetoclax-mediated cell death. These findings raise the possibility that interference in oxidative phosphorylation may enhance venetoclax efficacy in AML.

Knorr et al. observed that the NEDD8-activating enzyme (NAE) inhibitor pevonedistat (MLN4924)-induced Noxa upregulation in human myeloid leukemia cells, an effect that was

related to upregulation of c-Myc [60]. Knockdown of both c-Myc and Noxa diminished MLN4924-induced cell killing. The accumulation of Noxa was associated with degradation of Mcl-1, which significantly increased the antileukemic actions of both navitoclax and venetoclax. Together, these findings suggest a potential role for MLN4924 in enhancing venetoclax anti-leukemia activity and circumvention of resistance.

Inhibition of galectin 3 by a pharmacologic inhibitor (GCS-100) effectively sensitized both wild-type and FLT3-ITD mutant AML cells to venetoclax and navitoclax [61]. Interestingly, these events were not dependent upon Mcl-1 downregulation but were dependent upon p53 expression as p53 knockdown attenuated the activity of galectin antagonists.

The MDM2 antagonist idasanutlin was shown to enhance the antileukemic activity of venetoclax in p53 wild-type AML cells. This interaction was associated with perturbations in p53 signaling and cell-cycle regulatory pathways, as well as Mcl-1 downregulation [62]. Consistent with these findings, the idasanutlin/ venetoclax regimen demonstrated superior *in vivo* efficacy compared to single-agent treatment, suggesting a potential role for this strategy in p53 wild-type AML. Notably, a phase-I trial of venetoclax in combination with idasanutlin and the MEK1/2 inhibitor cobimetinib is currently underway in patients with relapsed/refractory AML (NCT02670044).

Analogous to previously cited results involving NHL systems, coadministration of a BET family bromodomain inhibitor (ABBV-075) with venetoclax synergistically induced cell death in AML model systems [47]. This interaction was associated with multiple changes in the expression of Bcl-2 family proteins, including downregulation of BCL-XL and MCL-1, and upregulation of Bim and PUMA. Similar results were obtained in multiple myeloma and NHL models. Collectively, these findings support a strategy in AML and potentially other hematopoietic malignancies combining BET inhibitors with venetoclax.

Multiple myeloma

In light of their importance in determining multiple myeloma cell survival and resistance to chemotherapy, members of the Bcl-2 family represent logical candidates for therapeutic intervention in this disorder. Because navitoclax (ABT-263) administration was associated with thrombocytopenia, presumably due to the dependence of platelets on Bcl-xL for survival [63], attention in myeloma has recently focused on the selective Bcl-2 antagonist venetoclax in this disease.

Studies comparing the activity of venetoclax to navitoclax in multiple myeloma cells revealed that venetoclax was at least as effective against these cells compared to its Bcl-xL-inhibiting counterpart [64]. This suggests that Bcl-xL may not be a critical survival factor for Mcl-1 cells. Interestingly, venetoclax, like navitoclax, was significantly more active against multiple myeloma cells displaying the t(11;14) translocation and high Bcl-2/Mcl-1 ratios. In contrast, it was relatively ineffective against high-risk patient samples (e.g. t(4;14) exhibiting low Bcl-2/Mcl-1 ratios. Collectively, these observations argue that Bcl-2 and Mcl-1 expression play a major role in determining multiple myeloma cell susceptibility to venetoclax. This same group subsequently reported that BH3 profiling revealed a

heterogeneous dependency on Bcl-2 family members in multiple myeloma, as well as sensitivity to BH3-mimetics such as venetoclax [27].

Bajpai et al. reported that interventions that depleted cellular glutamine resulted in diminished binding of Bim to Mcl-1 in multiple myeloma cells, and a significant increase in sensitivity to venetoclax [65]. Specifically, they found that the glutamine antagonist DON sharply increased venetoclax lethality in cultured and primary multiple myeloma cells, including those obtained from patients resistant to standard therapy.

Most recently, Punnoose et al. examined determinants of multiple myeloma cell resistance and sensitivity to venetoclax. They found that in multiple myeloma cell lines, sensitivity correlated with high Bcl-2 to Bcl-xL/Mcl-1 ratios, suggesting that Bcl-xL, like Mcl-1, may play a role in venetoclax-mediated cell death [28]. They also observed that cells coexpressing Bcl-2 and Bcl-xL were resistant to venetoclax, but this was reversed by a selective Bcl-xL inhibitor. In contrast, resistant cells co-expressing Bcl-2 and Mcl-1 were made more sensitive to venetoclax following exposure to bortezomib, which triggered upregulation of Noxa and subsequent down-regulation of Mcl-1. The authors concluded that Bcl-xL, in addition to Mcl-1, may represent a resistance factor to venetoclax in multiple myeloma.

Rational combination strategies with near-term translational potential

As is the case with essentially all targeted agents, resistance to mitochondrial-targeting drugs, either intrinsic or acquired, is likely to occur, making the chance of durable responses to single-agent therapy remote. Consequently, combination strategies will be required to circumvent this problem. These can take two forms: empiric combinations, for example, with standard cytotoxic agents, or rational combinations with other targeted agents based upon molecularly-based interactions. The large number of combination strategies cited in the preceding sections illustrate the numerous possibilities that exist in the case of the latter regimens. A brief description of examples of rational combination strategies involving targeted agents that have significant translational implications for hematologic malignancies, including resistant disease, in the near-term is provided below.

Histone deacetylase inhibitors (HDACIs)

HDACIs such as panobinostat have been approved, with bortezomib and dexamethasone, for the treatment of patients with relapsed/refractory multiple myeloma [66,67]. Among their numerous actions, HDACIs have been shown to upregulate expression of Bim through an E2F-dependent process [68]. Notably, HDACIs, when combined with BH3-mimetics such as ABT-737, effectively trigger apoptosis in multiple myeloma cells exhibiting either intrinsic or acquired resistance to bortezomib [25] through a process depending, at least in part, upon Bim upregulation. Whether similar effects occur in multiple myeloma cells exposed to venetoclax remains to be determined.

CDK inhibitors

A strong rationale exists for employing CDK inhibitors, particularly inhibitors of CDK9, to promote BH3-mimetic lethality in hematopoietic malignancies. As noted previously, Mcl-1

is a major determinant of resistance to BH3 mimetics and this short-lived protein is dependent upon active transcription for its persistence. Consequently, inhibitors of the pTEFb-related transcriptional regulatory apparatus can downregulate Mcl-1 and promote BH3-mimetic lethality [46]. In addition, CDK9 inhibitors may also upregulate BH3-only proteins such as Bim and Bik [69], which could potentiate the lethal effects of agents that inactivate antiapoptotic proteins such as Bcl-2 or Bcl-xL. Finally, CDK9 inhibitors may disrupt cytoprotective autophagy in response to Bcl-2 antagonists, leading to enhanced cell death [70]. In this context, it would be of significant interest to determine whether CDK9 inhibitors might overcome the resistance of certain high-risk myeloma subtypes (e.g. t;(4;14) to venetoclax. In this regard, as noted previously, the pan CDK inhibitor dinaciclib potentially enhanced the activity of venetoclax in NHL cells both *in vitro* and *in vivo* [46].

Proteasome inhibitors

While proteasome inhibitors can theoretically induce Mcl-1 upregulation by blocking degradation of this protein, multiple studies have shown that these agents can effectively downregulate this protein. This action most likely reflects induction of Noxa, a protein associated with Mcl-1 degradation [71]. Indeed, coadministration of proteasome inhibitors with BH3-mimetics leads to increased myeloma cell death, and the latter agents are active against proteasome inhibitor-resistant myeloma cells [72]. Given the established activity of proteasome inhibitors in multiple myeloma, as well as the potential of BH3-mimetics, such as ABT-199, at least in certain myeloma subtypes [64], such combination strategies warrant further consideration in this disease.

Conclusions and future directions

Given advances in our understanding of cell death mechanisms [73], agents that perturb the balance between pro- and antiapoptotic proteins represent logical therapeutic candidates in hematologic malignancies including acute myelogenous leukemia, non-Hodgkin lymphoma and multiple myeloma, diseases characterized by aberrant expression of Bcl-2 family members. In addition, such aberrations are likely to play key roles in both intrinsic and acquired forms of resistance in this disease. However, like most other targeted agents, venetoclax and related agents are unlikely to exhibit profound and sustained single-agent activity; instead, rational combination strategies will undoubtedly be required to achieve these goals. In addition, given the ability of venetoclax to induce durable molecular remissions, the possibility of circumventing the emergence of drug resistance through the use of discontinuous treatment schedules warrants consideration. In view of the increased acceptance of such approaches, it is likely that many of these novel strategies will be tested in relapsed/refractory multiple myeloma, AML, CLL, and non-Hodgkin lymphoma in the near future.

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