



Therapeutic development and current uses of BCL-2 inhibition

Andrew W. Roberts

Blood Cells and Blood Cancer Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; Department of Clinical Haematology, The Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Melbourne, Australia; Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, Australia; and Victorian Comprehensive Cancer Centre, Melbourne, Australia

B-cell lymphoma 2 (BCL2) is a key protein regulator of apoptosis. It is variably highly expressed in many hematological malignancies, providing protection from cell death induced by oncogenic and external stresses. Venetoclax is the first selective BCL2 inhibitor, and the first of a new class of anticancer drug (BH3-mimetics) to be approved for routine clinical practice, currently in chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML). To help understand the potential and limitations of this therapy, this brief review will touch on the history of development of venetoclax, dissect its mechanism of action, and summarize critical evidence for its approved use in the management of patients with CLL and AML. It will also consider recent data on mechanisms of resistance and explore concepts pertinent to its future development based on key lessons learned to date.

LEARNING OBJECTIVES

- Understand how venetoclax inhibits BCL2 to trigger apoptosis of CLL and AML cells and other blood cancers and how resistance can develop
- Understand the results of pivotal trials in CLL and AML and how tailored venetoclax combinations may prove effective in other diseases

Introduction: the discovery of BCL2 and its function

B-cell lymphoma 2 (*BCL2*) was the name given to a gene of unknown function discovered as the anonymous partner of the immunoglobulin heavy chain locus in the typical translocation seen in follicular lymphoma: t(14;18).¹ How it was oncogenic remained a puzzle, until it was revealed that rather than promoting proliferation, the BCL2 protein acted to protect cells from apoptosis when overexpressed.²⁻⁴ BCL2 was the first mammalian gene product associated with apoptosis, and recognition of its function led to an explosion of research into apoptosis and subsequently recognition that evasion of cell death is a hallmark of cancer. Avoidance of apoptosis is a prominent feature of many hematological malignancies.

We now recognize a large family of BCL2-related proteins, all operating by nonenzymatic protein:protein interactions to regulate the intrinsic or mitochondrial pathway to apoptosis, some protecting against apoptosis and some promoting it.⁵ In the prosurvival subfamily, MCL1, BCLxL (BCL2L1), BCL2A1 and BCLB, like BCL2, inhibit the initiation of apoptosis. Through direct binding they hold in check the 2

key cell death effector proteins, BAX and BAK, which when activated congregate on the outer membrane of the mitochondria and create pores which permeabilize and depolarize the organelle, releasing cytochrome C and activating caspases that execute the destruction of cells in a manner we recognize as apoptosis.^{5,6} BCL2 and other prosurvival proteins are naturally antagonized by the proapoptotic BH3-only protein subfamily comprising BIM, BID, NOXA, PUMA, BAD, HRK, BMF, and BIK. Although BIM, PUMA and BID can bind and neutralize the function of all prosurvival proteins, BAD only binds and inhibits BCL2, BCLxL and BCLW, and NOXA preferentially inhibits MCL1 and BCL2A1.⁷

The balance of activity between prosurvival proteins and BH3-only proapoptotic proteins determines whether a cell will live or undergo apoptosis, and collectively they serve to integrate the diverse extracellular and intracellular signals promoting either survival (eg, growth factors, nutrients) or death induced by stress (eg, oncogenic/proliferative, DNA damage, etc). This balance is deregulated in many hematological malignancies by altered expression of

BCL2 (or related proteins) or loss of BH3-only proteins or effector proteins.⁶ The various genetic mechanisms by which these abnormalities can occur are summarized elsewhere,⁸ but it is important to realize that high-level expression of prosurvival proteins can be an epigenetically regulated adaptive response to cellular stress. By way of general summary, malignant cells with upregulated BCL2 are being protected from undergoing apoptosis despite cellular stresses which would kill their normal counterpart cells. As described by Letai, these malignant cells are “primed for death,”⁶ and in theory should be highly susceptible to loss of BCL2’s protective function.

Targeting BCL2 with the BH3-mimetic, venetoclax

BH3-mimetics are a new class of anticancer drug that mimic the actions of BH3-only proteins in that they bind to prosurvival proteins like BCL2 in the same way (indeed the same groove) and inhibit BCL2’s ability to bind BAX or BAK.⁹ As BCL2 also exists

bound to native BH3-only proteins, BH3-mimetics can also displace these endogenous activators of apoptosis. Venetoclax is a BCL2-selective BH3-mimetic and its addition to BCL2-overexpressing cancer cells in vitro potently triggers apoptosis¹⁰ (Figure 1).

Extensive preclinical and in vivo patient data confirm that the principle mechanism by which venetoclax kills malignant blood cells is by induction of apoptosis.^{10,11} Killing is absolutely dependent on BAX/BAK and for most susceptible cells is very rapid in onset,¹² with permeabilization of the mitochondria occurring within minutes and death within hours, including in patients.^{10,11,13} In some less susceptible cells, mitochondrial permeabilization induced by venetoclax is insufficient to directly generate sufficient caspase activation for apoptosis, but disruption of mitochondrial energy production can prove lethal to vulnerable cells (eg, some AMLs)¹⁴⁻¹⁶ and release of mitochondrial DNA can trigger an antiviral like cell death response in others.¹⁷

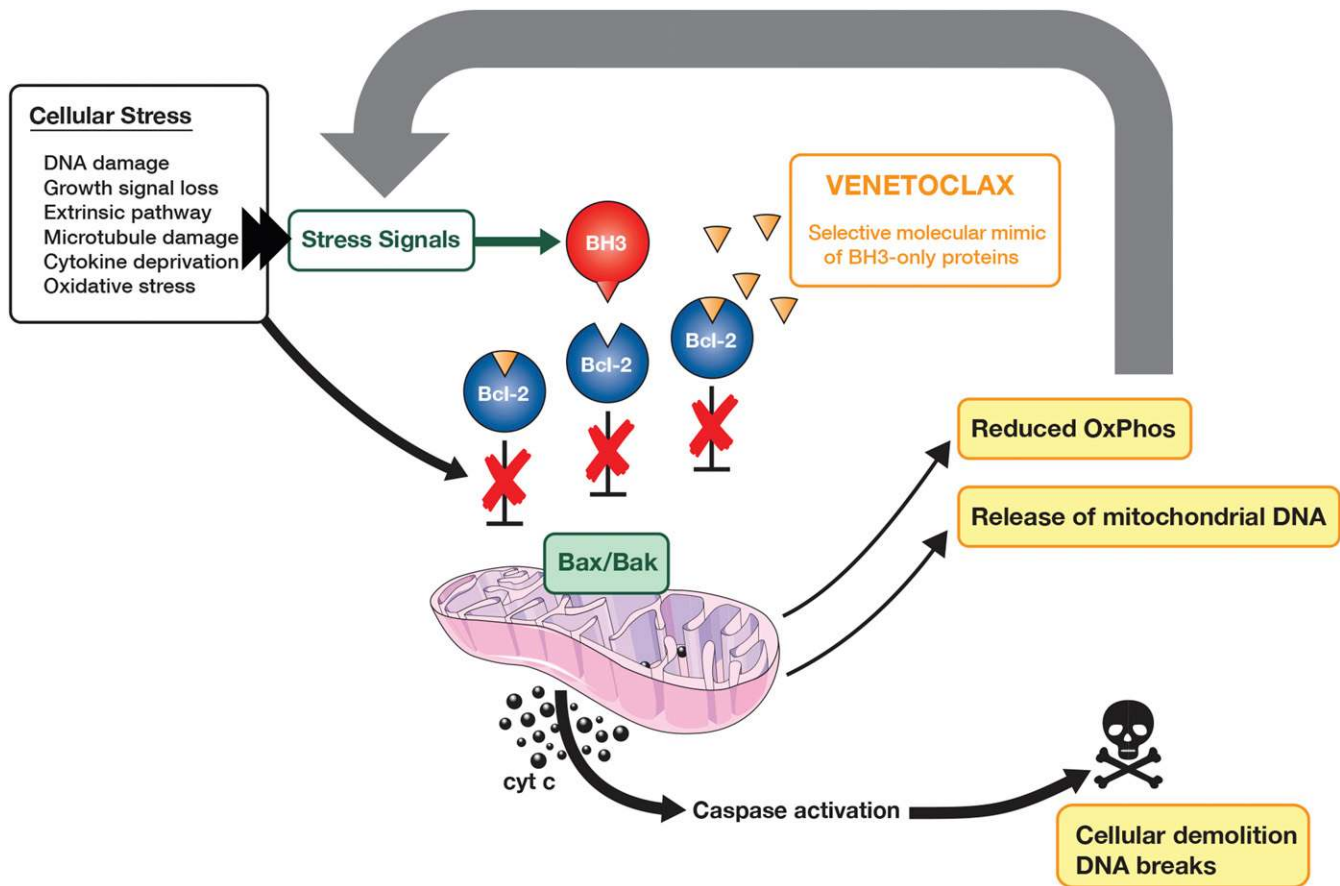


Figure 1. The mechanism of action of venetoclax, a BH3-mimetic that is a highly selective inhibitor of BCL2. The diagram illustrates in cartoon form how the small molecule venetoclax acts intracellularly in a BCL2-overexpressing leukemic cell to initiate apoptosis by mimicking the action of the endogenous antagonists of BCL2, the BH3-only proteins. Heightened expression of BCL2 protects leukemia cells from apoptosis by inhibiting activation of BAX and BAK, even when normally lethal cellular stresses induce prodeath BH3-only proteins such as BIM and NOXA. Venetoclax interacts with BCL2 selectively in the BH3-binding groove to directly and indirectly (via release of BIM) relieve repression of BAX/BAK which homodimerize or heterodimerize to permeabilize mitochondria,¹⁰ unleashing apoptosis through release of cytochrome C and subsequent caspase activation, which demolishes cellular organelles and the nuclear structures.⁵ Release of cytochrome C and caspase activation are generally considered the point of no return for cells. In some cells, apoptosis is not immediately fully established, but downstream effects such as disruption to oxidative phosphorylation^{14,16} or release of mitochondrial DNA¹⁷ amplify cellular stresses and complete commitment to apoptosis. Modified from Anderson et al⁵⁶ with permission.

Whether an individual cancer cell lives or dies after venetoclax therapy depends on the general dependence on BCL2 of that cell type (high for mature B lymphocytes, low for macrophages),¹⁸ the cell-intrinsic oncogenic stresses (high for Myc-driven, lower for kinase-driven tumors),¹⁹ the microenvironment in which the cell resides,²⁰ and the presence of other stressors (eg, additional therapy, such as DNA-damaging agents).¹⁰ Consequently, different hematological malignancies display varying susceptibility to BCL2 inhibition in preclinical testing (reviewed elsewhere).⁸

The current sum of preclinical and clinical data suggests that as a highly specific therapy, we should think of venetoclax as an agent that directly hits the "bullseye" (the Achilles' heel of the cancer) only in instances where direct inhibition of BCL2 rapidly induces cell death in the majority of cells (Figure 2A). In other cell types, this drug also hits the "target," but in a different way, with direct inhibition of BCL2 immediately setting off a wave of secondary consequences that encompass the "bullseye" and cause cell death (Figure 2B). This may be via triggering secondary inhibition of other prosurvival proteins (eg, MCL1, BCLxL) through displacement of previously bound BIM from BCL2, or reduction in oxidative phosphorylation by permeabilized mitochondria in AML, etc. For some diseases, such as AML, there will be heterogeneity in how cell populations respond, reflecting how BCL2-dependent individual cells are, and this will vary between patients depending on the genetic and epigenetic makeup of the cancer. The more cells that are impacted in "hammer-strike" fashion rather than an "arrow to the bullseye" fashion, the more important concomitant therapy will be to achieve a high degree of cell killing.

Venetoclax and CLL

BCL2 is highly expressed in all CLL cells in all patients, and the great majority of CLL cells appear dependent on BCL2 for

survival.^{10,12,21} Consistent with this, venetoclax is effective as monotherapy in ~75% to 80% of patients with relapsed CLL, and complete remission (CR; including CR with incomplete recovery [CRi]) can be expected in 15% to 20% of patients^{13,22} (Table 1). The rate of response, including CR, is independent of genetic subtypes, but the negative prognostic genetic markers del17p, TP53 mutations, and NOTCH1 mutations are associated with less durable responses in multivariable analyses.²³ Achieving CR and/or having undetectable minimal residual disease (uMRD; <10⁻⁴) in the peripheral blood (PB; achieved in 30% of patients) or bone marrow (BM) is associated with prolonged remissions.^{23,24} Venetoclax monotherapy is approved therapy for relapsed del17p CLL in many jurisdictions.

No randomized trials have been performed to determine whether addition of rituximab or other anti-CD20 antibodies increases response rate or durability of response. Nevertheless, rates of CR (51%) and uMRD (57% in BM) appeared higher in the first early phase combination trial with rituximab.²⁵ This combination trial also provided evidence that indefinite continuous daily venetoclax therapy was not required in CLL, with highly durable remissions continuing in patients who ceased in either CR or uMRD response.²⁶ These 2 characteristics led to the use of limited-duration combination venetoclax-anti-CD20 regimens in pivotal trials. Combination therapy with rituximab in the relapsed setting and with obinutuzumab in the frontline setting are associated with high rates of CR (27% and 50%, respectively) and PB uMRD (62% and 76%, respectively) at the end of combination therapy.^{27,28}

In randomized trials (Table 2), venetoclax-rituximab proved superior to bendamustine-rituximab for patients with relapsed CLL in terms of efficacy (progression-free survival [PFS] and overall survival) and toxicity (febrile neutropenia)²⁷; and venetoclax-obinutuzumab proved superior to chlorambucil-obinutuzumab for treatment-naïve older patients with comorbidities in terms of

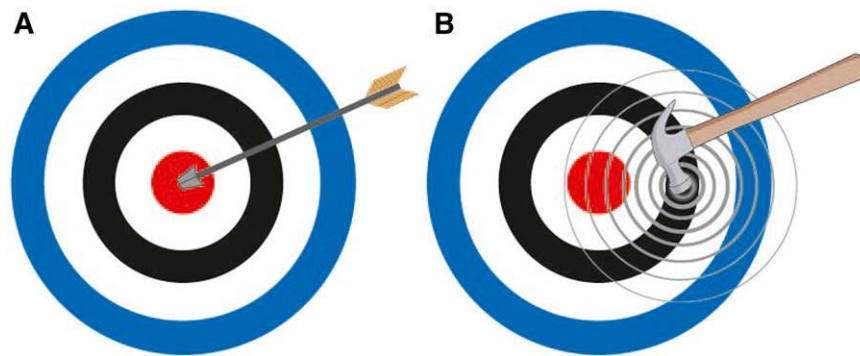


Figure 2. Schema for understanding variability in susceptibility of hematological malignancies to BCL2 inhibition. Venetoclax is a highly targeted therapy, binding almost exclusively to BCL2 when used in clinically achievable concentrations. (A) An illustration of the concept that venetoclax can hit the "bullseye" in malignancies such as CLL. In cells with invariably high expression of BCL2 and relatively minor expression of other prosurvival proteins, inhibition of BCL2 is sufficient to trigger apoptosis reliably in a high proportion of cells.^{10,11} Such cells can be considered BCL2-dependent, and we recognize these diseases clinically through their high rate of response and complete response to venetoclax monotherapy.^{13,24} (B) An illustration of the more common scenario, in which BCL2 is a target worth hitting, but inhibition of BCL2 directly is insufficient to "hit the bullseye." Cell killing by venetoclax is dependent on a wave of secondary events including secondary inhibition of other prosurvival proteins by displaced BH3-only proteins,^{8,57,58} or mitochondrial depolarization^{14,16} (see main text on this page). Such cells are not strictly BCL2-dependent. Additional stressors are likely to be required to maximize apoptosis and achieve high response rates in diseases in which the majority of cells are affected in this manner. Diseases such as AML and some lymphomas typically have a mix of malignant cells, some displaying susceptibility akin to panel A; others are vulnerable only through the secondary wave effect shown in panel B.

Table 1. Summary of indicative mature clinical trial data for venetoclax in hematological malignancies

| Monotherapy | | | | Combination | | | | |
|--|------------------|-----|----------------|--------------------------------|--|------------------|------------------|------------------------|
| Phase | Response rate, % | | Median PFS, mo | Partner drug(s) | Phase | Response rate, % | | Median PFS, mo† |
| | Overall | CR* | | | | Overall | CR* | |
| CLL | | | | | | | | |
| Relapsed/refractory | | | | | | | | |
| 1 ¹³ | 79 | 20 | 25 | + Rituximab | 1b ^{25,26} | 86 | 51 | 80% @ 2 y 56% @ 5 y |
| 2 ^{22,24} | 79 | 16 | 27 | + Rituximab | 3 ^{27,59} | 92 | 27 | 85% @ 2 y 71% @ 3 y |
| | | | | + Ibrutinib | 2 ²⁹ | 89 | 51 | 100% @ 1 y |
| First-line | | | | + Obinutuzumab + Ibrutinib | 3 ²⁸ 2 ³⁰ | 84.7 NR | 49.5 74‡ | 88% @ 2 y 98% @ 1 y |
| AML | | | | | | | | |
| Relapsed/refractory | | | | | | | | |
| 1b ³¹ | 38 | 19 | 2§ | + Aza/Decitabine | 2 ³⁵ 2 ³⁶ | 64 50 | 51 22.5 | 9 ? |
| First-line | | | | | | | | |
| (Elderly and/or unsuitable for standard induction) | | | | + Aza/Decitabine | 1b/2 ³⁴ | 62 | 60 | 11§ |
| | | | | + Azacitidine | 3 ³⁸ | 3 ³⁸ | 66 (61-72) | 10 (8-12)¶ |
| | | | | + Low-dose Ara-C | 1b/2 ³³ 3 ³⁷ | 64 NA | 62 48 (39-56) | 13.2 4.7¶ |
| (Fit, unsuitable for standard induction) | | | | + Ara-C/Idarubicin | 1b ⁴¹ | | 72 | 13.5 |
| Lymphoma (relapsed) | | | | | | | | |
| Follicular | | | | | | | | |
| 1 ⁴² | 38 | 14 | 11 | + Bendamustine/ Rituximab | 1b ⁶⁰ | 75 | 38 | NR @ 24 mo |
| Mantle cell | | | | | | | | |
| 1 ⁴² | 75 | 21 | 14 | + Ibrutinib | 2 ^{46,61} | 75 | 71 | 29 |
| Diffuse large B cell | | | | | | | | |
| 1 ⁴² | 18 | 12 | 1 | + Bendamustine/ Rituximab | 1b ⁶⁰ | 41 | 14 | 4 |
| Myeloma (relapsed) | | | | | | | | |
| All | | | | | | | | |
| 1 ⁴³ | 21 | 7 | 3# | + Bortezomib/ Dexamethasone | 1b ⁴⁹ 3 ^{50,62} | 67 84 | 20 29 | 9.5 23 |
| t(11;14) | 40 | 14 | 7# | + Bortezomib/ Dexamethasone | 1b ⁴⁹ 3 ^{50,62} | 78 95 | NA 55 | NA NR @ 29 mo |

Initial phase 1 and phase 2 trials and all phase 3 trials formally reported to date for CLL and AML are included here, but the table is incomplete for recent combination phase 1b and phase 2 trials in myeloma, lymphoma, and other malignancies. The first early phase combination trials have been selected to provide the most simple indirect comparisons with monotherapy activity.

Ara-C, cytosine arabinoside; Aza/Decitabine, azacitidine or decitabine; NA, not reported; NR, not reached; PFS, progression-free survival.

*CR indicates complete response (and/or CR with incomplete count recovery) as assessed by investigators as best response during trial.

†Where median PFS not reached, estimate at specific time point is provided.

*CR by intention to treat at time of reporting when many patients had not completed planned therapy.

§Duration of response.

||Leukemia-free survival for CR achievers.

¶Event-free survival reported.

#Time to progression.

Table 2. Key results of randomized trials related to FDA-approved indications

| Treatment | Durability of benefit | | | Overall survival | | | Toxicity | | | |
|---|-----------------------|------|-------|------------------|----------------|-------|-----------|----------------------------|--------------|---------------------------|
| | PFS/EFS* | HR | P | | HR | P | Nausea, % | ≥G3 febrile neutropenia, % | Pneumonia, % | Discontinued due to AE, % |
| CLL-relapsed^{27,59} | | | | | | | | | | |
| Ven-Ritux | 71% @ 3 y | 0.16 | <.001 | 88% @ 3 y | 0.50 (.3-.85) | .009 | 21 | 3.6 | 8.2 | 17 |
| Ben-Ritux | 15% @ 3 y | | | 80% @ 3 y | | | 34 | 9.6 | 8.0 | 16 |
| CLL-first-line²⁸ | | | | | | | | | | |
| Ven-Obin | 88% @ 2 y | 0.35 | <.001 | 92% @ 2 y | 1.24 (.64-2.4) | .52 | 19 | 17.5 | 4.7 | 22 [†] |
| Chl-Obin | 64% @ 2 y | | | 93% @ 2 y | | | 22 | 15.0 | 4.2 | 23 [†] |
| AML first-line (including sAML pretreated with HMA)³⁷ | | | | | | | | | | |
| Ven-LoDAC | 4.7* | 0.58 | .002 | 8.4 | 0.70 (.50-.99) | .04 | 42 | 32 | 13 | 9 |
| Pbo-LoDAC | 2.0* | | | 4.1 | | | 31 | 29 | 10 | 9 |
| AML-first-line (no prior HMA)³⁸ | | | | | | | | | | |
| Ven-Aza | 9.8* | | <.001 | 14.7 | 0.66 (.52-.85) | <.001 | 44 | 30 | 16 | NR |
| Pbo-Aza | 7.0* | | | 9.6 | | | 35 | 10 | 22 | NR |

≥G3, grade 3 or higher; AE, adverse event; Aza, azacytidine; Ben, bendamustine; Chl, chlorambucil; EFS, event-free survival; FDA, US Food and Drug Administration; HMA, hypomethylating agent therapy; HR, hazard ratio; LoDAC, low-dose cytosine arabinoside; NR, not reported; Obin, obinutuzumab; Pbo, placebo; PFS, progression-free survival; Ritux, rituximab; sAML, secondary AML; Ven, venetoclax.

*EFS for AML.

[†]All cause discontinuation excluding PD.

PFS, with similar toxicity.²⁸ These 2 regimens are approved in many jurisdictions as a standard of care.

In ongoing trials, venetoclax is being combined with ibrutinib and other BTK inhibitors, with and without anti-CD20 monoclonal antibodies. Preliminary data with ibrutinib-venetoclax combinations indicate high rates of CR (51% to 74%; Table 1) and PB uMRD (53% in the relapsed²⁹ and 61% in the frontline settings³⁰) after ~1 year of combined therapy, but efficacy and safety relative to currently approved regimens is unknown at this point.

A key unresolved question relates to the optimal duration of treatment with venetoclax, and whether this should be a fixed duration of therapy as currently approved or whether it should be informed by response assessment (ie, adaptive to depth and speed of response, with slow and incomplete responders receiving more prolonged therapy, and rapid responders less). Related to this is whether the use of time-limited therapy will reduce the emergence of resistant clones at progression, thereby enabling effective reuse of venetoclax-based regimens when progression occurs off therapy.

Venetoclax and AML

BCL2 is variably expressed in AML, and only a minority of patient samples show marked sensitivity in vitro.^{31,32} Consistent with this, single agent activity is evident in patients. In the phase 1 monotherapy trial in relapsed disease a minority demonstrated major reductions in blasts, and only 19% achieved CR/CRi.³¹ Even then the median duration of response was short (Table 1). These data reflect that inhibition of BCL2 in AML results in a

"hammer-strike" effect, with BCL2 being a significant target, but not a bullseye. Consequently, combination therapy is essential. The first partners evaluated in trials were low-dose cytosine arabinoside (LoDAC)³³ and the hypomethylating agents azacytidine and decitabine³⁴ (Table 1). In relapsed AML, the CR/CRi rate appears higher with these combinations than with monotherapy,^{35,36} but how much each drug is contributing and whether the antileukemic effects are subadditive, additive, or synergistic is unknown.

Not surprisingly, response rates in frontline therapy with the combinations are higher, with CR/CRi rates of 48% to 66% observed.^{33,34} There is little evidence for benefit in patients with AML who do not achieve CR. These venetoclax-combination regimens received provisional US Food and Drug Administration (FDA) approval as frontline treatments of elderly or unfit patients on the basis of early phase trial data, and placebo-controlled phase 3 trial data have just been reported in 2020 (Table 2).

With extended follow-up, the addition of venetoclax to LoDAC in patients with primary or secondary AML (20% pretreated with hypomethylating agents) and aged >75 years or unfit for intensive induction therapy modestly improved overall survival and event-free survival (EFS; HR 0.7 and 0.58, respectively).³⁷ CR rates were substantially higher with the venetoclax combination across prognostic categories (cytogenetic risk groups, primary or secondary, prior hypomethylating therapy, selected driver mutations) and similar magnitudes of relative survival benefit seen for the whole population were suggested in an underpowered exploratory analysis.

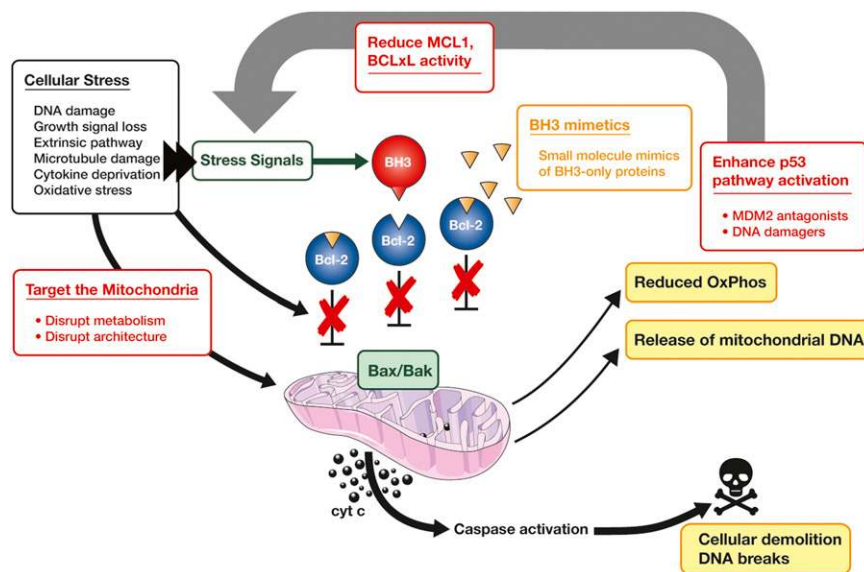


Figure 3. The anticancer effect of venetoclax theoretically can be enhanced through rational combination with other targeted therapies. This cartoon builds on the illustration of the mechanism of action of venetoclax in Figure 1 to highlight opportunities for enhancing apoptosis. A major avenue for amplifying the proapoptotic signal is to reduce the expression or activity of other pro-survival BCL2-like proteins (eg, MCL1 or BCLxL; red box, top center). This can be achieved directly by adding selective inhibitors of these proteins. Examples where this has been demonstrated preclinically^{32,54,63} and are being explored in clinical trials include AML, ALL, and mantle cell lymphoma. Reduction in prosurvival protein function can also be achieved indirectly via induction of their natural antagonists (eg, NOXA which antagonizes MCL1 and BCL2A1) by enhancing activity of the TP53 pathway through DNA damage or inhibition of MDM2⁶⁴ (red box, right side). These strategies are being explored clinically in lymphomas and AML. Preclinical evidence further indicates that killing can be augmented through direct targeting of mitochondrial structures and functions, such as energy production (red box, left side). This has been demonstrated particularly, but not only, in AML.^{15,16} A partial explanation of the enhanced efficacy of the azacitidine-venetoclax combination in AML includes disruption to energy metabolism.⁶⁵ The cartoon also depicts how combinatorial approaches can both amplify the proapoptotic effect upstream of BAX/BAK and also reduce the threshold for mitochondrial vulnerability to BAX/BAK activation. To maximize the therapeutic index for any of these combination approaches, each will need to be tailored to the specific vulnerabilities of individual diseases, and biomarkers may prove advantageous in this regard.

Addition of venetoclax to azacitidine in similar patients, but excluding those previously receiving hypomethylating agents for preceding myelodysplasia, also improved overall survival (HR, 0.66) and EFS.³⁸ In both trials, improvement in median overall survival was 4 to 5 months, with no plateau evident on survival curves and the majority of patients dying within 2 years. In both treatment-naïve and relapsed settings, well-established prognostic factors appear to still be relevant with venetoclax-based therapy. Lowest survival is still seen in high-risk cytogenetic subgroups and where *TP53* mutations are detected, and more favorable outcomes in intermediate cytogenetic risk AML with either *NPM1* or *IDH1/2* mutations.^{39,40} Mature follow-up and meta-analyses will be required to determine if any genetic marker is a true response-modifier that can be used to refine clinical decision-making.

A key question being addressed by several trials is whether venetoclax could have a role in treatment of patients fit for induction chemotherapy. Given that venetoclax induces selective killing of granulocytic progenitor cells in vitro and neutropenia in vivo, substantial additional bone marrow toxicity is anticipated, and scheduling issues are not yet resolved. Initial publications are expected during 2020, with an early trial indicating that venetoclax 600 mg per day for 14 days can be safely added to a 5+2 cytosine arabinoside/idarubicin regimen

and achieve high CR rates in a mixed population of patients >60 years⁴¹ (Table 1).

BCL2 inhibition in other hematological malignancies

Currently, venetoclax is being evaluated in >230 clinical trials in a wide range of hematological malignancies. Venetoclax has shown clinically meaningful single agent activity in selected lymphomas,⁴² multiple myeloma,⁴³ blastic plasmacytoid dendritic cell neoplasm,⁴⁴ and T-cell prolymphocytic leukemia.⁴⁵ It is also being evaluated in myelodysplasia using AML-style combinations and in relapsed acute lymphoblastic leukemia. Table 1 summarizes some illustrative published results for lymphoma and myeloma.

Mantle cell lymphoma is susceptible to single-agent BCL2 inhibition, with a 75% response rate in the relapse/refractory setting and durable responses particularly in the 21% achieving CR.⁴² Combination with ibrutinib appears additive at least, with PET-negative complete responses observed in >70% of patients, including 67% with uMRD, and in 50% of patients with *TP53*-aberrant disease.⁴⁶ At 30 months, 74% of responders remain relapse-free, and indefinite therapy is not necessarily required. Randomized trials are now comparing venetoclax-BTK inhibitor combinations with BTK inhibitor monotherapy.

Follicular lymphoma stands out as a disease with high and uniform expression of BCL2, yet only modest response rates with

venetoclax alone.⁴² This paradox remains to be resolved. Experience with venetoclax monotherapy in DLBCL was sobering, and the limited responses could not be associated with any specific pattern of BCL2 expression.⁴² In both these lymphoma types, combinations with DNA-damaging regimens and non-DNA-damaging regimens are being explored.

Multiple myeloma commonly expresses BCL2 at high, but variable, levels, as do normal plasma cells. However, responses to monotherapy are largely restricted to patients with the t(11;14) subclass,⁴³ as predicted preclinically,^{47,48} where BCL2 expression is highest. In some non-t(11;14) myeloma with high *BCL2/BCL2L1* (BCL-xL) expression ratios, responses can also be seen. Response rates and CR rates are higher when venetoclax is used in combination.⁴⁹ However, the therapeutic index of the venetoclax-bortezomib-dexamethasone combination in unselected patients with myeloma is problematic. Preliminary presentations of the randomized trial indicate increased antimyeloma activity but excess toxicity in patients whose myeloma lacks t(11;14) or high *BCL2/BCL2L1* expression ratio.⁵⁰

Lessons from clinical experience with venetoclax to date

As the first approved drug in this new class of anticancer therapy, experience with venetoclax has provided several key lessons that should help inform its ongoing development and that of future BH3-mimetics, for example, MCL1 inhibitors. First, because of its mechanism of action, venetoclax is a cytotoxic that kills vulnerable cells quickly,¹⁰⁻¹² with responses occur rapidly, typically with the first cycle.^{13,31} Second, durable benefit is predominantly seen in patients achieving CR, as seen in CLL,^{13,23} AML^{31,33} and sensitive lymphomas.⁴² Further in CLL, the most durable remissions are seen in patients who achieve MRD-negative remissions.^{23,24} Third, to achieve maximal tumor reduction, combination therapy is necessary. To date, venetoclax has been shown to be tolerable when combined with many different classes of drugs.

Fourth, among sensitive tumors, secondary clinical resistance may occur due to genetic or epigenetic changes in apoptosis regulators or by the acquisition of constitutive growth factor signaling. Changes that affect regulators of the intrinsic pathway to apoptosis have emerged as important in several lymphoid malignancies. Mutations in *BCL2* that encode proteins that maintain prosurvival function but have reduced (up to 180-fold) binding to venetoclax are prominent as a cause of late CLL relapse in long term venetoclax-treated patients.⁵¹ The most common is G101V, but several others have been described.^{52,53} MCL1 overexpression related to focal amplifications on chromosome 1q are also seen,¹⁶ as is upregulation of BCL-xL in CLL⁵¹ and in mantle cell lymphoma.⁵⁴ Importantly, each of these changes can co-occur in independent clones in the same patient. Data to date on secondary resistance in AML indicate that outgrowth of FLT3-ITD or RAS-MAPK pathway mutant subclones is common.^{39,40} Again, parallel emergence of clones with distinct mechanisms of resistance is observed in individual patients. Polyclonal heterogeneity is the norm for venetoclax-resistance, consistent with patterns now emerging for other highly targeted agents.⁵⁵

Finally, more translational research is urgently needed. Validated biomarkers are required to better select patients for venetoclax-based therapy in diseases where targeting BCL2 is not an "arrow through the bullseye." Similarly, rigorous pre-clinical experiments are required to guide improvement in overall response rates and length of remissions in AML, lymphomas and myeloma. Figure 3 provides suggestions as to how adding

targeted agents should amplify the apoptotic effect of BCL2 inhibition, based on recent insights into biology and mechanisms of resistance. It may be that we are just at the beginning of our understanding of how best to use BCL2 inhibitors like venetoclax.

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Conflict-of-interest disclosure

A.W.R. is an employee of the Walter and Eliza Hall Institute, which receives milestone and royalty payments related to venetoclax; receives a share of these royalties from the Institute; and has received research funding to his institutions from AbbVie, Janssen, and Servier for investigator-initiated clinical trials or laboratory research.

Off-label drug use

None disclosed.

Correspondence

Andrew W. Roberts, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; e-mail: roberts@wehi.edu.au.

References

1. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. *Science*. 1985;228(4706):1440-1443.
2. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature*. 1988;335(6189):440-442.
3. McDonnell TJ, Deane N, Platt FM, et al. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell*. 1989;57(1):79-88.
4. Strasser A, Harris AW, Bath ML, Cory S. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between myc and bcl-2. *Nature*. 1990;348(6299):331-333.
5. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*. 2002;2(9):647-656.
6. Letai AG. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. *Nat Rev Cancer*. 2008;8(2):121-132.
7. Chen L, Willis SN, Wei A, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol Cell*. 2005;17(3):393-403.
8. Roberts AW, Huang D. Targeting BCL2 with BH3 mimetics: basic science and clinical application of venetoclax in chronic lymphocytic leukemia and related B cell malignancies. *Clin Pharmacol Ther*. 2017;101(1):89-98.
9. Lessene G, Czabotar PE, Colman PM. BCL-2 family antagonists for cancer therapy. *Nat Rev Drug Discov*. 2008;7(12):989-1000.
10. Souers AJ, Levenson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med*. 2013;19(2):202-208.

11. Anderson MA, Deng J, Seymour JF, et al. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. *Blood*. 2016;127(25):3215-3224.
12. Vogler M, Dinsdale D, Dyer MJS, Cohen GM. ABT-199 selectively inhibits BCL2 but not BCL2L1 and efficiently induces apoptosis of chronic lymphocytic leukaemic cells but not platelets. *Br J Haematol*. 2013;163(1):139-142.
13. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2016;374(4):311-322.
14. Lagadinou ED, Sach A, Callahan K, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell Stem Cell*. 2013;12(3):329-341.
15. Jones CL, Stevens BM, D'Alessandro A, et al. Inhibition of amino acid metabolism selectively targets human leukemia stem cells. *Cancer Cell*. 2018;34(5):724-740 e.4.
16. Guizè R, Liu VM, Rosebrock D, et al. Mitochondrial reprogramming underlies resistance to BCL-2 inhibition in lymphoid malignancies. *Cancer Cell*. 2019;36(4):369-384.e13.
17. McArthur K, Whitehead LW, Heddleston JM, et al. BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science*. 2018;359(6378):eaa6047.
18. Khaw SL, Mérimo D, Anderson MA, et al. Both leukaemic and normal peripheral B lymphoid cells are highly sensitive to the selective pharmacological inhibition of prosurvival Bcl-2 with ABT-199. *Leukemia*. 2014;28(6):1207-1215.
19. Vandenberg CJ, Cory S. ABT-199, a new Bcl-2-specific BH3 mimetic, has in vivo efficacy against aggressive Myc-driven mouse lymphomas without provoking thrombocytopenia. *Blood*. 2013;121(12):2285-2288.
20. Thijssen R, Slinger E, Weller K, et al. Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors. *Haematologica*. 2015;100(8):e302-e306.
21. Mason KD, Khaw SL, Rayeroux KC, et al. The BH3 mimetic compound, ABT-737, synergizes with a range of cytotoxic chemotherapy agents in chronic lymphocytic leukemia. *Leukemia*. 2009;23(11):2034-2041.
22. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multi-centre, open-label, phase 2 study. *Lancet Oncol*. 2016;17(6):768-778.
23. Roberts AW, Ma S, Kipps TJ, et al. Efficacy of venetoclax in relapsed chronic lymphocytic leukemia is influenced by disease and response variables. *Blood*. 2019;134(2):111-122.
24. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax for patients with chronic lymphocytic leukemia with 17p deletion: results from the full population of a phase II pivotal trial [published correction appears in *J Clin Oncol*. 2019;37(25):2299]. *J Clin Oncol*. 2018;36(19):1973-1980.
25. Seymour JF, Ma S, Brander DM, et al. Venetoclax plus rituximab in relapsed or refractory chronic lymphocytic leukaemia: a phase 1b study. *Lancet Oncol*. 2017;18(2):230-240.
26. Brander DM, Seymour JF, Kipps TJ, et al. Durability of responses on continuous therapy and following drug cessation with venetoclax and rituximab: long-term follow-up analysis of a phase 1b study in patients with relapsed CLL [abstract]. *Blood*. 2019;134(suppl 1). Abstract 3036.
27. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med*. 2018;378(12):1107-1120.
28. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. *N Engl J Med*. 2019;380(23):2225-2236.
29. Hillmen P, Rawstron AC, Brock K, et al. Ibrutinib plus venetoclax in relapsed/refractory chronic lymphocytic leukemia: the CLARITY study [published correction appears in *J Clin Oncol*. 2020;38(14):1644]. *J Clin Oncol*. 2019;37(30):2722-2729.
30. Jain N, Keating M, Thompson P, et al. Ibrutinib and venetoclax for first-line treatment of CLL. *N Engl J Med*. 2019;380(22):2095-2103.
31. Konopleva M, Pollyea DA, Potluri J, et al. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. *Cancer Discov*. 2016;6(10):1106-1117.
32. Teh TC, Nguyen NY, Moujalled DM, et al. Enhancing venetoclax activity in acute myeloid leukemia by co-targeting MCL1. *Leukemia*. 2018;32(2):303-312.
33. Wei AH, Strickland SA Jr, Hou JZ, et al. Venetoclax combined with low-dose cytarabine for previously untreated patients with acute myeloid leukemia: results from a phase Ib/II study. *J Clin Oncol*. 2019;37(15):1277-1284.
34. DiNardo CD, Pratz KW, Letai A, et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol*. 2018;19(2):216-228.
35. Aldoss I, Yang D, Aribi A, et al. Efficacy of the combination of venetoclax and hypomethylating agents in relapsed/refractory acute myeloid leukemia. *Haematologica*. 2018;103(9):e404-e407.
36. Wang YW, Tsai CH, Lin CC, et al. Cytogenetics and mutations could predict outcome in relapsed and refractory acute myeloid leukemia patients receiving BCL-2 inhibitor venetoclax. *Ann Hematol*. 2020;99(3):501-511.
37. Wei AH, Montesinos P, Ivanov V, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. *Blood*. 2020;135(24):2137-2145.
38. DiNardo C JB, Pullarkat V, Thirman M, et al. A randomized, double-blind, placebo-controlled study of venetoclax with azacitidine vs azacitidine in treatment-naïve patients with acute myeloid leukemia ineligible for intensive therapy - VIALE-A. *N Engl J of Med*. 2020;383(7):617-629.
39. Chyla B, Daver N, Doyle K, et al. Genetic biomarkers of sensitivity and resistance to venetoclax monotherapy in patients with relapsed acute myeloid leukemia. *Am J Hematol*. 2018;93(8):E202-E205.
40. DiNardo CD, Tiong IS, Quagliari A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood*. 2020;135(11):791-803.
41. Chua CC, Roberts AW, Reynolds J, et al. Chemotherapy And Venetoclax in Elderly Acute myeloid leukemia Trial (CAVEAT): a phase 1b dose escalation study of venetoclax combined with modified intensive chemotherapy [published online ahead of print 20 July 2020]. *J Clin Oncol*. doi:10.1200/JCO.20.00572.
42. Davids MS, Roberts AW, Seymour JF, et al. Phase I first-in-human study of venetoclax in patients with relapsed or refractory non-Hodgkin lymphoma. *J Clin Oncol*. 2017;35(8):826-833.
43. Kumar S, Kaufman JL, Gasparetto C, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. *Blood*. 2017;130(22):2401-2409.
44. Montero J, Stephansky J, Cai T, et al. Blastic plasmacytoid dendritic cell neoplasm is dependent on BCL2 and sensitive to venetoclax. *Cancer Discov*. 2017;7(2):156-164.
45. Boidol B, Kornauth C, van der Kouwe E, et al. First-in-human response of BCL-2 inhibitor venetoclax in T-cell prolymphocytic leukemia. *Blood*. 2017;130(23):2499-2503.
46. Tam CS, Anderson MA, Pott C, et al. Ibrutinib plus venetoclax for the treatment of mantle-cell lymphoma. *N Engl J Med*. 2018;378(13):1211-1223.
47. Punnoose EA, Levenson JD, Peale F, et al. Expression profile of BCL-2, BCL-XL, and MCL-1 predicts pharmacological response to the BCL-2 selective antagonist venetoclax in multiple myeloma models. *Mol Cancer Ther*. 2016;15(5):1132-1144.
48. Touzeau C, Ryan J, Guerriero J, et al. BH3 profiling identifies heterogeneous dependency on Bcl-2 family members in multiple myeloma and predicts sensitivity to BH3 mimetics. *Leukemia*. 2016;30(3):761-764.
49. Moreau P, Chanan-Khan A, Roberts AW, et al. Promising efficacy and acceptable safety of venetoclax plus bortezomib and dexamethasone in relapsed/refractory MM. *Blood*. 2017;130(22):2392-2400.
50. Harrison S, Cavo M, De la Rubia J, et al. t(11;14) and high BCL2 expression are predictive biomarkers of response to venetoclax in combination with bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma: biomarker analyses from the phase 3 Bellini study [abstract]. *Blood*. 2019;134(suppl 1). Abstract 142.
51. Blombery P, Anderson MA, Gong JN, et al. Acquisition of the recurrent Gly101Val mutation in BCL2 confers resistance to venetoclax in patients with progressive chronic lymphocytic leukemia. *Cancer Discov*. 2019;9(3):342-353.
52. Tausch E, Close W, Dolnik A, et al. Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia. *Haematologica*. 2019;104(9):e434-e437.
53. Blombery P, Thompson ER, Nguyen T, et al. Multiple BCL2 mutations cooccurring with Gly101Val emerge in chronic lymphocytic leukemia progression on venetoclax. *Blood*. 2020;135(10):773-777.

54. Agarwal R, Chan YC, Tam CS, et al. Dynamic molecular monitoring reveals that SWI-SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. *Nat Med*. 2019;25(1):119-129.
55. Wei AH, Roberts AW. Polyclonal heterogeneity: the new norm for secondary clinical resistance to targeted monotherapy in relapsed leukemia? *Cancer Discov*. 2019;9(8):998-1000.
56. Anderson MA, Huang D, Roberts A. Targeting BCL2 for the treatment of lymphoid malignancies. *Semin Hematol*. 2014;51(3):219-227.
57. Touzeau C, Dousset C, Le Gouill S, et al. The Bcl-2 specific BH3 mimetic ABT-199: a promising targeted therapy for t(11;14) multiple myeloma. *Leukemia*. 2014;28(1):210-212.
58. Gong JN, Khong T, Segal D, et al. Hierarchy for targeting prosurvival BCL2 family proteins in multiple myeloma: pivotal role of MCL1. *Blood*. 2016;128(14):1834-1844.
59. Kater AP, Seymour JF, Hillmen P, et al. Fixed duration of venetoclax-rituximab in relapsed/refractory chronic lymphocytic leukemia eradicates minimal residual disease and prolongs survival: post-treatment follow-up of the MURANO phase III study. *J Clin Oncol*. 2019;37(4):269-277.
60. de Vos S, Swinnen LJ, Wang D, et al. Venetoclax, bendamustine, and rituximab in patients with relapsed or refractory NHL: a phase Ib dose-finding study. *Ann Oncol*. 2018;29(9):1932-1938.
61. Handunnetti SM, Anderson MA, Burbury K, et al. Three year update of the phase II ABT-199 (Venetoclax) and Ibrutinib in Mantle Cell Lymphoma (AIM) study [abstract]. *Blood*. 2019;134(suppl 1). Abstract 756.
62. Kumar S, Harrison S, Cavo M, et al. Updated results from Bellini, a phase III study of venetoclax or placebo in combination with bortezomib and dexamethasone in relapsed/refractory multiple myeloma [abstract]. *J Clin Oncol*. 2020;38(suppl 15). Abstract 8509.
63. Khaw SL, Suryani S, Evans K, et al. Venetoclax responses of pediatric ALL xenografts reveal sensitivity of MLL-rearranged leukemia. *Blood*. 2016;128(10):1382-1395.
64. Pan R, Ruvolo V, Mu H, et al. Synthetic lethality of combined Bcl-2 inhibition and p53 activation in AML: mechanisms and superior antileukemic efficacy. *Cancer Cell*. 2017;32(6):748-760.e6.
65. Pollyea DA, Stevens BM, Jones CL, et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat Med*. 2018;24(12):1859-1866.

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