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Review

BH3-Mimetic Drugs: Blazing the Trail for New Cancer Medicines

Delphine Merino,[1,2,3,4,9] Gemma L. Kelly,[1,4,9] Guillaume Lessene,[1,4,5,9] Andrew H. Wei,[6,9] Andrew W. Roberts,[1,4,7,8,9] and Andreas Strasser[1,4,9,*]
[1]The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia
[2]Olivia Newton-John Cancer Research Institute, Heidelberg, VIC 3084, Australia
[3]School of Cancer Medicine, La Trobe University, Melbourne, VIC 3086, Australia
[4]Department of Medical Biology, The University of Melbourne, Parkville, VIC 3010, Australia
[5]Department of Pharmacology and Therapeutics, The University of Melbourne, Parkville, VIC 3010, Australia
[6]Department of Haematology, Alfred Hospital and Monash University Melbourne, Melbourne, VIC 3004, Australia
[7]Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, VIC 3000, Australia
[8]Victorian Comprehensive Cancer Centre, Melbourne, VIC 3000, Australia
[9]These authors contributed equally
*Correspondence: strasser@wehi.edu.au
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Defects in apoptotic cell death can promote cancer and impair responses of malignant cells to anti-cancer therapy. Pro-survival BCL-2 proteins prevent apoptosis by keeping the cell death effectors, BAX and BAK, in check. The BH3-only proteins initiate apoptosis by neutralizing the pro-survival BCL-2 proteins. Structural analysis and medicinal chemistry led to the development of small-molecule drugs that mimic the function of the BH3-only proteins to kill cancer cells. The BCL-2 inhibitor venetoclax has been approved for treatment of refractory chronic lymphocytic leukemia and this drug and inhibitors of pro-survival MCL-1 and BCL-XL are being tested in diverse malignancies.

Introduction

The balance between survival versus death of cells is controlled by interactions among members of the three subgroups of the BCL-2 family of proteins (Adams and Cory, 2018). The pro-survival subgroup (BCL-2, BCL-XL, BCL-W, MCL-1, A1/BFL-1, and possibly BCL-B) promotes cell survival by inhibiting their pro-apoptotic relatives. The pro-apoptotic BAX/BAK-like proteins, including BOK, are the essential effectors of apoptosis, and the BH3-only proteins (BIM, PUMA, BID, NOXA, BID, BMF, BIK, and HRK) are the initiators of apoptosis. In healthy cells, the pro-survival BCL-2 proteins bind and inhibit BAX and BAK after they have been partially activated, impairing the ability of BAX/BAK to oligomerize and form pores to induce mitochondrial outer membrane permeabilization. The BH3-only proteins are induced transcriptionally or post-transcriptionally in response to diverse stresses and initiate apoptosis by either binding the pro-survival BCL-2 proteins, thereby unleashing BAX/BAK, or by directly activating these effectors of apoptosis (Czabotar et al., 2014; Green and Kalkavan, 2018). The various BCL-2 family proteins have differential specificity of binding to one another, resulting in a complex but ordered network of interactions governing cell fate (Czabotar et al., 2014).

The identification of the functions of the different BCL-2 family members, complemented by emerging insights into the structural interactions between pro-apoptotic and pro-survival family members, engendered the concept of killing cancer cells by targeting the pro-survival members with small molecules that mimic the function of the BH3-only proteins, now termed BH3-mimetics. Yet, 30 years on, and despite significant efforts, only six BH3-mimetic drugs have reached the clinic with only the BCL-2 inhibitor, venetoclax/ABT-199, currently approved (Figure 1A). This is testament to the multiple challenges encountered by medicinal chemists in this endeavor (Ashkenazi et al., 2017; Czabotar et al., 2014; Lessene et al., 2008), mostly linked to the nature of the targetable interfaces. Indeed, BH3-mimetics need to bind with very high affinities to the large and mostly hydrophobic grooves that underpin the protein-protein interactions between pro-survival BCL-2-like proteins and the BH3 domains of their pro-apoptotic relatives (Figure 1B). As a result, all advanced BH3-mimetics are characterized by relatively high molecular weight, lipophilicity, and chemical complexity (Ashkenazi et al., 2017).

AbbVie (previously Abbott Laboratories) in collaboration with Idun pioneered this field by developing the first validated BH3-mimetic, ABT-737 (Oltersdorf et al., 2005), that targets pro-survival BCL-2, BCL-XL, and BCL-W (Figure 1A). The closely related compound, ABT-263 (navitoclax), with the same binding profile but improved pharmacological properties, was the first BH3-mimetic to reach the clinic (Roberts et al., 2012; Wilson et al., 2010). Further structure-based drug discovery exploiting the subtle differences between the binding interfaces of BCL-2 versus BCL-XL led to the development of the BCL-2-selective inhibitor, ABT-199/venetoclax, by AbbVie, Genentech, and The Walter and Eliza Hall Institute (Souers et al., 2013). This drug demonstrated remarkable efficacy in the clinic, igniting enthusiasm for developing additional BH3-mimetic drugs.

The development of potent MCL-1-specific inhibitors has been much slower, partly because it is a more challenging target with fewer features on its surface and more rigid binding pockets. This made the identification of tractable lead compounds for drug discovery more difficult. However, in recent years, progress has accelerated, with the emergence of three distinct chemical series targeting MCL-1 now entering clinical trials. Servier first disclosed S63845 (Kotschy et al., 2016), and the

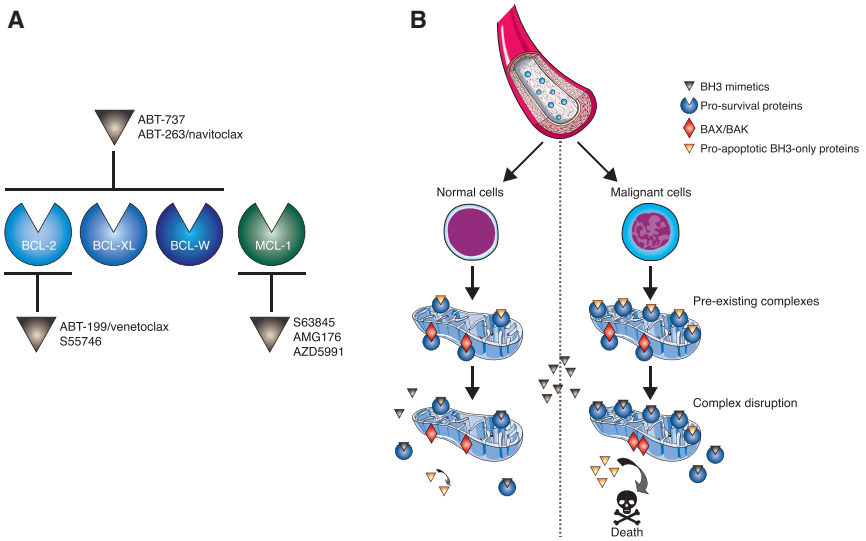

Figure 1. Mechanism of Action of BH3-mimetic Drugs

(A) Different BH3-mimetic drugs target different pro-survival BCL-2 family members.

(B) Priming of cancer cells: the increase in complexes between pro-survival BCL-2 family members and pro-apoptotic BH3-only proteins results in an increased sensitivity of cancer cells to BH3-mimetic drugs (and other anti-cancer agents). Unleashed or newly synthesized BH3-only proteins will be able to inhibit the pro-survival BCL-2 family members that are not targeted by the specific BH3 mimetic. This causes, indirectly or directly, activation of the effectors of apoptosis, BAX and BAK.

related compound, S64315/MIK665, is now being co-developed in the clinic with Novartis. Concomitantly, Amgen and AstraZeneca have disclosed their respective MCL-1 inhibitor programs and have initiated clinical trials with AMG 176 (Caenepeel et al., 2018; Nangia et al., 2018; Ramsey et al., 2018) and AZD5991 (Hird et al., 2017), respectively.

Highly potent and selective inhibitors of the pro-survival protein BCL-XL have been described (WEHI-539, A-1155463, and A-1331852) (Lessene et al., 2013; Leverson et al., 2015a; Tao et al., 2014). However, the clinical progression of these compounds has been challenging, largely due to the on-target impact on normal cells. This review will highlight the latest preclinical studies and ongoing clinical development of the BH3-mimetic drugs and address open questions in this rapidly evolving field.

Understanding the Functions of the Pro-survival BCL-2 Proteins in Normal Cells to Anticipate Side Effects of BH3-Mimetic Drugs

The role of the different BCL-2 pro-survival proteins in normal cells has been elucidated over many years using gene-targeted mice. As BH3-mimetics drugs are progressing into the clinic, the knowledge gained from these studies has attracted renewed interest to understand potential on-target toxicities and anticipate the spectrum of risks that could be encountered clinically.

The genetically engineered deletion of each pro-survival BCL-2 family member in mice has been associated with distinct impacts on physiology. Mice lacking BCL-2 succumb to polycystic kidney disease early in life because BCL-2 is critical for the survival of renal epithelial progenitor cells during embryogenesis. The BCL-2-deficient mice also have abnormally reduced numbers of mature, resting B and T lymphocytes, and gray prematurely because of the aberrant death of melanocytes (Bouillet et al., 2001; Nakayama et al., 1993, 1994; Veis et al., 1993; Yamamura et al., 1996). The loss of BCL-XL causes death of embryos around embryonic day 13.5 (E13.5) due to aberrant apoptosis of erythroid progenitors and certain neuronal populations (Motoyama et al., 1995). Moreover, B cell progenitors and immature $CD4^+CD8^+$ thymocytes also depend on BCL-XL for their survival (Ma et al., 1995; Motoyama et al., 1995). Loss of only one *Bcl2l1* allele (that encodes BCL-XL) impairs male fertility (Kasai et al., 2003) and reduces platelet numbers due to their shortened lifespan (Mason et al., 2007). The loss of BCL-W causes only minor defects in mice, restricted to male sterility (Print et al., 1998; Ross et al., 1998; Russell et al., 2001). Studies with gene-targeted mice have raised serious concerns about the on-target effects that MCL-1-specific BH3-mimetic drugs might have on normal cells. Mice lacking MCL-1 die before E3.5 prior to implantation (Rinkenberger et al., 2000). Cell-type-specific and/or temporally regulated deletion of MCL-1 in mice revealed that this pro-survival protein plays a critical role in diverse tissues, including hematopoietic stem cells (Opferman et al., 2005), thymic epithelial function (Jain et al., 2017), immature as well as mature B and T lymphoid cells (Opferman et al., 2003), activated T cells, activated germinal center B cells (Vikstrom et al., 2010), natural killer cells (Huntington et al., 2007; Sathe et al., 2014), plasma cells (Peperzak et al., 2013), certain myeloid cell subsets (Dzhagalov et al., 2007), cardiomyocytes (Thomas et al., 2013; Wang et al., 2013), neuronal cells (Arbor et al., 2008), alveolar cells in the lactating breast (Fu et al., 2015), and hepatocytes (Vick et al., 2009).

The Therapeutic Potential of BH3-Mimetic Drugs for Hematological and Solid Cancers—Lessons from Preclinical Models

Studies performed in mouse tumor models were fundamental in supporting the rationale to target BCL-2 for cancer therapy. Several cancers display abnormally high levels of BCL-2, including follicular lymphomas due to their t(14;18) chromosomal translocation (Fukuhara and Rowley, 1978; Tsujimoto et al., 1984), chronic lymphocytic leukemia (CLL) due to the loss of the microRNAs miR-15 and miR-16, which restrain BCL-2 expression (Cimmino et al., 2005) and a subset of small cell lung carcinomas (SCLCs) due to somatically acquired amplification of the *BCL2* gene locus on chromosome 18q21 (Ikegaki et al., 1994; Jiang et al., 1995; Stefanaki et al., 1998). As predicted, the BH3-mimetics, ABT-737 and navitoclax, which both inhibit BCL-2, as well as BCL-XL and BCL-W, were active against these cancer subtypes as single agents *in vitro* and in xenografts *in vivo* (Oltersdorf et al., 2005). Moreover, these compounds were effective against an even broader spectrum of

	Efficacy towards malignant cells	Toxicity towards normal cells (known or predicted)
BCL-2, BCL-XL, BCL-W inhibitor* ABT-263/navitoclax	CLL Lymphoma Solid tumours (including NSCLC, Ovarian, Colorectal, Breast cancers)	Platelets (Thrombocytopenia) Neutrophils (Neutropenia) Lymphocytes (Lymphopenia)
BCL-2 inhibitors* ABT-199/venetoclax S55746	CLL# Lymphoma Myeloma AML ER$^+$ Breast cancer^ ALL^	Neutrophils (Neutropenia) Lymphocytes (Lymphopenia)
MCL-1 inhibitors** S63845 AMG176 AZD5991	AML MM Lymphoma Melanoma TNBC/HER2$^+$ Breast cancer	Immune cells? Hepatocytes? Neuronal cells? Cardiomyocytes? Hematopoietic stem cells?

Figure 2. New BH3-Mimetic Drugs: Expected Efficacy and Predicted On-Target Side Effects

Prediction of efficacy in malignant cells and on-target side effects of BH3-mimetic drugs derived from experiments using mouse models as well as pre-clinical and clinical studies. *Efficacy demonstrated in clinical trials; for the majority of the diseases listed either mature phase 2 or 3 trial data, or data from multiple phase 1 trials have been reported; where efficacy results are preliminary (i.e., early results from phase 1 trials only, diseases are marked with an ^). **As predicted from experiments using conditional *Mcl1* knockout mouse models and pre-clinical studies. #FDA approved for patients with previously treated CLL.

tumors when combined with standard anti-cancer agents. In agreement with findings from gene-targeted mice (Ma et al., 1995; Motoyama et al., 1995), pre-clinical studies revealed that ABT-737 and navitoclax induced on-target death of platelets, immature B and T cells, and certain other BCL-XL-dependent cell types (Mason et al., 2007; Merino et al., 2012a). Despite promising findings in several blood cancers, particularly CLL, the progression of navitoclax was limited by its predicted on-target platelet toxicity (Mason et al., 2007; Roberts et al., 2012; Wilson et al., 2010). Phase 1 clinical trials in patients with lymphoid malignancies or SCLC did, however, indicate that thrombocytopenia might be attenuated by careful dosing (Gandhi et al., 2011; Roberts et al., 2012; Wilson et al., 2010). Of note, the platelet-suppressive activity of BCL-XL inhibitors could be leveraged to treat myeloproliferative disorders that are characterized by malignant thrombocytosis. In conclusion, there may be future potential for navitoclax or BCL-XL-selective BH3-mimetics in the clinic.

The development of the BCL-2-selective BH3-mimetic venetoclax (Souers et al., 2013) circumvented the thrombocytopenia associated with navitoclax (Figure 2). Venetoclax proved effective in killing diverse hematological cancer cells and certain solid cancer-derived cell lines, both *in vitro* and in xenografts *in vivo*, either alone or in combination with standard-of-care therapies (Khaw et al., 2014; Souers et al., 2013; Touzeau et al., 2014). With respect to toxicities predicted from knockout mice, venetoclax did not induce kidney disease, but it caused a reduction in mature B and T cells and early T cell progenitors in the thymus (Khaw et al., 2014), consistent with the high levels of BCL-2 expression in these lymphoid cells (Gratiot-Deans et al., 1993; Kelly and Strasser, 2011; Merino et al., 1994). Remarkably, venetoclax was efficacious and well-tolerated in patients with chemotherapy-refractory CLL, with only relatively minor side effects, such as neutropenia and an increased risk of respiratory tract infections after the initial challenge of tumor lysis had been overcome (Leverson et al., 2015a; Roberts et al., 2016) (Figure 2). Accordingly, the US Food and Drug Administration (FDA)-approved venetoclax for this indication on April 11th, 2016, followed by regulatory approval in other countries.

The evidence that many cancers are dependent on MCL-1 for their sustained growth is persuasive and has fueled efforts to develop MCL-1-specific BH3-mimetics. Analysis of large cancer genome datasets revealed that the genomic region containing *MCL1* is amplified in ~10% of cancers (Beroukhim et al., 2010). Of note, inducible gene deletion in pre-clinical murine models of cancer showed that MCL-1 is essential for the sustained expansion of acute myeloid leukemia (AML) (Glaser et al., 2012), c-MYC- or BCR-ABL-driven pre-B or B cell lymphomas (Kelly et al., 2014; Koss et al., 2013), T cell lymphomas driven by loss of p53 or other oncogenic lesions (Grabow et al., 2014; Spinner et al., 2016), and multiple myeloma (MM) (Gong et al., 2016; Morales et al., 2011). The development of S63845 provided the first opportunity to test an MCL-1-selective BH3-mimetic drug in pre-clinical cancer models (Kotschy et al., 2016). As a single agent, this MCL-1 inhibitor and the recently reported compounds AMG 176 and VU661013 were found to be effective at killing several leukemia-, lymphoma-, and MM-derived cell lines, including ones with genomic lesions that predict poor outcomes (Caenepeel et al., 2018; Kotschy et al., 2016; Ramsey et al., 2018). These data suggest that MCL-1-specific BH3-mimetic drugs could provide a treatment option for patients suffering from these cancer types. However, due to the severe impact of conditional loss of MCL-1 on many normal tissues, concerns remained about the safety of such therapies. Importantly, S63845 was tolerated in mice at doses that could achieve lymphoma regression (Kotschy et al., 2016). The normal hematopoietic system was largely unaffected, with a transient reduction in immature B cells in the bone marrow observed, and there were no detrimental effects on the liver, heart, lungs, or skeletal muscle. However, S63845, AMG 176, and possibly other MCL-1 inhibitors, have a weaker affinity for mouse, compared with human MCL-1 (Caenepeel et al., 2018; Kotschy et al., 2016). The recent development of two independent humanized MCL-1 mouse strains has permitted more accurate evaluations of the therapeutic potential of MCL-1 inhibitors and showed that, despite further pronounced effects on normal cells, a therapeutic window should be achievable (Brennan et al., 2018; Caenepeel et al., 2018).

Overall, BH3-mimetics have shown better tolerability in preclinical and clinical studies than would be predicted from studies of gene knockout mice. This discordance could be explained by the incomplete and only transient inhibition of pro-survival proteins achieved by BH3 mimetics, compared with gene deletion, which removes the protein function permanently. Furthermore, it has been reported that some pro-survival BCL-2 proteins may regulate non-apoptotic processes, such as mitochondrial respiration (Perciavalle et al., 2012), estrogen receptor (ER) calcium homeostasis (Chen et al., 2004; Ferdek et al., 2012; Vervliet et al., 2014), autophagy (Levine et al., 2008), cell cycling (Zinkel et al., 2006), and necroptosis (Hitomi et al., 2008). Such functions may not be impacted by BH3-mimetic drugs yet would be expected to be ablated in the gene knockout mice, potentially explaining the more severe phenotypes in the latter. It should, however, be noted that cell lines deficient for all pro-survival BCL-2 proteins could be generated as long as BAX and BAK were removed beforehand, and these cells were able to grow in culture comparably with their parental counterparts (O'Neill et al., 2016; Zhang et al., 2016). Moreover, the regulation of autophagy by pro-survival BCL-2 proteins was shown to be secondary to promoting cell survival through constraining BAX and BAK (Lindqvist et al., 2014). Thus, functions of pro-survival BCL-2 family members outside of the regulation of apoptosis may largely be indirect and therefore not of concern with respect to on-target effects of BH3-mimetic drugs to healthy tissues.

Impact of Combinations of BH3-Mimetics with Standard-of-Care Therapeutics

The potential to further improve patient outcomes by combining BH3-mimetic drugs with standard-of-care therapeutics is under intense investigation, with clinical trials of venetoclax alongside standard and novel anti-cancer agents in hematological malignancies leading the way. For solid organ-derived cancers, preclinical studies indicate that BH3-mimetic therapy will likely only be effective when combined with standard chemotherapeutics or inhibitors of oncogenic kinases (Cragg et al., 2009).

Navitoclax synergized with several chemotherapeutics in triple-negative breast cancer models (Oakes et al., 2012; Panayotopoulou et al., 2017) and non-small-cell lung carcinoma (NSCLC) (Kim et al., 2017) and this is likely due to the targeting of BCL-XL (Leverson et al., 2015b; Xiao et al., 2015). Interestingly, a recent report found that navitoclax and venetoclax synergize with chemotherapeutics that can reduce MCL-1 levels (Inoue-Yamauchi et al., 2017), however, the underlying molecular mechanisms explaining the efficacy of combinations may vary with different drugs. ABT-737 and navitoclax, both targeting BCL-2, BCL-XL, and BCL-W, were also shown to synergize with inhibitors of oncogenic kinases that cause upregulation of pro-apoptotic BH3-only proteins BIM, PUMA, and BMF that can neutralize the pro-survival BCL-2 family members that are not targeted by these BH3 mimetics (Cragg et al., 2007, 2008; Kuroda et al., 2006). The potential of the combination therapy of venetoclax and cyclophosphamide for pediatric and young adult patients with neuroblastoma or certain other malignancies will be tested in a clinical trial (NCT03236857).

In the case of MCL-1 inhibitors, single agent tolerability will need to be established before combination approaches can be considered. The observation that loss of only one allele of *Mcl1* significantly impaired hematopoietic recovery after exposure to high doses of 5-fluoro-uracil or γ-radiation (Delbridge et al., 2015) raised concerns that MCL-1 inhibitors could be hazardous alongside agents that induce DNA damage. However, it was recently shown that $Mcl1^{+/-}$ heterozygous mice, that have 30%–50% reduced MCL-1 protein levels in their cells (mimicking the impact of MCL-1 inhibitor treatment) were able to tolerate several cytotoxic drugs, including ones that cause DNA damage, at doses clinically relevant for leukemia and lymphoma patients (Brinkmann et al., 2017). This suggests that, with careful clinical management, combinations of an MCL-1 inhibitor with standard chemotherapy could be a viable therapeutic avenue.

Combining BH3-mimetics with targeted therapies, such as inhibitors of oncogenic kinases may also be an attractive strategy for cancer therapy. Kinase inhibition increases expression of pro-apoptotic BH3-only proteins, such as BIM and PUMA, thereby inhibiting pro-survival BCL-2 proteins that are not targeted by the BH3-mimetics (Cragg et al., 2007, 2008; Rohrbeck et al., 2016). Accordingly, combinations of MCL-1 inhibitors, such as S63845 and AMG 176 (and the related compound AM 8621), with inhibitors of EGFR, MEK, or B-RAF were shown to efficiently diminish the *in vitro* and even the *in vivo* growth of cell lines derived from NSCLC (Kotschy et al., 2016; Leverson et al., 2015b; Nangia et al., 2018; Song et al., 2005; Zhang et al., 2011), lung squamous cell carcinoma (Weeden et al., 2018), hepatocarcinoma, or glioblastoma (Karpel-Massler et al., 2017). Of note, ~85% of ER-positive breast cancers express high levels of BCL-2 (Dawson et al., 2010) and, accordingly, venetoclax and navitoclax synergized with tamoxifen in inhibiting the growth of patient-derived xenografts in mice (Vaillant et al., 2013). MCL-1 inhibitors, such as S63845, were also shown to efficiently inhibit the growth of triple-negative as well as HER-2-positive breast cancers when combined with chemotherapy or HER2-targeted therapies (Kotschy et al., 2016; Leverson et al., 2015b; Merino et al., 2017; Xiao et al., 2015; Young et al., 2016). Neuroblastoma growth was attenuated by inhibition of either BCL-2 or MCL-1 (Bate-Eya et al., 2016; Tanos et al., 2016), and the combination of venetoclax with the aurora kinase A inhibitor MLN8237 achieved complete remission in a patient-derived xenograft of *MYCN*-amplified neuroblastomas (Ham et al., 2016). Inhibitors of the mammalian target of rapamycin/phosphatidylinositol 3-kinase pathway have also been shown to enhance the impact of navitoclax or venetoclax in diverse cancer-derived cell lines (Bean et al., 2013; Faber et al., 2014; Muranen et al., 2012; Potter et al., 2016; Vaillant et al., 2013). Collectively, these reports provide the foundation for clinical trials combining venetoclax and, possibly, soon also MCL-1 inhibitors, with standard-of-care chemotherapy or inhibitors of oncogenic kinases.

Killing Cancer Cells with BH3-Mimetic Drugs—Notions of Priming and Dependence on Select Pro-survival BCL-2 Family Members

The potential for BH3-mimetics to selectively kill cancer cells over normal cells has been explained using the concept of "priming" (Certo et al., 2006; Montero and Letai, 2018; Potter et al., 2016). Cancer cells usually develop a dependency on specific pro-survival BCL-2 proteins due to multiple factors, such as their tissue of origin, impact of the oncogenic lesions that drove

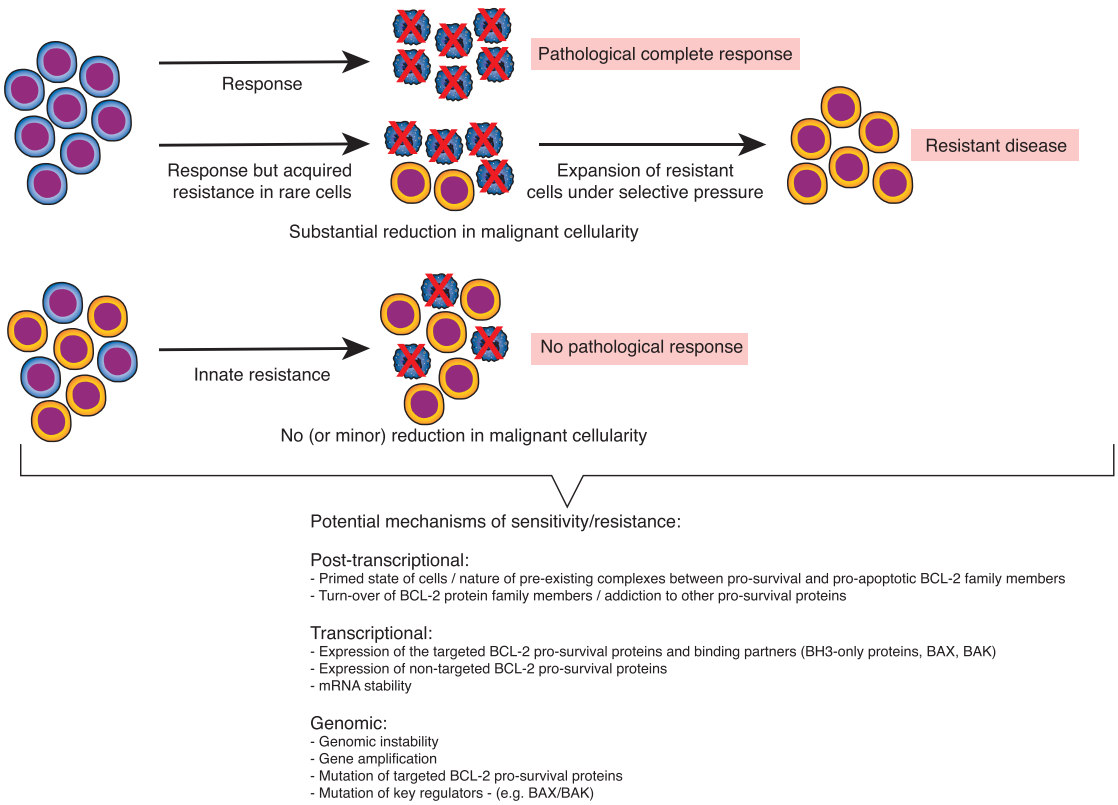

Figure 3. Factors Impacting Sensitivity versus Resistance of Tumors to BH3-Mimetic Drugs
At the molecular level, several factors impact the response versus resistance (innate or acquired) of tumor cells to BH3-mimetic drugs, including the cell of origin of the tumor, the oncogenes driving malignant transformation, the selective pressure exerted by therapy or the composition of the tumor microenvironment. Understanding these factors will inform the rational design of combination therapies that should improve responses in patients with malignant disease.

tumorigenesis, and/or factors produced by the tumor stroma. High levels of pro-survival BCL-2 family members are often accompanied by an accumulation of BH3-only proteins that are short-lived in their unbound state but are stabilized when bound to the pro-survival proteins (Jorgensen et al., 2007; Merino et al., 2012b). In addition, the levels of the BH3-only proteins can be increased by oncogenic transcription factors, such as E2F1 (Hershko and Ginsberg, 2004) or tumor suppressors, such as p53 (Nakano and Vousden, 2001; Oda et al., 2000; Yu et al., 2001). This increases the number of complexes between pro-survival and pro-apoptotic proteins in cancer cells. Such primed cells are therefore more sensitive to BH3-mimetics (and other anti-cancer agents) compared with their normal counterparts (Figure 1B). Of note, the ratios between the pro-survival BCL-2 family members and the pro-apoptotic BH3-only proteins were found to correlate with sensitivity to BH3-mimetic drugs (Punnoose et al., 2016; Roberts et al., 2012; Touzeau et al., 2014). Since at least some BCL-2 family members can be regulated post-translationally, the challenge will be to develop assays able to measure not only the amounts but also the molecular states/activities of the different BCL-2 family members in cells to predict sensitivity to BH3-mimetic drugs. The so-called "dynamic BH3 profiling" is one of the strategies proposed to identify dependencies on anti-apoptotic BCL-2 family members in tumor cells (Montero and Letai, 2018). This can also be achieved by using an inducible CRISPR/Cas9 platform (Aubrey et al., 2015) or simply by culturing malignant cells from the patient with different BH3-mimetic drugs (Kotschy et al., 2016).

While certain cancers, mostly leukemias and lymphomas, appear addicted to a single pro-survival protein, the survival of others, in particular solid tumors, is often safeguarded by multiple pro-survival BCL-2 family members (Caenepeel et al., 2018; Kotschy et al., 2016; Ramsey et al., 2018). Although not universal, the differential addiction of certain cancer cells to anti-apoptotic BCL-2, BCL-XL, or MCL-1 has been reported to correlate with the relative expression levels of the respective proteins (Inoue-Yamauchi et al., 2017). Of note, this phenomenon plays a pivotal role in dictating sensitivity versus primary resistance to BH3-mimetic drugs (Figure 3), and therefore assays that could accurately identify the pro-survival protein(s) essential for sustained tumor expansion would be beneficial.

From Clinical Trials to Standard-of-Care

The initial indication for venetoclax was treatment of relapsed CLL with 17p deletion (FDA), with somewhat broader indications in other jurisdictions. Table 1 summarizes key outcomes of major trials of venetoclax reported to date.

Recurring themes are evident. Firstly, venetoclax can induce remarkable responses in patients whose cancer has failed standard chemo-immunotherapy, such as in CLL and mantle cell lymphoma, where complete response (CR) rates of ~20% are observed with venetoclax as monotherapy. Secondly, venetoclax

Table 1. Summary of Mature Clinical Trial Data for Venetoclax in Hematological Malignancies

	Monotherapy				Combination				
	Phase	Response Rate		Median PFS		Phase	Response Rate		Median PFS[b]
Disease	(Reference)	Overall (%)	CR (%)	(Months)	Partner Drug(s)	(Reference)	Overall (%)	CR (%)	(Months)
CLL (relapsed)	1 (Roberts et al., 2016)	79	20	25	+ rituximab	1b (Seymour et al., 2017)	86	51	80% at 2 years
	2 (Stilgenbauer et al., 2016, 2018)	79	9/16[a]	27	+ rituximab	3 (Seymour et al., 2018)	92	8/27[a]	85% at 2 years
Lymphoma (relapsed)									
Follicular	1 (Davids et al., 2017)	38	14	11	+ bendamustine/ rituximab	1b (de Vos et al., 2018)	75	38	NR at 24 months
Mantle cell	1 (Davids et al., 2017)	75	21	14	+ ibrutinib	2 (Tam et al., 2018)	75	71	57% at 18 months
Diffuse large B cell	1 (Davids et al., 2017)	18	12	1					
Myeloma (relapsed)									
Total	1 (Kumar et al., 2017)	21	7	TTP 3	+ bortezomib/ dexamethasone	1b (Moreau et al., 2017)	67	20	9.5
t(11; 14)		40	14	TTP 7			78	NA	
Acute Myeloid Leukemia									
Relapsed/ refractory	1b (Konopleva et al., 2016)	38	19	2 (DOR)					
First-line elderly					+ azacitidine or decitabine	1b/2 (DiNardo et al., 2018a)	62	60	11 (DoR)
					+ low-dose ara-C	1b/2 (Wei et al., 2017a)	64	62	13.2

PFS, progression-free survival; TTP, time to progression; DOR, duration of response; NA, not reported; NR, not reached.
[a]As assessed by investigators as best response during trial. The first number is the percentage CR as assessed by an independent review committee.
[b]Where median PFS not reached, the estimate at a specific time point is provided where stated.

kills cancer cells independently of whether the p53 tumor-suppressor pathway is intact or not (Anderson et al., 2016; Tam et al., 2018). CR rates were the same in CLL patients with or without del(17p) (loss of *TP53*), explaining the remarkable single-agent efficacy in this traditionally poor-prognosis patient subgroup. Thirdly, there is marked variability in the durability of benefit when BH3-mimetic monotherapy is used in a given disease. While some patients with CLL have remained in remission for >4 years, others progress within 6–12 months. Genomic instability manifesting as complex karyotype and resistance to prior fludarabine therapy are the most powerful predictors of failure of venetoclax monotherapy in CLL (Anderson et al., 2016). Fourthly, in diseases where monotherapy is effective, regardless of whether the response rates (RRs) are modest or high, combination therapy clearly increases the likelihood of achieving a response, especially a CR, and prolongs the durability of benefit for patients. This principle is well exemplified in CLL, where combining venetoclax with six doses of the anti-CD20 monoclonal antibody, rituximab, increases the CR rate from 20% to 51%, and achieves a clearance of so-called minimal residual disease (MRD) in 57% of patients. Consequently, patients who achieve a so-called deep response (MRD-negative CR) appear able to cease all therapy and still sustain durable remission. This strategy is now being extended to mantle cell lymphoma, where the combination of venetoclax with the BTK inhibitor, ibrutinib, is highly effective *in vitro* (Zhao et al., 2015) and in patients, achieving MRD-negative CRs (Tam et al., 2018). Fifthly, predictive biomarkers are needed to prospectively identify which patients with BCL-2-expressing cancers (CLL, B cell lymphomas, myeloma, and AML) will respond to venetoclax. High level BCL-2 expression itself is required for a response, but this is not per se predictive (Davids et al., 2017; Kumar et al., 2017; Moreau et al., 2017). Further work is needed to identify markers, and it appears likely these may vary across diseases. Most progress has been made in myeloma, where patients with t(11;14) myeloma are most responsive to venetoclax monotherapy, and often experience durable responses, and this seems associated with a signature of high BCL-2 protein and low *BCLXL* mRNA expression (Kumar et al., 2017). Using flow cytometry and/or Cytof to measure the levels and molecular states of several proteins, including pro-survival BCL-2 family members and pro-apoptotic BH3-only proteins, as well as upstream signal transducers in several pathways (e.g., activated AKT, nuclear factor κB), may lead to future progress in this area. Such identification of biomarkers that predict vulnerabilities in cancer cells will be critical for the optimal stratification of patient populations for treatment with existing, and yet to be developed, BH3-mimetic drugs and to define the drugs to best partner them with.

Clinical progress in AML has been an elusive goal, particularly among elderly patients not able to tolerate intensive chemotherapy. Overexpression of BCL-2 in $CD34^+$ AML progenitors was noted (Delia et al., 1992), and cases with a low BAX to BCL-2 ratio were found to be associated with a lower remission rate and reduced survival after chemotherapy (Del Poeta et al., 2003). The risk of drug-induced thrombocytopenia diverted initial

clinical development of navitoclax away from patients with AML. However, pre-clinical studies demonstrating sensitivity of leukemic progenitors to BH3-mimetics targeting BCL-2 paved the way for a single-arm phase 2 clinical trial with venetoclax in 2013 among 32 patients with relapsed and refractory AML (Vo et al., 2012). Although the side effect profile was satisfactory, the overall RR in advanced AML was only 19% (Konopleva et al., 2016). Two back-to-back papers in 2006 reported enhanced anti-leukemic activity *in vitro* when the proof-of-concept BH3-mimetic ABT-737 was combined with approaches that reduce the levels of MCL-1 (Konopleva et al., 2006; van Delft et al., 2006). This suggested that the survival of AML cells was likely safeguarded by two or more pro-survival BCL-2 proteins. Pre-clinical studies confirmed the importance of MCL-1 as a key survival factor in AML (Glaser et al., 2012), supporting attempts to enhance venetoclax activity by adding chemotherapeutics that can indirectly neutralize MCL-1 (Bogenberger et al., 2014; Niu et al., 2016; Teh et al., 2018).

Two parallel phase 1b/2 studies were developed to explore the potential of venetoclax in combination with either hypomethylating agents (HMA) or low-dose cytarabine (LDC) in elderly patients with AML unfit for intensive chemotherapy. The results were promising, catapulting venetoclax into prominence as a therapeutic advance in elderly AML (Table 1). The studies demonstrated a highly encouraging RR of 62%–68% (DiNardo et al., 2018b; Wei and Tiong, 2017) compared with 11%–28% in historical studies using either HMA or LDC alone (Dombret et al., 2015; Kantarjian et al., 2012). The median clinical response and survival durations exceeded 12 months. With protocol-mandated prophylactic measures implemented, the risk of clinical tumor lysis syndrome was very low. Based on these impressive results, venetoclax was awarded "breakthrough designation" by the US FDA in combination with HMA (January 2017) and LDC (July 2017). Preliminary molecular correlations from these clinical studies suggested that patients with *NPM1* mutations were highly responsive to venetoclax-containing regimens, whereas inferior outcomes were noted among patients with adverse cytogenetic features (Wei et al., 2017b). Randomized studies for venetoclax in combination with HMA or LDC completed accrual in 2018. Positive outcomes from these studies will likely lead to a practice change and establishment of venetoclax-containing regimens as a new standard-of-care for elderly patients with AML.

Durable remission is thought to necessitate elimination of residual leukemic stem cell (LSC) populations. Although it has been proposed that inhibitors of BCL-2 have potential to eliminate quiescent LSCs (Lagadinou et al., 2013), these cells may also express other pro-survival proteins, such as MCL-1 and BCL-XL, raising the potential for primary or emergent drug resistance (Konopleva and Zhao, 2002; Yoshimoto et al., 2009). Clinical studies showed that the combination of venetoclax with HMA or LDC has reduced efficacy in patients with chemotherapy-relapsed or refractory AML (Aldoss et al., 2018; DiNardo et al., 2018b). An intriguing approach to enhance the activity of venetoclax in such resistant AML cases may involve directly targeting both BCL-2 and MCL-1, a strategy shown to be effective in pre-clinical AML model systems *in vivo* (Caenepeel et al., 2018; Moujalled et al., 2018; Ramsey et al., 2018; Teh et al., 2018). The emergence of potent MCL-1 inhibitors has made this feasible, and phase 1 clinical studies verifying the safety of S63845 and AMG 176 in humans are currently underway. Successful delivery of dual BH3-mimetic regimens to patients with AML may represent a step toward a chemotherapy-free future for patients with this aggressive cancer.

Encouraging results in CLL, mantle cell lymphoma, myeloma, and AML are paving the way to test BCL-2 inhibitors in additional hematological cancers, such as T cell prolymphocytic leukemia (Boidol et al., 2017), acute lymphoblastic leukemia (Khaw et al., 2016), and blastic plasmacytoid dendritic cell neoplasm (Montero et al., 2016). Several clinical trials are also currently ongoing in solid tumors, in particular breast and lung cancers (for venetoclax, NCT02391480 and ISRCTN98335443). While none of the BH3-mimetic drugs have shown efficacy in pre-clinical studies as single agents for various subtypes of breast cancer, synergy was observed with standard-of-care (Merino et al., 2016; Oakes et al., 2012; Vaillant et al., 2013). This led to an mBEP study investigating the effect of venetoclax plus tamoxifen combination treatment in patients with ER-positive breast cancer (ISRCTN98335443), with early encouraging effects reported (Lindeman et al., 2017). In lung cancer, including SCLC, venetoclax or navitoclax were found to be inefficient as single agents and therefore combinational therapies are currently being tested (NCT00445198). Results from phase I and II clinical trials indicate that navitoclax had limited efficacy against advanced and recurrent SCLC (Gandhi et al., 2011; Rudin et al., 2012), but epidermal growth factor receptor or BET inhibition may enhance the efficiency of navitoclax in clinical settings (Niederst et al., 2015) (NCT02520778 and NCT02391480).

Conclusions and Perspectives

While BH3-mimetics are showing real promise in the clinic for a range of tumor types, challenges still remain. Currently, clinically important susceptibility to BH3-mimetics as single agents is limited to a relatively small number of disease subtypes. Therefore, most development strategies are focused on combination therapy approaches, which increase the potential for toxic complications as well as efficacy. Furthermore, the mechanisms of primary and acquired resistance that apply clinically for each of the different BH3-mimetics appear complex and remain to be unraveled (Figure 3). Studies with leukemia-derived cell lines have identified several potential mechanisms of resistance to BH3-mimetic drugs. Mutations that reduce the binding of venetoclax to BCL-2 have been reported in resistant cell lines (Fresquet et al., 2014; Tahir et al., 2017), but have so far not been reported in patient samples. Other mechanisms of resistance to venetoclax include reduced expression of the BH3-only protein BIM or the apoptosis effector BAX (Fresquet et al., 2014; Huang et al., 2017). Moreover, it was reported that navitoclax (inhibits BCL-XL, BCL-2, and BCL-W) fails to kill at least some BCL-XL-addicted cancer cells that express only low levels of BH3-only proteins due to the inability of navitoclax to disrupt BCL-XL/BAK complexes (Inoue-Yamauchi et al., 2017; Merino et al., 2012a; Rooswinkel et al., 2012). The combined loss of BIM, PUMA, and BID, the most potent BH3-only proteins, renders cancer cells profoundly resistant to diverse conventional anticancer agents (Chen et al., 2015; Erlacher et al., 2006; Happo et al., 2010; Valente et al., 2016), and therefore probably also to BH3-mimetic drugs. There is also evidence that acquired

resistance to BH3-mimetic drugs may be conferred by upregulation of non-targeted BCL-2 pro-survival proteins (Caenepeel et al., 2018; Kotschy et al., 2016; Leverson et al., 2015b; Merino et al., 2017; Ramsey et al., 2018; van Delft et al., 2006; Vogler et al., 2009; Yecies et al., 2010) (Figure 3). For example, BCL-2-dependent cell lines selected for the ability to grow in the presence of venetoclax were found to express increased levels of BCL-XL or MCL-1. These malignant cells could be killed by a combination of venetoclax plus BH3-mimetics targeting either BCL-XL or MCL-1, showing that the ability of targeting BCL-2 had remained intact (Tahir et al., 2017). Combining two or even more BH3-mimetic drugs to inhibit several pro-survival BCL-2 proteins, either simultaneously or sequentially, may be an attractive approach to cancer therapy (Caenepeel et al., 2018; Khaw et al., 2016; Moujalled et al., 2018; Ramsey et al., 2018; Teh et al., 2018). However, it will be important to ensure that such strategies will not cause unacceptable on-target side effects, since combination targeting of MCL-1 and BCL-XL was reported to be lethal in mouse models, due to acute liver toxicity (Weeden et al., 2018). However, careful dosing and timing of drug administration may still make it possible to establish a therapeutic window. An intriguing concept to deliver single or multiple BH3-mimetic payloads preferentially (and thus more safely) to cancer cells is to conjugate them to target-directing antibodies (e.g., patent no. US2016/0158377, "BCL-XL Inhibitory Compounds and Antibody Drug Conjugates Including the Same," priority date December 12th, 2014, assigned to AbbVie). With the increasing number of clinical trials involving BH3-mimetics, alone or in combination with other chemotherapeutics, it will soon emerge which mechanisms will drive treatment failure in patients. The incorporation of correlative biological analyses linked to detailed genomic profiling of patient samples are therefore becoming increasingly valuable for the identification of biomarkers and of emerging mechanisms of drug resistance.

It has taken decades of research to develop BH3-mimetic drugs and evaluate how they activate the intrinsic apoptotic program in cancer cells without causing unacceptable damage to healthy tissues. While some tumors, such as CLL, appear to rely predominantly on a single pro-survival BCL-2 family member, underpinning the success of the BCL-2 inhibitor venetoclax as a single agent, other malignancies appear reliant on several BCL-2-like proteins for survival. The priority therefore must be on the rational design of drug combinations and discovery of predictive biomarkers to identify patients most likely to benefit from therapies containing BH3-mimetic drugs, taking into consideration tumor type, oncogenic drivers, and tumor heterogeneity.

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DECLARATION OF INTERESTS

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