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Toward Targeting Antiapoptotic MCL-1 for Cancer Therapy

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Abstract

Apoptosis is critical for embryonic development, tissue homeostasis, and the removal of infected or otherwise dangerous cells. It is controlled by three subgroups of the BCL-2 protein family—the BH3-only proteins that initiate cell death; the effectors of cell killing, BAX and BAK; and the antiapoptotic guardians, including MCL-1 and BCL-2. Defects in apoptosis can promote tumorigenesis and render malignant cells refractory to anticancer therapeutics. Activation of cell death by inhibiting antiapoptotic BCL-2 family members has emerged as an attractive strategy for cancer therapy, with the BCL-2 inhibitor venetoclax leading the way. Large-scale cancer genome analyses have revealed frequent amplification of the locus encoding antiapoptotic *MCL-1* in human cancers, and functional studies have shown that MCL-1 is essential for the sustained survival and expansion of many types of tumor cells. Structural analysis and medicinal chemistry have led to the development of three distinct small-molecule inhibitors of MCL-1 that are currently undergoing clinical testing.

INTRODUCTION

Apoptosis is a genetically programmed form of cell death that removes superfluous, damaged, or dangerous cells, such as those infected with pathogens or undergoing neoplastic transformation (Green 2019, Strasser et al. 2000). Accordingly, defects in apoptosis can promote tumorigenesis, particularly if they occur in conjunction with oncogenic lesions that drive aberrant cell division (Strasser et al. 1990, Vaux et al. 1988). Many cancers overexpress inhibitors of cell death or lack initiators of apoptosis, and these aberrations diminish the sensitivity of malignant cells to commonly used multitargeted anticancer agents, including targeted inhibitors of oncogenic kinases (Cragg et al. 2009, Strasser et al. 1991). Therefore, there has been great interest in developing drugs that can directly activate the apoptosis machinery for use in cancer therapy (Letai 2008, Merino et al. 2018). These so-called BH3-mimetics, small-molecule drugs that bind and inhibit select antiapoptotic BCL-2 family members, are emerging as promising new therapeutics for hematological and possibly also solid cancers, with the BCL-2-specific inhibitor venetoclax leading the way. Targeting the related antiapoptotic protein MCL-1 is of particular interest in the field because a substantial fraction of human cancers carry somatically acquired copy number amplifications of the genomic region containing its gene. Furthermore, functional investigations using inducible loss of *Mcl-1* have revealed that the protein is critical for both the development and sustained survival and growth of diverse tumor cells. However, given that the (inducible) loss of MCL-1 also kills several vital normal cell types, such as cardiomyocytes (Thomas et al. 2013, Wang et al. 2013) and neurons (Arbour et al. 2008), there is concern that targeting MCL-1 for cancer therapy may not be safe. In this review, we describe the known functions of MCL-1 in nontransformed and malignant cells, and we discuss the development and ongoing preclinical testing of the first small-molecule inhibitors of MCL-1 (Caenepeel et al. 2018, Kotschy et al. 2016, Ramsey et al. 2018, Tron et al. 2018), in anticipation of the first disclosure of data from the clinical trials of these drugs.

THE APOPTOSIS MACHINERY IS CONTROLLED BY THE BCL-2 PROTEIN FAMILY

Apoptotic cell death is regulated by three subsets of proteins of the BCL-2 family (**Figure 1**) (Czabotar et al. 2014, Green 2019, Singh et al. 2019). The antiapoptotic proteins BCL-2, BCL-XL, MCL-1, BCL-W, and A1/BFL1 safeguard cell survival. The proapoptotic effectors BAX, BAK, and possibly BOK are essential for the execution of apoptosis by creating pores in the outer mitochondrial membrane, allowing apoptogenic factors such as cytochrome c and SMAC/DIABLO to be released into the cytosol, which then drive the demolition of the doomed cells (Green 2019). The proapoptotic BH3-only proteins—BIM, PUMA, BID, NOXA, BMF, BAD, BIK, and HRK—initiate apoptosis signaling. In surviving cells, the apoptosis effectors BAX and BAK are restrained by the antiapoptotic BCL-2 family members. Cell stress, such as that elicited by deprivation of nutrients or growth factors, activation of oncogenes, or treatment with chemotherapeutic drugs, causes transcriptional or posttranscriptional upregulation of BH3-only proteins, such as BIM or PUMA. These initiators of apoptosis bind with high affinity to the antiapoptotic BCL-2 family members, such as BCL-2 or MCL-1, and this liberates BAX and BAK to unleash the effector phase of apoptosis (**Figure 1**). Certain BH3-only proteins, such as BIM, BID, and PUMA, have been reported to also activate BAX and BAK by interacting with them directly (**Figure 1**). The interactions between members of the three subgroups of the BCL-2 protein family and hence the regulation of apoptosis are complex; for example, some BH3-only proteins (BIM, BID, and PUMA) can bind with high affinity to all antiapoptotic BCL-2 family members and are thus

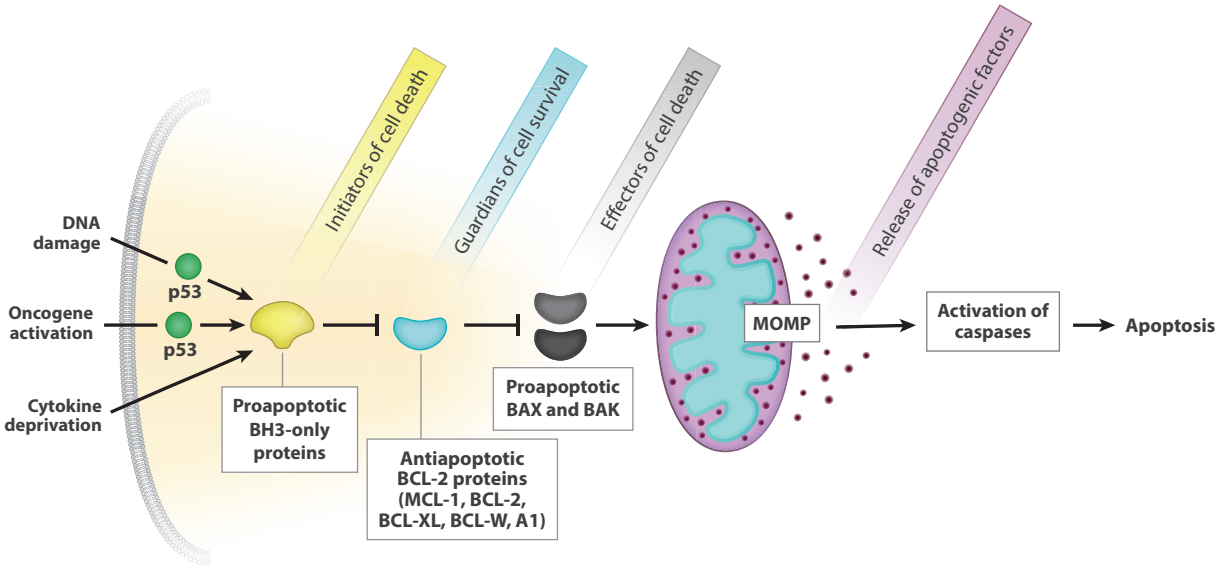

Figure 1

The intrinsic apoptotic pathway is controlled by three groups of proteins, all belonging to the BCL-2 family of proteins. The proapoptotic BH3-only proteins are the initiators of cell death since they become upregulated in response to cytotoxic stimuli, including DNA damage, oncogene activation, and cytokine deprivation. They can bind and inhibit the antiapoptotic BCL-2 proteins, which are the guardians of cell survival. This leads to release and activation of the effectors of cell death, BAX and BAK, which can oligomerize and lead to mitochondrial outer membrane permeabilization (MOMP). Additionally, select BH3-only proteins have been reported to also directly activate BAX and BAK. Following MOMP, the release of apoptogenic factors activates caspases that mediate the demolition of the cell. Activation of BAX or BAK leading to MOMP is considered the point of no return in apoptosis signaling.

highly potent initiators of apoptosis, whereas others can only bind a subset of the antiapoptotic proteins (e.g., NOXA binds to MCL-1, whereas BAD binds to BCL-2, BCL-XL, and BCL-W) (**Figure 2*a***) (Chen et al. 2005, Kuwana et al. 2005).

Different cellular stresses lead to upregulation of certain BH3-only proteins that then initiate apoptosis. For example, studies using knockout mice revealed that Puma and to a lesser extent Noxa, both direct transcriptional targets of the tumor suppressor p53, are critical for the killing of cells by DNA damage–inducing drugs (**Figure 1**) (Jeffers et al. 2003, Villunger et al. 2003). Conversely, Bim is needed for cell killing induced by nutrient/growth factor deprivation, glucocorticoids, ER stress, or inhibitors of oncogenic kinases (Bouillet et al. 1999, Kuroda et al. 2006, Puthalakath et al. 2007). The apoptosis effectors BAX and BAK have largely overlapping functions; accordingly, mice lacking either *Bax* or *Bak* have only minor defects, whereas *Bax/Bak* double-knockout and *Bax/Bak/Bok* triple-knockout mice have significant developmental abnormalities and their cells are highly resistant to all apoptotic stimuli tested (Ke et al. 2018, Lindsten et al. 2000). Thus, activation of BAX or BAK (or maybe BOK) constitutes the point of no return in apoptotic cell death signaling.

CONTROL OF MCL-1 EXPRESSION

The expression of MCL-1 and the other antiapoptotic BCL-2 family proteins is controlled at several levels, permitting tight control over cell fate. The expression of the genes that encode the antiapoptotic BCL-2 family proteins can be transcriptionally upregulated in response to cellular

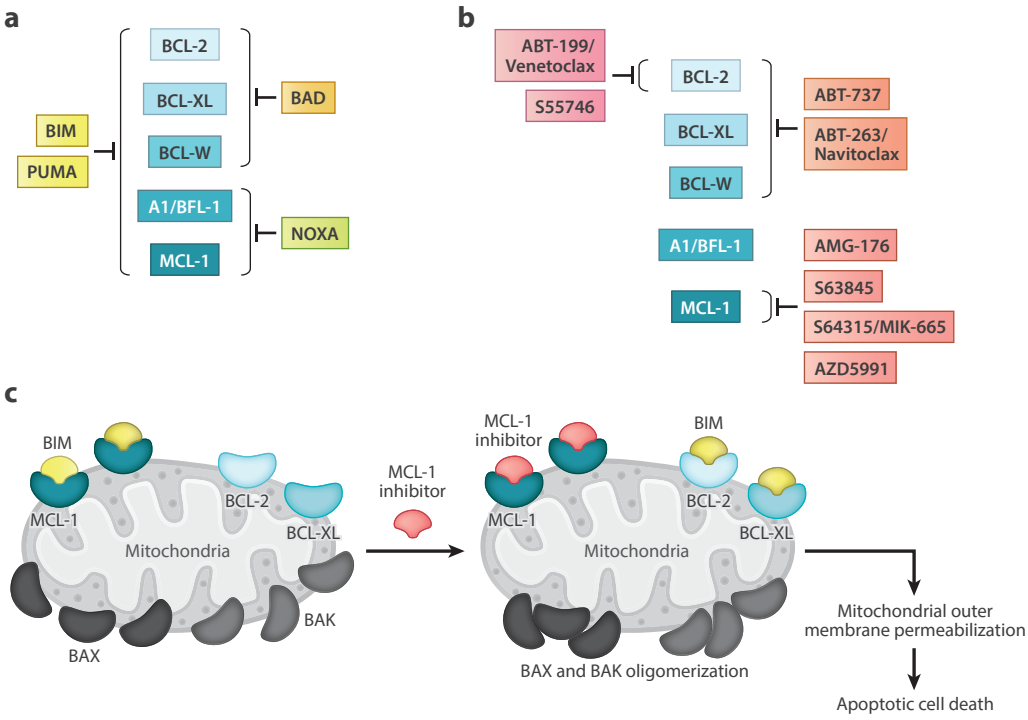

Figure 2

(*a*) Specificities of different BH3-only proteins for the different antiapoptotic BCL-2 family proteins. (*b*) Specificities of different BH3-mimetic drugs for the different antiapoptotic BCL-2 family proteins. (*c*) Mode of action of an MCL-1-specific BH3-mimetic drug to kill cells. The MCL-1 inhibitor can bind to MCL-1, preventing it from binding to BAX and BAK and causing displacement of BH3-only proteins such as BIM from MCL-1, allowing BIM to bind to and inhibit other antiapoptotic BCL-2 proteins present in the cell (that are not targeted by the MCL-1 inhibitor), such as BCL-2 and BCL-XL. Overall, these events unleash BAX and BAK from the restraint of MCL-1 and the other antiapoptotic BCL-2 family members present in the cell, allowing them to be activated and oligomerize, forming holes in the outer mitochondrial membrane and initiating the caspase cascade that demolishes the cell.

Erratum >

stimulation with a broad range of growth factors (e.g., IL-3, IL-5, VEGF, SCF) and downregulated following growth factor withdrawal in a cell-type-dependent manner (Chao et al. 1998, Huang et al. 2000, Le Gouill et al. 2004, Wang et al. 2003). Several cellular transcriptional pathways are capable of regulating *MCL-1* expression, including the JAK/STAT, PI3K/AKT, MEK/ERK, and p38/MAPK pathways (Akgul et al. 2000, Wang et al. 2003). Select microRNAs (miRs) are reported to posttranscriptionally regulate expression of antiapoptotic BCL-2 family members (reviewed by Villanova et al. 2018); for example, *MCL-1* transcripts are targeted by miR-29b and miR-125b (Gong et al. 2013, Mott et al. 2007). An additional level of control over the expression and function of the antiapoptotic BCL-2 family proteins is exerted at the posttranslational level. The stability of the MCL-1 protein can be controlled by polyubiquitin ligases, including MULE (MCL-1 ubiquitin ligase E3), SCF^{Fbw7}, and $SCF^{B\text{-}TrCP}$, that target it for proteasomal degradation (Ding et al. 2007, Inuzuka et al. 2011, Warr et al. 2005, Zhong et al. 2005), and this is counteracted by the deubiquitinases USP9X and USP13 that can stabilize MCL-1 protein expression (Schwickart et al. 2010, Zhang et al. 2018). Phosphorylation of MCL-1 at residues within the PEST domain by protein kinases, such as GSK-3, can also affect the stability of MCL-1 (reviewed by Thomas et al. 2010).

DIFFERENT ANTIAPOPTOTIC BCL-2 FAMILY MEMBERS ARE CRITICAL TO SAFEGUARD DISTINCT CELL TYPES

Studies using whole-body and tissue-restricted knockout of genes in mice have identified overlapping roles of the different antiapoptotic Bcl-2 family members in the survival of certain cell types. Bcl-2 is critical for the survival of progenitor cells in the embryonic kidney, melanocyte progenitors, and mature B and T lymphocytes (Bouillet et al. 2001, Veis et al. 1993). Bcl-xL is required for the survival of erythroid progenitors (both in embryos and adults), certain neuronal populations, and platelets and lymphoid cells at select stages of differentiation and activation (Mason et al. 2007, Motoyama et al. 1995, Wagner et al. 2000). Of all the antiapoptotic Bcl-2 family members, the loss of Mcl-1 has the most dramatic impact. Constitutive loss of *Mcl-1* in all tissues causes embryonic lethality prior to implantation (embryonic day 3.5) (Rinkenberger et al. 2000), and conditional deletion of *Mcl-1* was shown to deplete hematopoietic stem/progenitor cells (Opferman et al. 2005), B cell–committed and T cell–committed progenitors (Opferman et al. 2003), activated B cells (Vikstrom et al. 2010), antibody-secreting plasma cells (Peperzak et al. 2013), neuronal cells (Arbour et al. 2008) and cardiomyocytes (Thomas et al. 2013, Wang et al. 2013). The dramatic impact of the genetic loss of *Mcl-1* on so many cell types has raised significant concerns with regard to targeting this antiapoptotic protein for cancer therapy. Much less severe defects were observed following the loss of some other antiapoptotic Bcl-2 proteins. Bcl-w loss only causes defects in spermatogenesis (Print et al. 1998), and so far no defects have been reported for mice deficient for A1 (Schenk et al. 2017).

Studies using cells in which two antiapoptotic Bcl-2 family members were inactivated, either through genetic deletion in mice or using targeted inhibitors on cultured cells (see below), revealed that these proteins can have substantially overlapping functions in the survival of many cell types. For example, the combined hepatocyte-restricted loss of *Mcl-1* and *Bcl-x* causes considerably greater liver damage in mice than loss of either antiapoptotic gene alone (Hikita et al. 2009). Moreover, BH3-mimetic drug–mediated inhibition of Mcl-1, Bcl-2, and Bcl-xL impaired the survival in culture of B and T lymphoid cells and certain myeloid cell populations to a greater extent than inhibition of only one or two of these proteins (Vikstrom et al. 2016). In at least certain cell types, there appears to be a delicate balance between survival and death. For example, the combined loss of only one allele of *Mcl-1* with one allele of *Bcl-x* causes severe craniofacial abnormalities during mouse embryonic development, and remarkably, these defects can be prevented by the additional loss of one allele of the proapoptotic BH3-only gene *Bim* (Grabow et al. 2018). The above studies highlight the impact of perturbations in apoptosis on the survival of normal cells of diverse cellular types, and in particular, they underscore the necessity of Mcl-1 expression for the survival of many normal cell types, which may affect the use of MCL-1-inhibiting drugs for cancer therapy.

ABERRANT EXPRESSION OF MCL-1 AND OTHER ANTIAPOPTOTIC BCL-2 FAMILY MEMBERS IN HUMAN CANCER

BCL-2, the first regulator of programmed cell death to be recognized in any organism (Vaux et al. 1988), was discovered owing to the chromosomal translocation of its gene into the *IGH* (immunoglobulin heavy chain) locus (t[14;18]), with consequent deregulated overexpression of BCL-2 in almost all cases of human follicular center B cell lymphoma (Tsujimoto et al. 1984). Chromosomal translocations involving the genes encoding other prosurvival BCL-2 proteins have not been reported, but somatically acquired copy number amplifications of the genomic regions containing the *MCL-1* or *BCL-X* gene have been found in ~10% or ~3% of human cancers overall, respectively (Beroukhim et al. 2010), and these amplifications were also apparent

in TCGA (The Cancer Genome Atlas) data through cBioPortal (Cerami et al. 2012) and in data summarized by Campbell & Tait (2018). For a small number of cell lines with such somatically acquired gene copy number amplifications, it was shown that knockdown of MCL-1 or BCL-XL using RNA interference technology can induce apoptosis and curtail their growth in culture. This provides evidence that it is specifically the increase in expression of these antiapoptotic proteins, as opposed to the gain of another protein encoded by a gene residing within the amplified region of the genome, that contributed to neoplastic transformation and malignant growth (Beroukhim et al. 2010). Notably, genetic loss or epigenetic silencing of genes encoding proapoptotic BCL-2 family members has also been observed in diverse cancers, including loss of BIM in mantle cell B lymphoma (Tagawa et al. 2005) and renal cell carcinoma (Zantl et al. 2007) or loss of BAX in DNA mismatch repair–defective colon cancer (Rampino et al. 1997). Collectively, these findings show that defects in apoptosis caused by deregulated overexpression of antiapoptotic BCL-2 family members or abnormally reduced expression of their proapoptotic relatives are present in a substantial fraction of cancers of diverse origins.

IMPACT OF GENETIC LOSS OF MCL-1 OR OTHER ANTIAPOPTOTIC BCL-2 FAMILY MEMBERS ON TUMOR DEVELOPMENT

Very early in cell death research it was established that abnormal overexpression of Bcl-2 (Strasser et al. 1990, Vaux et al. 1988) or its antiapoptotic family members such as Mcl-1 (Campbell et al. 2010) can promote tumorigenesis. However, the importance of antiapoptotic BCL-2 proteins when expressed under normal endogenous control in tumor development remained an open question. Bcl-2 overexpression greatly accelerates lymphoma development driven by deregulated expression of the oncogene *c-Myc*, which promotes cell proliferation and metabolism (Soucek & Evan 2010). However, this was not predictive of a role for endogenously expressed Bcl-2 in the development of c-Myc-driven lymphomas, since homozygous loss of *Bcl-2* had no impact on tumor incidence or latency (Kelly et al. 2007). By contrast, loss of just a single allele of *Mcl-1* could almost completely abrogate c-Myc-driven lymphomagenesis (Grabow et al. 2016). Endogenous Bcl-xL expression could also contribute to c-Myc-driven lymphoma development but to a lesser extent than Mcl-1 since complete loss of Bcl-xL was required to inhibit c-Myc-driven lymphoma development and loss of only one allele of *Bcl-x* had no impact (Kelly et al. 2011). This indicates that Mcl-1 is more important for the development of these tumors than Bcl-xL. Importantly, c-Myc-driven lymphoma development could be restored in mice lacking one allele of *Mcl-1* or in those completely deficient for *Bcl-x* by concomitant loss of just a single allele of proapoptotic *Bim* and to a lesser extent by loss of *Puma* (Delbridge et al. 2015, Grabow et al. 2016). This reveals that a major role of Mcl-1 in c-Myc-driven lymphomagenesis (and possibly in the development of tumors of other cell lineages and of tumors driven by other oncogenes) must be to safeguard the survival of cells during early stages of their neoplastic transformation when the stresses emanating from the activation of oncogenes cause an increase in the levels of apoptosis-initiating BH3-only proteins. Additional experiments using transgenic mice or retroviruses to express various oncogenes in mice revealed that Mcl-1 (expressed under its normal endogenous control) is also critical for the development of thymic T cell lymphoma driven by loss of p53 (Grabow et al. 2014) or gain-of-function mutations in Notch (Spinner et al. 2016), BCR-ABL-driven pre-B cell acute lymphocytic leukemia (pre-B ALL), and acute myeloid leukemia (AML) driven by MLL-AF9, AML-ETO, or certain other oncogenic fusion proteins (Glaser et al. 2012). There is increasing evidence that MCL-1 expression is also required for the development of certain solid cancers, including a mouse model of breast cancer where there was strong selection to retain Mcl-1 expression (Campbell et al. 2018). Collectively, these findings demonstrate a critical role for MCL-1

in the development of several types of cancers with a variety of oncogenic drivers. However, it will be of interest to expand these investigations, with a particular focus on the role of MCL-1 and its antiapoptotic relatives in an extended panel of solid cancer types.

IMPACT OF INDUCIBLE LOSS OF MCL-1 OR OTHER ANTIAPOPTOTIC BCL-2 FAMILY MEMBERS ON SUSTAINED TUMOR GROWTH

While the above-described studies helped identify the roles of MCL-1 and related proteins in the development of tumors, they provided no direct insight into the more clinically relevant questions concerning which antiapoptotic BCL-2 family member is critical for the sustained survival and growth of a given cancer and therefore which is a potentially attractive therapeutic target. These questions were addressed using several methods to reduce or abrogate expression of distinct antiapoptotic BCL-2 family members within tumor cells. The tools and resources used include (*a*) genetically engineered mouse tumor models in which *loxP*-flanked antiapoptotic *Bcl-2* family genes (*Mcl-1*, *Bcl-x*, and *Bcl-2*) can be deleted at will using a tamoxifen-regulatable Cre recombinase ($CreER^{T2}$), (*b*) inducible expression of short hairpin RNA vectors targeting a particular antiapoptotic gene, and (*c*) inducible expression of BH3-only proteins (initiators of apoptosis) or variants thereof that bind and inhibit select antiapoptotic BCL-2 family members (**Figure 2*a***). The loss or inhibition of BCL-XL or BCL-2 inhibited the survival and expansion of certain tumor-derived cell lines [e.g., the impact of targeting BCL-XL in certain cell lines derived from multiple myeloma, AML, or melanoma (Gong et al. 2016, Lee et al. 2019, Pan et al. 2014)]. However, the loss or inhibition of MCL-1 had by far the greatest impact on the sustained growth and survival of several cancers, including AML driven by several oncogenic fusion proteins (e.g., MLL-ENL, MLL-AF9, AML-Eto9a) (Glaser et al. 2012), BCR-ABL-driven pre-B ALL (Koss et al. 2013), c-Myc-driven pre-B or B cell lymphoma (Kelly et al. 2014), T cell lymphoma driven by loss of p53 (Grabow et al. 2014) or mutations in Notch (Spinner et al. 2016), multiple myeloma (Gong et al. 2016), and melanoma (Lee et al. 2019). In several of these studies, loss of *Mcl-1* was shown to markedly delay or even abrogate tumor growth within mice (e.g., Glaser et al. 2012, Kelly et al. 2014, Koss et al. 2013). Remarkably, in the case of c-Myc-driven pre-B or B cell lymphoma, the loss of just a single allele of *Mcl-1* was sufficient to cure mice of these tumors, unless they had acquired a mutation in the tumor suppressor p53 (Kelly et al. 2014). This was a very important discovery considering earlier studies examining the impact of loss of Mcl-1 on normal cells that showed that severe defects followed loss of both alleles of *Mcl-1* in normal tissues (see above) but that loss of a single allele of *Mcl-1* was tolerable for normal cells (Brinkmann et al. 2017, Rinkenberger et al. 2000). Accordingly, these observations provided hope that a therapeutic window might be established for drugs that inhibit MCL-1. Collectively, these findings provided evidence that targeting MCL-1 with a small-molecule inhibitor might be efficacious and tolerable for cancer therapy.

DEVELOPMENT AND TESTING OF MCL-1-SPECIFIC BH3-MIMETIC DRUGS

The finding that proapoptotic BH3-only proteins that initiate apoptosis signaling are the natural inhibitors of MCL-1 and the other antiapoptotic BCL-2 family members (Huang & Strasser 2000) led to the idea that apoptosis could be induced in tumor cells by small-molecule compounds that could mimic the action of the BH3-only proteins, i.e., so-called BH3-mimetic drugs (**Figure 2*b***). Developing such drugs has been challenging because of the requirement for these BH3-mimetics to disrupt protein-protein interactions. The first bona fide BH3-mimetic

compound to be published was ABT-737, which potently binds to and inhibits BCL-2, BCL-XL, and BCL-W (Oltersdorf et al. 2005). This compound could potently kill certain cancer-derived cell lines (leukemia, lymphoma, and lung and colon cancer) either as a single agent or when used in combination with other anticancer agents in culture or within tumor-grafted mice. Of note, ABT-737 was efficacious for several more tumor-derived cell lines when used in combination with standard-of-care anticancer agents (Oltersdorf et al. 2005). A related compound that also inhibits BCL-2, BCL-XL, and BCL-W (**Figure 2*b***), ABT-263, was the first BH3-mimetic drug to progress to clinical trials (Roberts et al. 2012, Wilson et al. 2010). Although ABT-263 had a marked impact in patients with chronic lymphocytic leukemia (CLL), a BCL-2-dependent cancer, its progression in the clinic is hampered by its on-target toxicity to platelets, which require BCL-XL for their survival (Mason et al. 2007). This prompted the development of the BCL-2-specific BH3-mimetic drug ABT-199, also called venetoclax or venclexta (**Figure 2*b***), which is well tolerated in patients and due to its remarkable efficacy has been approved in several countries for the treatment of refractory CLL (Roberts et al. 2016, Souers et al. 2013) and AML (Konopleva et al. 2016, Vo et al. 2012). This drug is currently in ~50 clinical trials as a single agent or in combination with additional anticancer drugs (e.g., BTK inhibitors or CD20 antibodies) for several hematopoietic malignancies and breast cancer (reviewed by Merino et al. 2018).

The success of venetoclax, combined with the compelling preclinical results presented in the abovementioned publications and many others, encouraged pharmaceutical and biotechnology companies and academic institutions to develop inhibitors of MCL-1 for cancer therapy. The first compounds to be published, A-1210477 and the series of MCL-1 inhibitors developed by the Fesik laboratory, bound MCL-1 with ~100-nM affinity but had limited potency in killing tumor-derived cell lines in culture (in part due to substantial binding to serum proteins and relatively poor membrane permeability); no results regarding in vivo efficacy were reported for these compounds (Friberg et al. 2013, Leverson et al. 2015). In contrast, the tool compound S63845 developed by Servier binds to MCL-1 with an ~1-nM (or higher) affinity (**Figure 2*b***), has good membrane permeability, and has low binding to serum proteins (Kotschy et al. 2016). Accordingly, this compound can effectively kill a broad range of cancer-derived cell lines as a single agent in culture. This included many human multiple myeloma and AML cell lines, where sensitivity did not correlate with the genetic lesion (e.g., chromosomal translocation) driving their neoplastic transformation, as well as some B and T cell lymphoma lines (Kotschy et al. 2016). For all cell lines tested, the clearest predictor was an inverse correlation between the levels of *BCL-X* mRNA and sensitivity to S63845 (Kotschy et al. 2016). Remarkably, treatment with S63845 substantially delayed the growth of human multiple myeloma- or AML-derived cell lines in immune-deficient mice and could cure ~70% of immune-competent mice bearing c-Myc-driven mouse lymphomas (Kotschy et al. 2016). Of note, doses of S63845 that were effective at inhibiting tumor growth in mice were readily tolerated, with no overt damage seen in tissues in which the genetic (complete) loss of *Mcl-1* causes severe and often fatal pathology (Kotschy et al. 2016). This may be explained by the fact that treatment with an inhibitor for a limited amount of time does not have as much of an impact as complete irreversible loss of this antiapoptotic protein in genetic studies.

Amgen recently published their own inhibitors of MCL-1: the clinical compound AMG-176 and the related analog compound AM-8621, which also have very high affinity for their target (**Figure 2*b***), good membrane permeability, and low serum binding (Caenepeel et al. 2018). Accordingly, AM-8621 also showed excellent on-target activity in a broad panel of hematological cancer–derived cell lines in culture, and for some cancer-derived lines, this was also documented using AMG-176 in vivo in tumor xenograft studies (Caenepeel et al. 2018). Similarly, two other MCL-1 inhibitors, AZD5991 from AstraZeneca and VU661013, recently demonstrated efficacy toward hematological cancer cell lines (Tron et al. 2018) and AML cell lines (Ramsey et al. 2018),

respectively, both in vitro and in vivo (**Figure 2*b***). The MCL-1 inhibitors S63845, AM-8621, and AZD5991 had only modest impact as single agents against a broad panel of solid cancer–derived cell lines (including ones derived from breast, colon, and lung cancers and melanomas). However, S63845, AM-8621, and AMG-176 synergized potently with inhibitors of oncogenic kinases (e.g., inhibitors of MEK, EGFR, or BRAF) in killing a collection of solid cancer–derived cell lines in culture and in vivo in tumor xenograft studies (Caenepeel et al. 2018, Kotschy et al. 2016, Nangia et al. 2018).

S63845, AMG-176, and AZD5991 have considerably higher (~6- to 1000-fold) affinity for human MCL-1 than mouse Mcl-1. Thus, testing these drugs using mouse tumor–derived cell lines or regular mice may underestimate their efficacy and, perhaps more importantly, their on-target toxicity. To overcome these limitations, two groups independently generated mice in which the coding regions for mouse Mcl-1 were replaced with those for human MCL-1 (*huMcl-1* mice). Initial experiments conducted in *huMcl-1* mice bearing tumors expressing human MCL-1 reaffirmed that a therapeutic window could be established for these two MCL-1 inhibitors, even in combination with the commonly used chemotherapeutic agent cyclophosphamide (Brennan et al. 2018, Caenepeel et al. 2018). Collectively, the results from these extensive preclinical tests supported the entry of three MCL-1 inhibitors—S64315/MIK-665 from Servier and Novartis, AMG-176 from Amgen, and AZD5991 from AstraZeneca—into clinical trials (**Table 1** summarizes the ongoing clinical trials with these drugs). An alternative approach to targeting MCL-1 for cancer therapy would be to use PROTAC-like degraders of MCL-1 protein; however, these are currently in an earlier stage of development than BH3-mimetic drugs (Papatzimas et al. 2019).

Table 1 Summary of the current clinical trials using MCL-1 inhibitor drugs[a]

ClinicalTrials.gov identifier	Clinical trial phase	MCL-1 inhibitor	Sponsor	Combination drugs	Malignancies
NCT02675452	Phase I	AMG-176	Amgen	N/A	Relapsed or refractory multiple myeloma or acute myeloid leukemia
NCT02979366	Phase I	S64315 (also referred to as MIK-665)	Servier	N/A	Acute myeloid leukemia, myelodysplastic syndrome
NCT02992483	Phase I	MIK-665 (also referred to as S64315)	Novartis	N/A	Refractory or relapsed multiple myeloma or lymphoma
NCT03218683	Phase I	AZD5991	AstraZeneca	N/A	Relapsed or refractory hematologic malignancies: non-Hodgkin's lymphoma, Richter syndrome, chronic lymphocytic leukemia, small lymphocytic leukemia, T cell lymphoma, multiple myeloma
NCT03672695	Phase I	S64315 (also referred to as MIK-665)	Servier	Venetoclax (BCL-2 inhibitor)	Acute myeloid leukemia
NCT03797261	Phase I	AMG-176	AbbVie	Venetoclax (BCL-2 inhibitor)	Relapsed or refractory acute myeloid leukemia or non-Hodgkin's lymphoma/diffuse large B cell lymphoma

[a]In November 2019, the US Food and Drug Administration put a temporary hold on clinical trials of MCL-1 inhibitors.

MECHANISMS BY WHICH MCL-1-SPECIFIC BH3-MIMETIC DRUGS KILL CANCER CELLS

MCL-1 promotes the survival of cells by restraining BAX and BAK (see above; **Figure 1**). Therefore, its inhibition by a BH3-mimetic drug or its genetic loss is predicted to kill cells by activating BAX/BAK-mediated apoptosis. As predicted, the combined loss of BAX and BAK, using CRISPR/Cas9 technology, rendered all tumor-derived cell lines tested fully resistant to the MCL-1 inhibitors S63845 (Kotschy et al. 2016) and AMG-176 (Caenepeel et al. 2018). However, simple prevention of MCL-1-mediated restraint of BAX/BAK probably does not fully account for the mechanism of tumor cell killing by the MCL-1-specific BH3-mimetic drugs. Genetic and biochemical studies revealed that liberating BH3-only proteins (particularly BIM and NOXA) from MCL-1, thus allowing them to bind to and inhibit the other antiapoptotic BCL-2 family members present in a tumor cell, also contributes to these drugs' mechanism of action (**Figure 2*c***) (Nangia et al. 2018). This is consistent with the notion that for BAX or BAK to become activated to unleash the effector phase of apoptosis, all antiapoptotic proteins present within a cell must be inhibited. This also explains why in tumor cells that express readily detectable amounts of two (or more) antiapoptotic BCL-2 family members, treatment with a BH3-mimetic drug that targets only one of them is usually insufficient to cause substantial killing. Achieving efficient killing requires treating such cells with, for example, two BH3-mimetic drugs to target MCL-1 plus BCL-2 (Caenepeel et al. 2018, Kotschy et al. 2016, Moujalled et al. 2019, Tron et al. 2018) or a single-BH3-mimetic drug in combination with an additional anticancer agent (e.g., cyclophosphamide or inhibitors of oncogenic kinases; see above) (Kotschy et al. 2016, Nangia et al. 2018); these other anticancer agents cause an increase in BH3-only proteins (particularly, BIM, PUMA, and NOXA) that can inhibit the antiapoptotic BCL-2 family members that are not targeted by the BH3-mimetic drug used (**Figure 2*c***).

In addition to inhibiting apoptosis, MCL-1 also promotes energy production by acting in the intermitochondrial space (Perciavalle et al. 2012). It is therefore conceivable that treatment with MCL-1-specific BH3-mimetic drugs or genetic loss of *Mcl-1* can inhibit the survival and growth of malignant (and nontransformed) cells not only by inducing BAX/BAK-mediated apoptosis but also by impairing energy production in mitochondria. However, the loss of the BH3-only protein BIM or the combined loss of the apoptosis effectors BAX and BAK was shown to render cell lines derived from AML, multiple myeloma, pre-B/B cell lymphoma, or certain other tumors fully resistant to the induced complete genetic deletion of *Mcl-1* or treatment with MCL-1-specific BH3-mimetic drugs (Caenepeel et al. 2018, Glaser et al. 2012, Gong et al. 2016, Kotschy et al. 2016). This indicates that, at least in malignant cells, genetic loss of *Mcl-1* or inhibition of MCL-1 mediated by BH3-mimetic drugs impairs survival and growth solely by unleashing apoptosis. Whether this is also the case in normal cells remains to be fully determined, and answering this question is very important for predicting and managing on-target toxicities of MCL-1-specific BH3-mimetic drugs for healthy tissues. Studies have sought to address this using *Bak*-deficient mice, where either *Mcl-1* alone or *Mcl-1* plus *Bax* were inducibly genetically deleted in cardiomyocytes using a tissue-restricted tamoxifen-activatable Cre recombinase. Deletion of *Mcl-1* in *Bak*-deficient cardiomyocytes led to substantial death and fatal damage to the heart. Combined inducible deletion of *Mcl-1* and *Bax* on the $Bak^{-/-}$ background caused less, but still marked, damage to the heart (Thomas et al. 2013, Wang et al. 2013). It was therefore concluded that the genetic loss of *Mcl-1* caused damage to the heart not only by unleashing Bax/Bak-mediated apoptosis in cardiomyocytes but also through additional mechanisms. However, when interpreting these findings, it is important to bear in mind that Mcl-1 protein has a very short half-life (20–30 min) whereas Bax protein has a long half-life (~20 h). Therefore, with inducible CRE-mediated deletion of both *Mcl-1* and

Bax, cardiomyocytes will experience a time when they completely lack Mcl-1 protein but still contain substantial Bax protein. Thus, the incomplete protection of cardiomyocytes observed could be due to the induction of apoptosis through the residual Bax present in these cells early after inducible genetic deletion of *Mcl-1* and *Bax*. This is an important issue to resolve and could be done by sequential genetic removal of *Bax* before *Mcl-1* on a $Bak^{-/-}$ background using two gene recombination systems (e.g., FLP and CRE recombinases). Perhaps even more importantly, researchers need to understand in detail the dose-limiting toxicities of MCL-1-specific BH3-mimetic drugs and the mechanisms causing them to make progress with these drugs in the clinic.

CONCLUSIONS AND PERSPECTIVES

Following the success of the BCL-2 inhibitor venetoclax in the clinic (Roberts et al. 2016, Souers et al. 2013), MCL-1 inhibitors have been the latest BH3-mimetics to enter into clinical trials. Information on the potential of these drugs as anticancer agents is eagerly awaited and will emerge as the clinical trials progress. From preclinical work it is predicted that these MCL-1 inhibitors will have the most impact and benefit across diverse tumor types when used in combination with other therapeutics, perhaps including BH3-mimetics targeting other antiapoptotic BCL-2 proteins (e.g., venetoclax to inhibit BCL-2), chemotherapeutic agents that induce expression of the proapoptotic BH3-only proteins, or drugs that target the oncogenic drivers of tumor cell proliferation. Future efforts will therefore need to focus on testing the efficacy and tolerability of rational drug combinations in tumor models, ideally using *huMcl-1* mice to overcome the issues of preferential binding of these drugs to human MCL-1 versus mouse Mcl-1. Additionally, to date there are very few identified markers of response or resistance to MCL-1 inhibitors and other BH3-mimetic drugs. The identification of prognostic indicators that could be used to stratify patients would therefore likely improve the clinical application of this exciting emerging class of anticancer agents.

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