REVIEW

MCL-1 inhibition in cancer treatment

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Abstract: Myeloid cell leukemia-1 (MCL-1), a member of antiapoptotic BCL-2 family proteins, is a key regulator of mitochondrial homeostasis. Frequent overexpression of MCL-1 in human primary and drug-resistant cancer cells makes it an attractive cancer therapeutic target. Significant progress has been made in the development of small-molecule MCL-1 inhibitors in recent years, and three MCL-1 selective inhibitors have advanced to clinical trials. This review briefly discusses recent advances in the development of small molecules targeting MCL-1 for cancer therapy.

Keywords: BCL-2, BAX, BAK, apoptosis, BH3 mimetics

Introduction

MCL-1 is an antiapoptotic member of BCL-2 family proteins, which include the following three groups classified by their functions: antiapoptotic, proapoptotic, and BH3-only proteins.[1] Antiapoptotic BCL-2 proteins include MCL-1, BCL-2, BCL-W, BCL-XL, BCL-B, and BFL-1/A1. Proapoptotic proteins include BCL-2 homologous antagonist killer (BAK) and BCL-2 associated X protein (BAX). BH3-only proteins include BCL-like protein 11 (BIM), BCL-2-associated death (BAD), BH3 interacting-domain death agonist (BID), phorbol-12-myristate-13-acetate-induced protein 1 (NOXA), and p53 upregulated modulator of apoptosis (PUMA). The interactions between these three groups of proteins determine the fate of cells.

MCL-1 is widely expressed in human tissues[2] and is primarily located in the mitochondria in cells, in which it inserts into the mitochondrial membrane via a hydrophobic tail.[3] The antiapoptotic function of MCL-1 is essential to cell survival and homeostasis.[4,5]

Amplification and overexpression of MCL-1 have been reported in various human tumors, including hematological malignancies and solid tumors (eg, non-small-cell lung cancer, breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer).[6–11] An analysis of 3,131 cancer specimens has revealed that 36% of breast cancer and 54% of lung cancer specimens exhibit elevated levels of MCL-1 expression.[12] MCL-1 amplification and overexpression are also frequently associated with poor prognosis and resistance to anticancer drugs. As an example, breast cancer, especially triple-negative breast cancer, is characterized with the amplification of *MCL-1* gene loci (~20%).[13,14] Also, high MCL-1 expression level in breast tumors correlates with high tumor grade and poor prognosis in patients.[7,15] Similarly, in esophageal squamous cell carcinoma, the copy number of *MCL-1* and its variants is correlated with patient's poor prognosis.[16,17]

MCL-1 has been shown to be both an intrinsic and acquired resistance factor that limits the efficacy of various antitumor agents, including taxol, cisplatin, erlotinib, and other standard anticancer drugs.[13,18,19] Not surprisingly, downregulation of MCL-1

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expression increases cancer cell sensitivity to drug treatment. For instance, MCL-1 knock down in neuroblastoma cell lines increases their sensitivities to etoposide, doxorubicin, and ABT-737 by 2–300-folds.[20] Depletion of MCL-1 reverses cisplatin and doxorubicin chemoresistance in osteosarcoma cell lines *in vitro* and xenograft tumors *in vivo*.[21]

MCL-1 and apoptosis

MCL-1 exerts its antiapoptotic function by sequestering proapoptotic proteins BAK/BAX through the BH3 domain containing hydrophobic groove. In the prosurvival mode (Figure 1A), BAK/BAX interacts with antiapoptotic BCL-2 proteins and is unable to execute the apoptotic program, thereby allowing cells to maintain homeostasis. In the apoptotic mode (Figure 1B), BAK/BAX can be activated through intrinsic pathways such as stress and upstream signals that act on the BH3-only proteins.[22,23] These BH3-only proteins bind the BCL-2 prosurvival proteins releasing BAK/BAX. Unlike BAK, which attaches to the membrane of mitochondria, BAX is located in the cytosol. After the activation of the BH3-only protein BID, BAX is translocated to the mitochondria to exert its apoptotic function; however, this BAX translocation mechanism remains to be fully understood.[24,25] After dissociation from prosurvival proteins, BAK/BAX

exposes its BH3 domain through which it forms oligomers. The oligomers are able to breach the outer membrane lipid bilayer of mitochondria that leads to mitochondrial herniation and mitochondrial DNA efflux, followed by the release of cytochrome *c* and the activation of initiator caspases.[26,27]

BH3-only proteins, BIM, PUMA, BAD, NOXA and BID, restore BAX/BAK activities through interruption of the MCL-1:BAK/BAX complexes.[28] There are two proposed BH3-only protein rescuing mechanisms. The first mechanism is the substrate swap model, in which BH3-only proteins bind to MCL-1 to displace BAK/BAX from the MCL-1:BAK/BAX heterodimer.[1,29] This model is supported by the evidence that some BH3-only proteins bind tightly to MCL-1 at the site MCL-1 uses to bind with BAK/BAX; thus, some BH3-only proteins retain MCL-1 and prevent it from binding to BAK/BAX. However, this is contested by the reports, which show that when the BH3-only proteins are absent, BAK can still be activated by losing MCL-1 in nontransformed cells.[29,30] The second mechanism proposes that BH3-only proteins, competing against MCL-1, instead bind with BAK/BAX and the complexes formed by BH3-only proteins and BAK/BAX activate the apoptotic program. Based on the proposed mechanisms, the BH3-only proteins are also divided into two types, namely sensitizers and activators.[31,32] The sensitizer

BCL-2: BCL-2, BCL-XL, MCL-1, BCL-B, BFL-1/A1
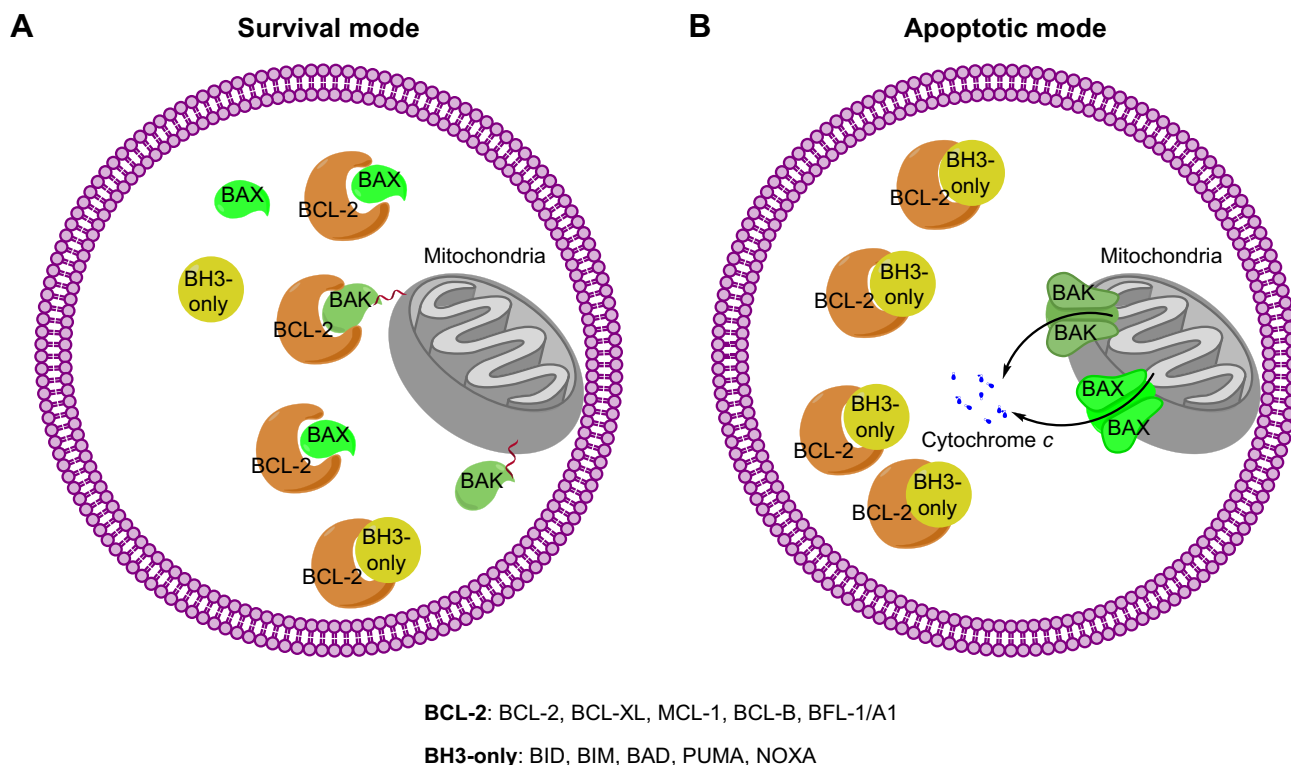
BH3-only: BID, BIM, BAD, PUMA, NOXA

Figure 1 The balance between pro- and antiapoptotic BCL-2 proteins plays a critical role in maintaining cellular homeostasis.
Note: Perturbation of this balance drives the homeostatic balance toward either survival (**A**) or apoptosis (**B**).

BH3-only proteins maintain cellular homeostasis and can displace BAK/BAX from the MCL-1:BAK/BAX complex by binding to MCL-1. The activators BH3-only proteins not only bind to MCL-1 but also bind to BAK/BAX directly to facilitate BAK/BAX oligomerization by forming macropores embodied on the mitochondrial membrane. This model has been illustrated by monitoring the fluorescence complex of BIM, PUMA, and NOXA with BAK/BAX in living HeLa cells.[33]

Targeting MCL-1 for cancer therapy

Given the critical roles of BCL-2 family proteins in maintaining cellular homeostasis, perturbation of the complexes between pro- and antiapoptotic BCL-2 proteins or their levels of expression could alter the cellular homeostatic balance and lead to overcoming apoptosis. Such imbalances can lead to the immortalization of cancers. To achieve the effects of controlling cell fate, small molecules have been developed to compensate for the imbalance between pro- and antiapoptotic BCL-2 proteins and restore the apoptotic pathway (Table 1). Several MCL-1 targeting small molecules have advanced into clinical trials (Table 2).

Small molecules targeting MCL-1

MCL-1, and other antiapoptotic BCL-2 proteins, interact with BAK/BAX through the conserved BH3 domain binding groove in MCL-1. BH3-only proteins bind to MCL-1 using the same site to displace BAK/BAX. However, selectivity of BH3-only proteins to different antiapoptotic BCL-2 proteins exists.[34,35] For example, BIM and PUMA bind indiscriminately to all antiapoptotic BCL-2 proteins at low nanomolar K_d value. BAD does not bind to MCL-1 but binds tightly to BCL-2, BCL-XL, and BCL-W. BID binds to MCL-1 in the micromolar range but is an activator of BAX for its translocation. NOXA selectively binds to MCL-1 in sub-nanomolar concentration without significant binding to BCL-2 and BCL-XL up to micromolar concentrations. In summary, BIM and PUMA are pan prosurvival BCL-2 binders, BAD is a non-MCL-1 prosurvival BCL-2 protein binder, and NOXA is a MCL-1 selective binder (Figure 2).[36]

BH3 mimetics derived from BH3-only proteins are also able to release BAK/BAX sequestered by prosurvival proteins, therefore leading to apoptosis in cancer cells. Each BH3 mimetic has a similar spectrum of selectivity toward antiapoptotic BCL-2 proteins to its parent BH3-only protein.[37] For example, NOXA mimetics demonstrate MCL-1 selectivity over BCL-2, BCL-XL, and BCL-W, whereas BIM and PUMA mimetics are pan-BCL-2 family inhibitors.

Table 1 Approaches of targeting MCL-1 for cancer therapy

Category	Mechanism of action	Compound	Category/activity	In vivo properties	References
Small molecules targeting MCL-1 protein	Pan-BCL-2 family inhibitor with anti-MCL-1 activity	AT101 (R-(−)-gossypol)	K_i=0.32 μM (BCL-2) K_i=0.48 μM (BCL-XL) K_i=0.18 μM (**MCL-1**)	Phase II CLL patients: C_{max}=565 ng/mL at 3.1 hours 30 mg/day; 1%–15% CLL cells apoptosis at 30 mg/day; 18%–45% CLL cells apoptosis at 80 mg/day	41–43
		TW-37	K_i=0.29 μM (BCL-2) K_i=1.11 μM (BCL-XL) K_i=0.26 μM (**MCL-1**)	OSCC3 mouse model: tumor was 5.7-fold smaller than untreated at 15 mg/kg ip	44, 45
		Gambogic acid	IC_{50}=0.66 μM (BCL-B) IC_{50}=0.79 μM (**MCL-1**) IC_{50}=1.06 μM (BFL-1/A1) IC_{50}=1.21 μM (BCL-2) IC_{50}=1.47 μM (BCL-XL) IC_{50}=2.02 μM (BCL-W)	PK in rats: C_{max}=12.8 ng/mL; $t_{1/2}$=1 hour 6.25 mg/kg po; H22 mouse model: 45% tumor inhibition at 2 mg/kg ip	46–48
		Sabutoclax (BI-97C1)	IC_{50}=0.32 μM (BCL-2) IC_{50}=0.31 μM (BCL-XL) IC_{50}=0.20 μM (**MCL-1**) IC_{50}=0.20 μM (BFL-1/A1)	Prostate cancer mouse model: >80% tumor inhibition at 10 mg/kg SQ and 60% tumor inhibition at 10 mg/kg ip	49–51

(Continued)

Table I (*Continued*)

Category	Mechanism of action	Compound	Category/activity	In vivo properties	References
	Selective MCL-1 inhibitor	Marinopyrrole A (maritoclax)	IC$_{50}$ =10.1 μM (MCL-1)	PK in mouse: C$_{max}$ =1,536 ng/mL; t$_{1/2}$=3.5 hours 10 mg/kg ip; U937 mouse model: 59% tumor cells response to 25 mg/kg ip	52, 58
		UMI-77	K$_i$=490 nM (MCL-1)	PC3 mouse model: 65% tumor inhibition at 60 mg/kg iv MDA-MB-468 mouse model: inhibit tumor growth at 60 mg/kg iv	7, 53
		A-1210477	K$_i$=0.454 nM (MCL-1) IC$_{50}$ =26.2 nM (MCL-1)	Inhibit mice ESCC formation in a dose-dependent manner	59, 61
		Fesik's compounds	K$_i$<10 nM (MCL-1)		55, 56, 62
		MIK665/S64315 S63845	K$_i$<1.2 nM (MCL-1) K$_d$=0.19 nM (MCL-1)	At 25 mg/kg iv achieved tumor regression in AMO1, H929, and MV4-11 mice models	42, 63
		AMG176	K$_i$<1 nM (MCL-1)	Tumor regression at 20–60 mg/kg, PO, and QD on OPM2 mouse model	64
		AZD5991	IC$_{50}$ <3 nM (MCL-1)	Regression on multiple mouse models[a]	57
Small molecules modulating MCL-1 expression	Inhibiting MCL-1 transcription	Flavopiridol Roscovitine CR8 Voruciclib (PI446A-05)	CDKs' inhibitor		73, 74, 94
		Cardiac glycosides UNBS1450	Na$^+$/K$^+$-ATPase		63, 75
	Inhibiting MCL-1 translation	Benzyl isothiocyanate	Dephosphorylation of eukaryotic initiation factor 4G		53, 64
		BAY43-9006	EGFR/VEGFR inhibition		76
		BEZ235 AZD8055	PI3K/mTOR inhibitor		77
	MCL-1 proteasome degradation	Arsenic trioxide Bufalin	Activation of GSK-3β		78, 79

Notes: [a]Undisclose mouse models. MCL-1 is bold to highlight MCL-1 among other BCL-2 targets.
Abbreviations: PK, pharmacokinetics; ip, intraperitoneal injection; po, per os (oral adminstartion); iv, intravenous; SQ, subcutaneous; IC$_{50}$, half maximal inhibitory concentration; CLL, chronic lymphocytic leukemia; ESCC, esophageal squamous cell carcinoma; QD, quaque die (once a day).

Table 2 Clinical trials of MCL-1-targeting small molecules[a]

MCL-1 targeting small molecules	Phase	Disease	NCT number	Start time	Status
AT-101	Phase I	Combined with temozolomide in glioblastoma	NCT00390403	October, 2006	Completed
	Phase I/II	Single agent in men with hormone refractory prostate cancer	NCT00288806	February, 2006	Completed
	Phase I	Combined with cisplatin and etoposide in advanced NSCLC	NCT00544596	October, 2007	Completed
	Phase I	Combined with erlotinib in NSCLC	NCT00934076	July, 2009	Withdrawn
	Phase I	Combined with paclitaxel and carboplatin in advanced lymphoma	NCT00891072	April, 2009	Completed
	Phase II	Single agent in B cells' malignancies	NCT00275431	January, 2006	Completed
	Phase II	Combined with erlotinib in lung cancer	NCT00988169	October, 2009	Terminated
	Phase II	Single agent in chronic lymphocytic leukemia	NCT00286780	February, 2006	Completed
	Phase I/II	Single agent in advanced esophageal or gastroesophageal cancer	NCT00561197	November, 2007	Terminated
	Phase I/II	Combined with docetaxel and prednisone in prostate cancer	NCT00286793	February, 2006	Completed
	Phase II	Single agent in advanced adrenocortical carcinoma	NCT00848016	February, 2009	Completed
	Phase I/II	Combined with lenalidomide in lymphocytic leukemia	NCT01003769	October, 2009	Recruiting
	Phase II	Combined with bicalutamide in advanced prostate cancer	NCT00666666	April, 2008	Completed
	Phase II	Combined with docetaxel in NSCLC	NCT00544960	October, 2007	Completed
	Phase II	Combined with prednisone and docetaxel in hormone refractory prostate cancer	NCT00571675	December, 2007	Completed
	Phase II	Combined with docetaxel, cisplatin, and carboplatin in laryngeal cancer	NCT01633541	July, 2012	Recruiting
	Phase II	Combine with docetaxel in squamous cell carcinoma	NCT01285635	January, 2011	Terminated
	Phase I/II	Combined with topotecan in small cell lung cancer	NCT00397293	November, 2006	Completed
	Phase II	Combined with biomarker in glioblastoma multiforme	NCT00540722	October, 2007	Completed
	Phase II	Combined with rituximab in follicular lymphoma	NCT00440388	February, 2007	Completed
	Phase I/II	Combined with dexamethasone and lenalidomide in myeloma	NCT02697344	March, 2016	Recruiting
	Phase II	Single agent in advanced small cell lung cancer	NCT00773955	October, 2008	Completed
MIK665/S64315	Phase I	Single agent in lymphoma or myeloma	NCT02992483	December, 2016	Recruiting
	Phase I	Single agent in AML or myelodysplastic syndrome	NCT02979366	December, 2016	Recruiting
AMG176	Phase I	Single agent in AML and multiple myeloma	NCT02675452	February, 2016	Recruiting
AZD5991	Phase I	Single agent in relapsed or refractory hematologic malignancies	NCT03218683	July, 2017	Recruiting

Note: [a]From www.ClinicalTrials.gov.
Abbreviations: AML, acute myeloid leukemia; NSCLC, non-small cell lung cancer.
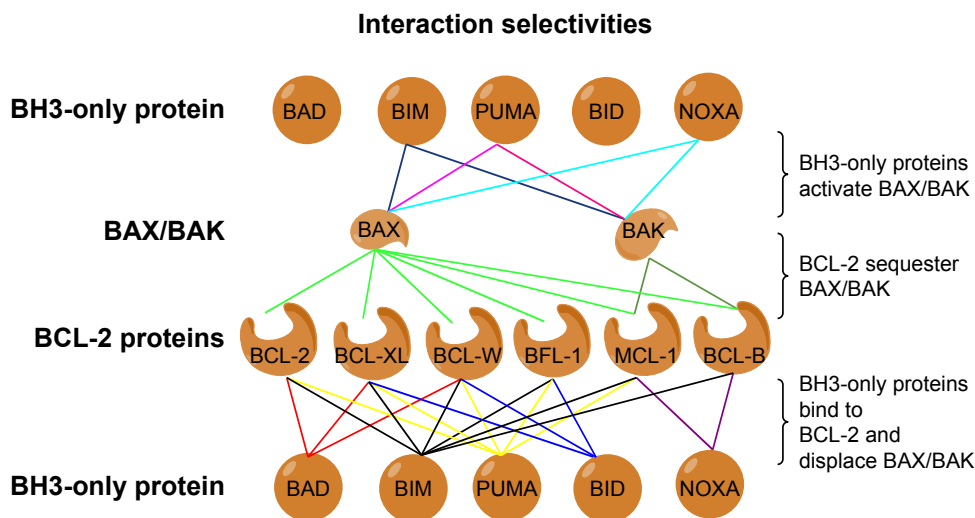
Interaction selectivities

Figure 2 Interactions between BCL-2 family proteins.
Note: Lines indicate the interactions between proteins, and different colors of lines illustrate different cohorts of protein interactions.

BH3 mimetics have been extensively investigated and more than two dozen small-molecule BH3 mimetics have shown promising antitumor activities in preclinical studies. Some of these BH3 mimetics have been extensively reviewed elsewhere.[38,39] Only those with anti-MCL-1 activity will be discussed here.

Pan-BCL-2 inhibitors with anti-MCL-1 activity

The development of potent and selective MCL-1 inhibitors has proven challenging, in part due to key structural differences between the BH3-binding grooves of MCL-1 and other BCL-2 proteins. Several pan-BCL-2 family protein inhibitors, such as AT-101, TW-37, sabutoclax (BI-97C1), marinopyrrole A, and gambogic acid (GA), have been reported to possess anti-MCL-1 activities (Figure 3).

AT-101

AT-101, the *R*-(−) enantiomer of gossypol acetic acid, binds with BCL-2, BCL-XL, and MCL-1 with the K_i of 0.32, 0.48, and 0.18 µM, respectively, in cell-free assays.[40] In NSCLC and myeloma mouse models, AT-101 demonstrates potent antitumor activities either administered alone or in combination with gefitinib and cisplatin.[41,42] In a Phase II clinical trial in CLL, with a single 80 mg dose of AT-101, apoptosis of CLL cells was detected in 18%–45% of cells by Annexin V flow cytometry analysis in four of the six patients.[43] As a single agent or in combination with the standard of care chemotherapies, AT-101 has been evaluated in more than 20 Phase I and II clinical trials in different types of cancers (Table 2).

AT-101 is the first small molecule with potent anti-MCL-1 activity that has been evaluated in clinical trials.

TW-37

TW-37 is a rationally designed small molecule targeting the BH3-binding groove in BCL-2 where proapoptotic BCL-2 proteins bind and has a higher affinity and selectivity for MCL-1 and BCL-2 over BCL-XL with the K_i values of 0.26, 0.29, and 1.11 µM, respectively.[44] TW-37 exerts strong antiproliferative effect in de novo chemoresistant lymphoma cells without a significant effect on normal peripheral blood lymphocytes. Mechanistically, TW-37 disrupts heterodimer complexes between BAX and MCL-1 or BCL-2, resulting in apoptotic cell death. Pre-exposure of lymphoma cells to TW-37 significantly enhances the antitumor activity of cyclophosphamide–doxorubicin–vincristine–prednisone regimen both *in vitro* and *in vivo*. A Kaplan–Meier analysis reveals that the combination of TW-37 with AT-101 significantly extends the time to the failure of human oral squamous cell carcinoma OSCC-3 tumors in severe combined immunodeficiency disease mice as compared with monotherapies with TW-37 or cisplatin.[45]

GA

GA is a caged xanthone derived from *Garcinia hanburyi* and has a strong cytotoxic activity in human cancer cell lines. Employing BFL-1/A1 as a target for screening of a library of natural products, Reed's group identified GA as a competitive inhibitor of BFL-1 in a fluorescence polarization (FP) assay.[46] Analysis of competition for the BH3 peptides binding revealed that GA competitively inhibits BCL-B, MCL-1,
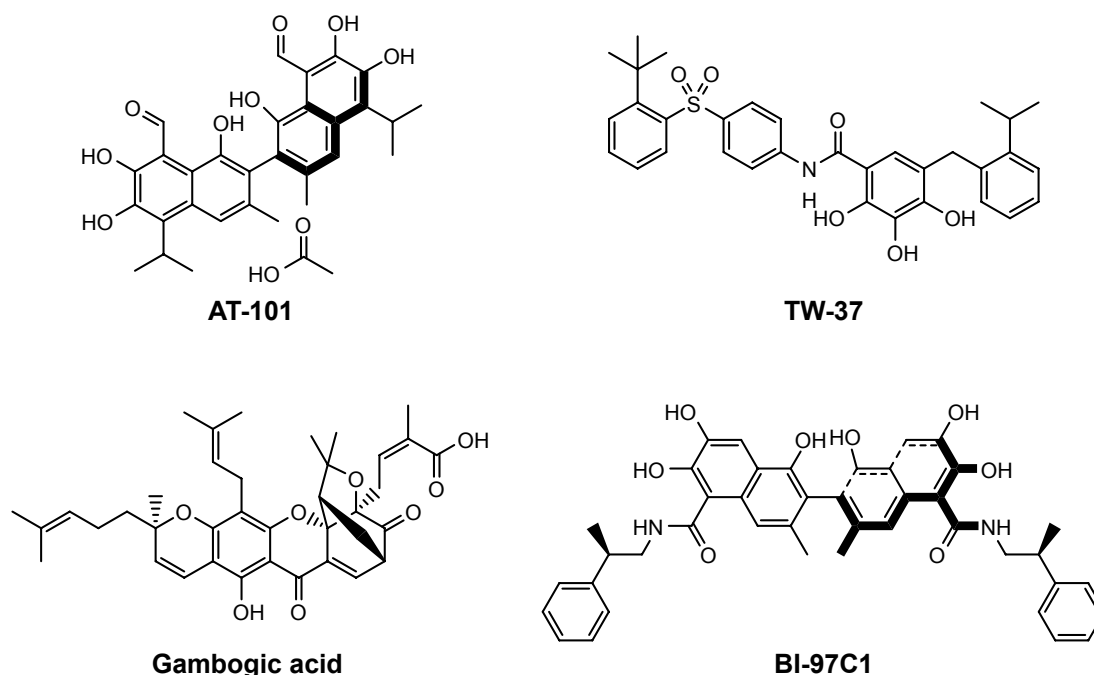
Figure 3 Pan-BCL-2 inhibitors with anti-MCL-1 activity.

BFL-1/A1, BCL-2, BCL-XL, and BCL-W, with the IC$_{50}$ of 0.66, 0.79, 1.06, 1.21, 1.47, and 2.02 μM, respectively. However, GA retains a cytotoxic activity against *BAX$^{-/-}$/BAK$^{-/-}$* cells, indicating that additional targets contribute to the cytotoxic activity of GA. GA has a half-life of 1 hour and a relative low *C*$_{max}$ of 12.8 ng/mL in rats after 6.25 mg/kg oral administration.[47] GA also exerts moderate efficacy in H22 xenograft tumors with only 45% tumor inhibition after 2 mg/kg ip dosing.[48]

Sabutoclax (BI-97C1)

Sabutoclax, an apogossypol derivative, binds to BCL-2, BCL-XL, MCL-1, and BFL-1/A1 with the IC$_{50}$ values of 0.32, 0.31, 0.20, and 0.20 μM, respectively, measured by a BIM FP competitive assay. Sabutoclax also demonstrates low micromolar IC$_{50}$ values in various breast cancer and prostate cancer cell lines.[49] *In vivo* studies demonstrated that sabutoclax possesses the ability to overcome drug resistance in breast cancer and mda-7/IL-24-mediated prostate cancer. Sabutoclax treatment effectively reduces the tumor sizes in both xenograft tumor models and transgenic mouse models of prostate cancer.[50,51]

Selective MCL-1 small-molecule inhibitors

The recent few years have witnessed the significant progress in the development of selective MCL-1 inhibitors. A cohort of highly potent and selective small molecule MCL-1 inhibitors has been reported. These include maritoclax,[52] UMI-77,[53] A-1210477,[54] a diverse collection of compounds from Fesik's group at Vanderbilt University,[55,56] AMG176,[16] AZD5991,[57] and the MIK665/S64315 and S63845 series (Figure 4).[42]

Maritoclax

Maritoclax is the renamed natural product of marinopyrrole A. It is reported to selectively bind MCL-1 over BCL-XL by the ELISA and blocks the interaction between MCL-1 and BIM in a biochemical assay.[52] Maritoclax selectively kills MCL-1-overexpressed but not BCL-2- or BCL-XL-overexpressed K562 leukemia cells. Moreover, maritoclax induces MCL-1 degradation via the proteasome system, which is associated with the proapoptotic activity of maritoclax.[58]

UMI-77

UMI-77 was identified by high-throughput screening using an FP-based binding assay.[53] It binds to MCL-1, BFL-1/A1, BCL-W, BCL-2, and BCL-XL with the *K*$_i$ of 0.49, 5.33, 8.19, 23.83, and 32.99 μM, respectively. UMI-77 blocks the heterodimerization of MCL-1/BAX and MCL-1/BAK in cells, thus antagonizing the MCL-1 function. UMI-77 inhibits cell growth and induces intrinsic apoptosis in pancreatic cancer cells. In a pancreatic cancer cell line BxPC-3 xenograft mouse model, UMI-77 (60 mg/kg iv) exhibits tumor growth inhibition activity without overt toxicity. UMI-77 also demonstrated
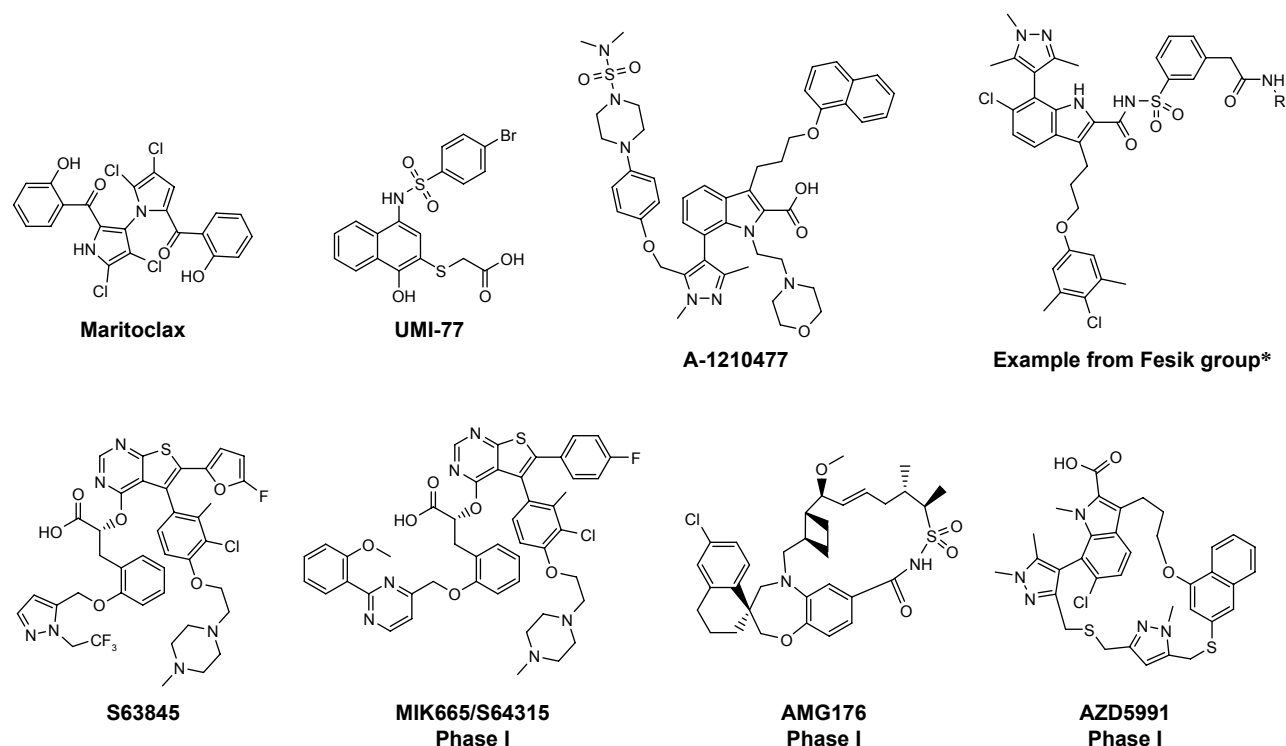
Figure 4 Selective MCL-1 inhibitors.
Note: *R stands for different substitutions based on Fesik's papers.

tumor inhibitory activity in triple-negative breast cancer cell line MDA-MB-468 xenograft mouse model.[53]

A-1210477

A-1210477, developed by the AbbVie team, which also developed highly selective and potent BCL-2-specific and BCL-XL-specific small-molecule inhibitors, is a highly potent and selective MCL-1 inhibitor with the K_i and IC_{50} values of 0.454 and 26.2 nM, respectively, and gives >100-fold selectivity over other BCL-2 family members.[59] A-1210477 potently induces apoptosis and inhibits MCL-1-dependent cell viability in a panel of non-small-cell lung cancer cell lines. Moreover, A-1210477 synergizes with navitoclax (ABT-263) to kill various cancer cell lines. In SKBR3 breast cancer cells, A-1210477 inhibits MCL-1:BIM interaction and induces intrinsic apoptosis.[60] A recent study shows that A-1210477 suppresses the formation of carcinogen 4-nitroquinoline oxide-induced esophageal squamous cell carcinoma in mice.[61]

Fesik's compounds

Stephen Fesik's group at Vanderbilt University has discovered a series of potent and selective MCL-1 inhibitors with picomolar to low nanomolar binding affinities utilizing fragment-based drug design and structure-based drug design approaches.[55,56,62]

Most of these compounds bear a benzo-fused bicyclic and a dimethyl chloro phenyl ring as exemplified in Figure 4. Further structural rigidification has yielded compounds with reduced human serum albumin binding and improved physicochemical properties. Importantly, these compounds bind to the hydrophobic groove of MCL-1 and exhibit cell killing activities in MCL-1-dependent cell lines *in vitro*.

MIK665/S64315

MIK665/S64315, developed by Servier, Vernalis, and Novartis, belongs to the same series of compounds as S63845.[42] Unlike S63845, very little information on S64315 has been disclosed. S63845 initiates BAK/BAX-dependent apoptosis following its binding to the BH3 binding groove of MCL-1. It demonstrates high binding selectivity for human MCL-1 (FP K_i<1.2 nM, and surface plasmon resonance K_d=0.19 nM) over human BCL-2 and BCL-XL (both FP K_i>10,000 nM). It has single-digit to two-digit nanomolar IC_{50} values in most myeloma, myeloid leukemia, and acute myeloid leukemia (AML) cell lines. However, solid tumor cell lines such as non-small cell lung cancer (NSCLC) cell lines can circumvent S63845 inhibition by overexpressing BCL-XL. In a mouse toxicity study, S63845 is well tolerated at an efficacious dose (25 mg/kg), but significant mice weight loss is observed at a 60 mg/kg dosage. In efficacy studies,

S63845 at 25 mg/kg, iv dosing, achieves tumor regression in AMO1, H929, and MV4-11 xenograft tumor models.[63] For S64315, two Phase I clinical trials, one in AML and myelo-dysplastic syndrome (MDS) (NCT02992483) and the other in multiple myeloma (MM) and lymphoma (NCT02979366), have started patient recruitment to determine the tolerated dosage and the toxicity profile.

AMG176

AMG176 is selected from more than a thousand compounds during lead optimization by Amgen.[64] AMG176 has K_i<1 nM to MCL-1 in biochemical assays and IC_{50} <0.1 µM in cel-lular assays and is a selective MCL-1 inhibitor. A profiling of >200 cell lines treated with AMG176 reveals its robust effects on the viability of hematological cell lines (MM, AML, and non-Hodgkin's lymphoma) and a subset of solid tumor cell lines (breast cancer and non-small-cell lung cancer cell lines). AMG176 was shown to disrupt the MCL-1:BAK complex, leading to the rapid induction of intrinsic apoptosis and the loss of cell viability. Oral administration of AMG176 to mice bearing MM OPM-2 xenografts results in a dose-dependent increase in activated BAK. Treatment of AMG176 at 20–60 mg/kg oral, once a day, demonstrates robust tumor growth inhibition, whereas complete tumor regression has been achieved at an elevated dose. AMG176 is currently evaluated in Phase I clinical trials in relapsed or refractory MM and myeloid leukemia (NCT02675452).

AZD5991

AZD5991, developed by AstraZeneca, possesses high MCL-1 selectivity with sub-nanomolar affinity for MCL-1 in biochemical assays and induces rapid apoptosis in MCL-1-dependent myeloma cell lines with half concentration of growth inhibition as low as 10 nM.[57] Mechanism studies reveal that AZD5991 disrupts the MCL-1:BAK complex to exert its antitumor activity. As such, depletion of BAK by siRNA blocks the activity of AZD5991. AZD5991 achieves excellent antitumor activity in mouse xenograft models of AML, non-Hodgkin's lymphoma, and MM. Ex vivo studies in primary tumor samples from leukemia patients suggest that leukemia patients would respond favorably to AZD5991 treatment. AZD5991 is now in Phase I clinical trials in patients with relapsed or refractory hematologic cancers (NCT03218683).

Targeting MCL-1 expression

MCL-1 is a short-lived protein,[65] and its expression is tightly regulated at transcriptional, translational, and post-translational levels. MCL-1 can be upregulated by trophic factor cytokines, including interleukins IL-3,[65] IL-6,[66] IL-10,[67] and IL-15,[68] and growth factors, such as EGF,[69] vascular endothelial growth factor (VEGF),[70] and platelet-derived growth factor (PDGF) (Figure 5).[71] STAT3 and STAT5, activated by upstream interleukins (IL-3, -6, -10, and -15) and Janus kinases (JAKs), bind to the promoter region of MCL-1 and potentiate MCL-1 transcription. EGF activates MCL-1 translation through RAS–RAF–MEK–ERK and ELK1 pathway. EGF and VEGF upregulate MCL-1 translation either through PI3K–AKT or through PI3K–mTOR signal transduction pathways. PDGF–β-catenin–HIF1α pathway increases MCL-1 mRNA via functional hypoxia response elements in the promoter region of MCL-1. Activation of GSK-3β phosphorylates MCL-1 and causes proteasomal degradation of MCL-1.[72]

Inhibition of MCL-1 upstream signal pathways can downregulate MCL-1 expression by decreasing transcription and translation. For example, CDK9 inhibitors, roscovitine and CR8, and Na^+/K^+-ATPase inhibitor cardiac glycosides UNBS1450 effectively inhibit MCL-1 transcription.[73–75] Benzyl isothiocyanate inhibits the phosphorylation of eukary-otic initiation factor 4G, resulting in a decreased MCL-1 translation, followed by cell cycle arrest and apoptosis in leukemia cells.[53,64] EGFR/VEGFR inhibitor BAY43-9006 downregulates MCL-1 translation cofactor ELK-1 via RAS/RAF–MEK–ERK pathway and inhibits MCL-1 expression.[76] Inhibition of PI3K/mTOR pathway by BEZ235/AZD8055 also results in reduced MCL-1 translation.[77] Pharmacologi-cal promotion of MCL-1 degradation is another approach to reduce cellular MCL-1 protein levels. In this case, activation of GSK-3β phosphorylation by arsenic trioxide and bufalin enhances MCL-1 degradation.[78,79]

BAK/BAX agonist

During the apoptotic process, BAK/BAX undergoes confor-mational changes, which is followed by homo-oligomerization to form macropores in the membrane of mitochondria.[80] In this regard, direct activation of BAK/BAX to potentiate macropore formation by bypassing MCL-1 inhibition offers another promising approach to target MCL-1-dependent tumors for treatment. The hydrophobic groove formed by the α2-α5 helices in BAK/BAX interacts with BH3-only proteins that are required for BAX/BAK activation during apoptosis. Besides the pocket formed by the α2-α5 helices, multiple sites on BAK/BAX have also been targeted by dif-ferent approaches. For example, monoclonal antibody Fab 7D10 recognizes the α1 loop of BAK/BAX and its binding
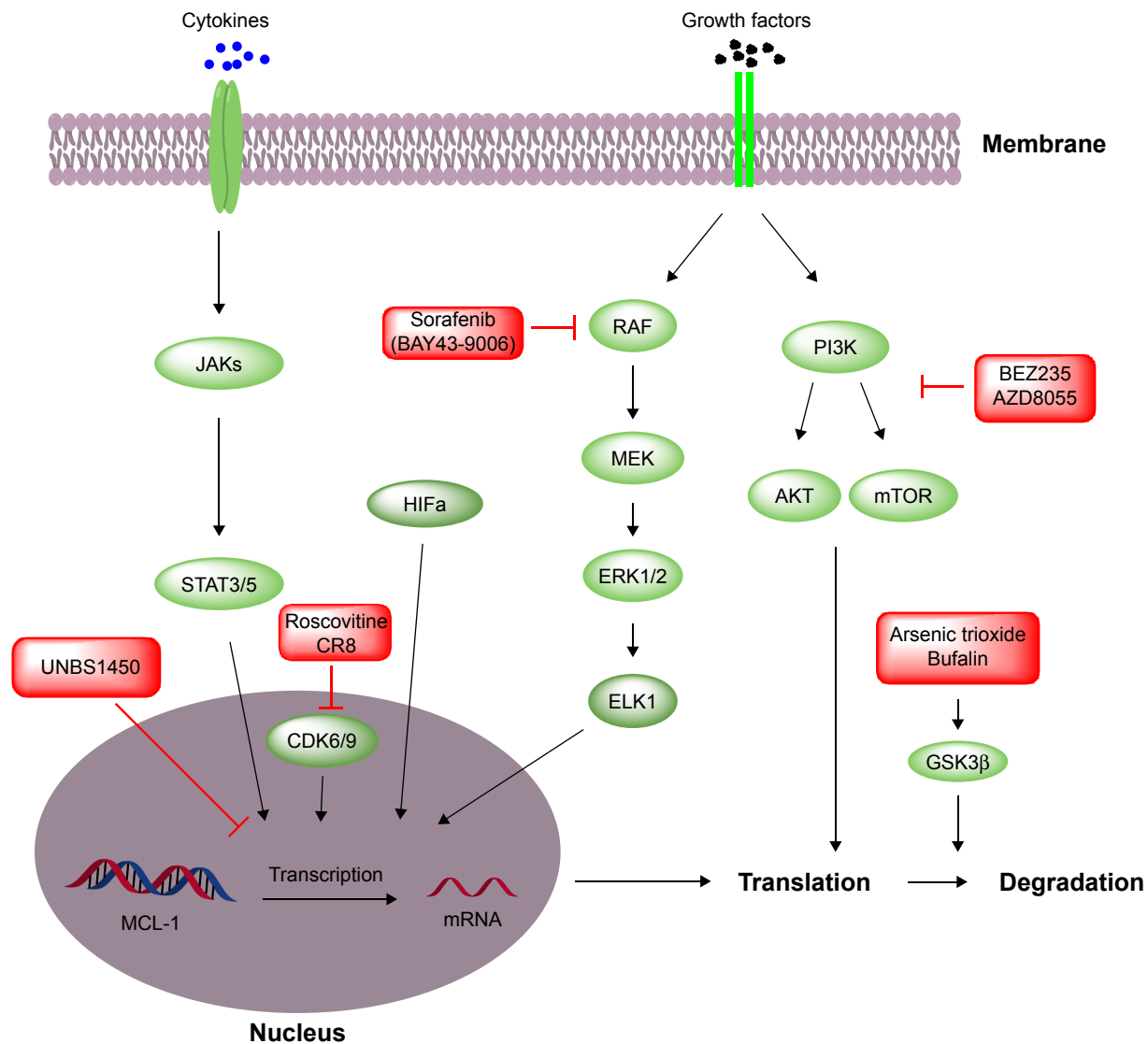
Figure 5 Regulation of MCL-1 transcription, translation, and degradation through different upstream pathways.
Notes: Signal transduction pathways are highlighted in lizard green and black arrows. Drugs targeting these pathways are in red squares.

with BAK/BAX leads to BAK/BAX oligomerization and apoptosis. The α1-α2 loop of BAK/BAX was shown to be another potential druggable site.[81] The α6 helix of BAK is also identified as another site recruited by BH3-only proteins to facilitate BAK homo-oligomerization.[82] Unlike the sites on the α1-α2 loop, the α6 site on BAK/BAX requires the BH3-only proteins binding to activate BAK/BAK. Small molecules SMBA1–3 identified by virtual screening bind to the BAX S184 pocket and potentiate BAX function by blocking S184 phosphorylation without affecting the binding of BCL-2 antiapoptotic family protein to BAX.[83]

While these aforementioned strategies have been explored to antagonize the antiapoptotic functions of MCL-1, studies to directly compare these approaches for their therapeutic effects are lacking. Most of the earlier reported BH3 mimetics are neither very selective nor potent enough to achieve significant tumor growth inhibition in preclinical models and, thus, are mainly used as tool compounds. Most recently discovered BH3 mimetics are highly selective and able to achieve tumor regression in xenograft tumor models. Drugs targeting MCL-1 upstream signaling are highly unlikely to selectively modulate the expression of MCL-1 without affecting other genes. Yet mechanistic studies of these drugs' actions may shed light on the development of combination therapies. Although BAK/BAX agonists showed promising future, it still needs significant medicinal chemistry efforts to identify drug-like molecules.

Drugs' combination

Depletion of MCL-1 combined with standard chemotherapies is tolerable in mice, attesting the feasibility of combining

Table 3 Selective MCL-1 inhibitors evaluated in preclinical drug combination studies

MCL-1 inhibitors	Drug	Model system	Purpose	Reference
Maritoclax	ABT-737	Hematologic cancer cell lines	Overcome resistance	52
A-1210477	ABT-199	Acute myeloid leukemia cells	Overcome resistance	85
A-1210477	ABT-263	Cervical cancer cell lines	Synergistic effect	86
UMI-77	Irradiation	BxPC-3 and Panc-1 cells	Synergistic effect	87
AMG176	Selective BCL-2 inhibitors, MAPK pathway inhibitors, proteasome inhibitors[a]	Cell-based assay[b]	Synergistic effect	64
S63845	Docetaxel, lapatinib, trastuzumab	Triple-negative and HER2-amplified breast cancer cell lines and PDX mouse model	Synergistic effect	42
AZD5991	Standard of care chemotherapeutic agents[a]	Mouse model	Synergistic effect	57

Notes: [a]Undisclosed drugs. [b]Undisclosed cell-based assays.

MCL-1 inhibitors with other anticancer drugs.[84] However, the overlapping toxicities from MCL-1 inhibitors and standard chemotherapies need to be carefully monitored and addressed. Nevertheless, pan-BCL-2 inhibitor AT-101 in combination with a variety of anticancer drugs has been evaluated in multiple Phase I and II clinical trials (Table 2).

Aberrant MCL-1 expression has been linked to anticancer drug resistance in almost every tumor type. Overcoming MCL-1-mediated drug resistance necessitates combination therapies of MCL-1 inhibitors with other anticancer drugs (Table 3). In a study using ABT-199-resistant acute myeloma lymphoma cell lines, MCL-1 selective inhibitor A-1210477 combined with ABT-199 exerts synergistic apoptotic effects and circumvents ABT-199 resistance.[85] Another study shows that selective MCL-1 inhibitor maritoclax increases the efficacy of ABT-737 against multiple drug-resistant hematologic cancer cell lines.[52]

In addition to overcoming drug resistance, synergistic effect is another benefit of MCL-1 combination therapies (Table 3). A-1210477,[86] UMI-77,[87] AMG176,[64] and S63845[42] have been shown to synergize with other anticancer drugs. The synergistic effects between S63845 and docetaxel, lapatinib, and trastuzumab and between AZD5991 and standard anticancer agents were observed in mouse tumor models.[42,57]

Assessment of small-molecule MCL-1 inhibitors

A specific and bona fide MCL-1 inhibitor should 1) selectively bind to MCL-1 in homogeneous biochemical assays and engage with MCL-1 in cellular context, 2) dissociate the heterodimeric interactions between MCL-1 and proapoptotic BCL-2 family proteins in the cellular context, 3) cause changes in mitochondrial outer membrane permeabilization

(MOMP) and the release of cytochrome c in cells, and 4) induce BAX/BAK-dependent apoptosis.

In cellular context, target engagement of MCL-1 small molecules can be conveniently evaluated by a cellular thermal shift assay[88,89] or pull down assay using a biotin conjugated MCL-1 ligand. The dissociation of MCL-1 with proapoptotic BCL-2 family proteins can be detected by immunoprecipitation assay. A key feature for the regulation of apoptosis by the BCL-2 family proteins is to control MOMP and release of cytochrome c, which can be evaluated by conventional cellular and biochemical assays. Orthogonal approaches, such as the application of small-interfering RNA-mediated knockdown or clustered regularly interspaced short palindromic repeats-mediated knockout can be used to determine the on-target cytotoxicity of MCL-1 inhibitors.

Mechanisms of resistance to MCL-1 targeted therapies

While several previous studies have revealed that the sensitivity to the inhibition or depletion of MCL-1 is inversely correlated with the expression levels of BCL-XL,[14,60,90–92] there are very few studies on the resistant mechanisms to small-molecule MCL-1 inhibitors. With the recent advancement of multiple selective MCL-1 inhibitors to clinical trials, more studies on the resistant mechanisms to small-molecule MCL-1 inhibitors are expected in the near future.

Conclusion

MCL-1 is frequently overexpressed in human cancers and identified as the linchpin of cancer drug resistance in a variety of tumor types, making it an attractive therapeutic target. This stresses the necessity to develop MCL-1 inhibitors that can be used either as a single agent or in combination regimens. Efforts to develop MCL-1 inhibitors have been predominantly focused

on BH3-mimetics, and now several small molecules with high MCL-1 affinities and selectivity have been discovered. However, most of the MCL-1 inhibitors are still at the stages of preclinical or early clinical development. Beside the MCL-1 inhibitors, newly identified druggable sites on BAK/BAX may offer an alternative approach to target MCL-1-dependent cancers. Furthermore, the emerging proteolysis targeting chimera technology to pharmacologically induce MCL-1 degradation may provide a novel strategy to target MCL-1.[93]

Disclosure

The authors report no conflicts of interest in this work.

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