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STATE-OF-THE-ART REVIEW

# Understanding MCL1: from cellular function and regulation to pharmacological inhibition

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Myeloid cell leukemia-1 (MCL1), an antiapoptotic member of the BCL2 family characterized by a short half-life, functions as a rapid sensor that regulates cell death and other relevant processes that include cell cycle progression and mitochondrial homeostasis. In cancer, MCL1 overexpression contributes to cell survival and resistance to diverse chemotherapeutic agents; for this reason, several MCL1 inhibitors are currently under preclinical and clinical development for cancer treatment. However, the non-apoptotic functions of MCL1 may influence their therapeutic potential. Overall, the complexity of MCL1 regulation and function represent challenges to the clinical application of MCL1 inhibitors. We now summarize the current knowledge regarding MCL1 structure, regulation, and function that could impact the clinical success of MCL1 inhibitors.

## Introduction

Myeloid cell leukemia-1 (MCL1), a member of the BCL2 (B-cell lymphoma 2) family of proteins, was initially identified as an early gene induced in human myeloblastic leukemia (ML-1) cells upon phorbol ester (TPA) exposure. In this case, MCL1 upregulation triggered the differentiation of ML-1 cells into monocytes or macrophages; however, MCL1 downregulation induced apoptosis, thereby suggesting a role for MCL1 in cell survival. Protein homology studies provided evidence for a primary function of the BCL2 family of proteins in regulating cell death and, in particular, in mitochondrial outer membrane permeabilization (MOMP) [1–3].

BCL2 proteins modulate apoptosis, a form of programmed cell death essential for the control of homeostasis, and are characterized by the presence of BCL2 homology (BH) domains and a carboxy-terminal transmembrane domain (TMD) present in most members. BCL2 proteins are classified into three groups according to their function and the number of BH domains—the antiapoptotic proteins (BCL2, BCL-xL, BCLW, MCL1, A1, and BCLB), the proapoptotic *BH3-only* 'sensor' proteins (including BAD, BID, BIM, BMF, PUMA, and NOXA), and the proapoptotic 'executors' (BOK, BAX, and BAK). A complex network of interactions between proapoptotic and antiapoptotic members of this protein family governs MOMP, which first induces the release of apoptogenic factors such as cytochrome *c* into the cytosol, where it

**Abbreviations**
A1, BCL2-related protein A1; BAD, BCL2-associated agonist of cell death; BAK, BCL2 homologous antagonist/killer; BAX, BCL2-associated X protein; BCL2, B-cell lymphoma 2; BCLB, BCL2-like protein 10; BCLW, BCL2-like protein 2; BCL-xL, BCL-extra-large; BID, BH3-interacting domain death agonist; BIM, BCL2-like protein 11; BMF, BCL2-modifying factor; BOK, BCL-2-related ovarian killer; MCL1ES, myeloid cell leukemia-1 extra short form; MCL1L, myeloid cell leukemia-1 long form; MCL1S, myeloid cell leukemia-1 short form; NOXA, phorbol-12-myristate-13-acetate-induced protein 1; PUMA, BCL2-modifying factor; TIM/TOM, translocase of inner/outer membrane.


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binds to apoptosis protease-activating factor 1 (APAF1) and procaspase-9 in a macromolecular complex known as the apoptosome. Subsequently, activated procaspase-9 activates the procaspase-3 effector protease that finally dismantles the cell [4,5].

MCL1 has three confirmed BH domains (BH1–BH3), a putative BH4 domain, a C-terminal TMD, and a large N-terminal region [1–170 amino acids (aa)] (Fig. 1). The large N-terminal region differs from the other BCL2 family members and contains large numbers of proline (P), glutamic acid (E), serine (S), and threonine (T) residues (or 'PEST' sequences). PEST enrichment and arginine pairs represent typical features of labile proteins; consequently, MCL1 displays a short half-life and is highly regulated, thereby providing a means by which MCL1 can modulate apoptosis in response to rapidly changing cellular contexts. Intense recent efforts have led to the description of new small-molecule drugs that block MCL1 function in diseases such as cancer, in which MCL1 plays an inhibitory role in apoptosis. Importantly, MCL1 also plays important nonapoptotic roles in mitochondrial homeostasis, embryonic development, autophagy, and cell cycle.

Given the availability of tools that modulate MCL1 function, the coming years may provide an avalanche of preclinical studies and clinical trials that will require a deeper molecular understanding of this critical protein.

## MCL1 structural features that impact function and treatment strategies

The human *MCL1* gene located on chromosome 1p21.2 contains three exons and encodes a 350 aa pro-survival protein called MCL1 or MCL1L [6]. Alternative splicing generates two additional shorter forms, MCL1S and MCL1ES (Fig. 1).

The MCL1S variant (271 aa) lacks exon two and produces a protein without BH1, BH2, and the TMD —as a result, MCL1S behaves like a *BH3-only* sensor protein, induces apoptosis, selectively forms heterodimers with MCL1L, and displays a predominantly cytosolic localization [but exists to a lesser extent in the endoplasmic reticulum (ER)] [7,8]. Thus, a balance between MCL1L/MCL1S expression may regulate the machinery controlling mitochondrial fusion and fission [9].

The shortest MCL1ES variant (1–179 aa) possesses a truncated exon one, which results in the loss of the N-terminal section of the PEST sequences. MCL1ES binds to MCL1L, localizes to the outer mitochondrial membrane (OMM), and induces BAK- and BAX-independent mitochondrial cell death [10,11]. Evidence suggests that the formation of MCL1L-MCL1ES oligomers triggers MOMP and apoptotic signaling.

The first reported structure of MCL1 in solution, corresponding to residues 152–308 of mouse MCL1, was determined in 2005 [12]. Like all MCL1 structures published to date, it lacked the large PEST domain located in the N-terminal region and the C-terminal TMD; however, the publication of the human protein structure soon after established a significant similarity to the mouse MCL1 protein [13]. Important differences included a negative charge distribution in the binding groove of the helix α3 region of human MCL1, a region which is positively charged in the mouse protein [14]. Since the publication of these studies, many MCL1 structures complexed with *BH3-only* proteins or inhibitors have been described.

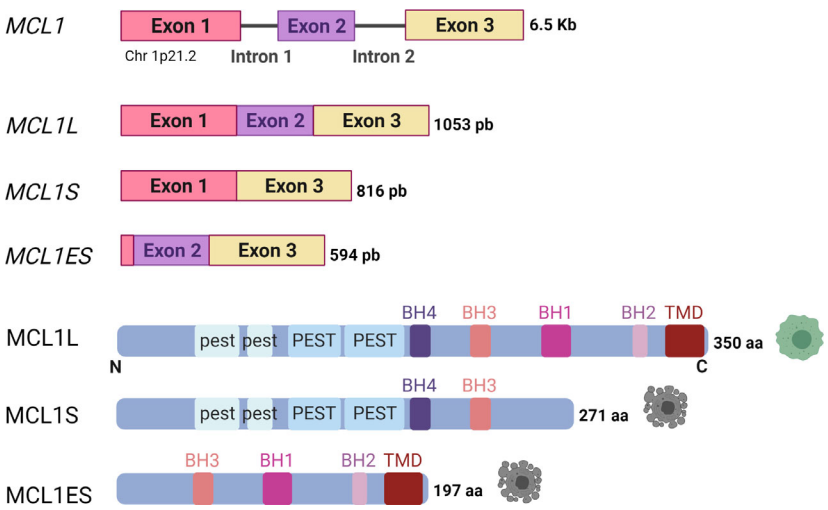


**Fig. 1.** Schematic representation of MCL1 gene, mRNAs, and protein variants. MCL1S and MCL1ES display proapoptotic behavior while MCL1L is antiapoptotic.


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Human and mouse MCL1 share a helical core structure consisting of eight helices [in which a set of amphipathic helices surrounds a hydrophobic central helix (α5)] with other BCL2 antiapoptotic proteins. The folded structure of MCL1 generates a hydrophobic region known as the 'BH3-binding groove', which is formed by residues from helices two, three, and four; meanwhile, helices five and eight comprise the groove base (Fig. 2A). The BH3-binding groove establishes interactions with BH3 domains in other BCL2 protein family members (Fig. 2B). Of note, this interaction has been studied in-depth due to its relevance as a pharmacological intervention point for the modulation of apoptosis.

A conserved pattern within BH3 domains, comprising four positions (h1–h4) occupied by hydrophobic residues located on the same face of the helix, controls interactions with the BH3-binding groove (Fig. 3A). An aspartic acid residue from the BH3 domain forms a salt bridge with arginine 263 of the MCL1-binding groove [15–18].

The MCL1-binding groove can also be subdivided into four pockets (P1-P4) that interact respectively with residues h1-h4 from *BH3-only* proteins (Fig. 3B). Exhaustive structural comparisons of different protein complexes have identified P2 and P3 pockets as the location for the 'hot spot' residues for protein–protein interactions in MCL1, which is unlike antiapoptotic proteins such as BCL2 (P2 and P4/P1) or BCL-xL (P2 and P4) [19,20]. The structure of MCL1 P2 is shorter and wider compared with the BCL2 P2 [20], while the MCL1 groove displays a more open conformation when compared to other antiapoptotic BCL2 proteins. Moreover, the surface properties of BCL2 family proteins differ significantly despite high levels of structural homology. MCL1 has abundant lysine and histidine residues, which generate an electropositive surface that influences drug interactions with the binding groove. Together, these differences explain the interaction specificities of different *BH3-only* proteins and contribute to the design of novel specific MCL1 inhibitors and an appreciation of their mechanism of action.

Song *et al.* [21] recently identified a conserved domain formed by residues Q221, R222, and N223 (QRN motif) in the BH3 domain of MCL1, that undergoes a conformational switch (to a helix) following NOXA binding that facilitates protein ubiquitination by MCL1 ubiquitin ligase E3 (MULE, also called LASAU1, ARF-BP1, or HUWE1). Interestingly, binding of the BIM BH3 domain stabilizes the nonhelical structure of this motif to avoid MCL1 ubiquitination and degradation. Thus, inhibitors designed to target the QRN motif could interfere with BH3 binding and facilitate protein degradation, thereby increasing apoptosis.

As noted previously, the MCL1 protein plays additional apoptosis-independent roles, including the inhibition of senescence. Mutational and structural studies have demonstrated that this senescence-inhibiting function does not depend on the BH3 binding domain [22]. Instead, a loop formed by G203, P198, K197, and K1974 (Fig. 3C) controls the MCL1-mediated inhibition of senescence. Interestingly, peptides derived from this so-called senescence-regulating domain counteracted MCL1 function in vitro and in vivo in a colon cancer model of doxorubicin-induced senescence [23].

## The complex regulation of MCL1 expression

MCL1 expression is controlled at the transcriptional and post-transcriptional levels and the translational and post-translational levels. Overall, MCL1 displays low tissue and cell-specific expression and broad intracellular localization. While localizing primarily to the mitochondria membranes [24], MCL1 can be found in light membrane fractions, such as the ER [9] and the nucleus [25,26].

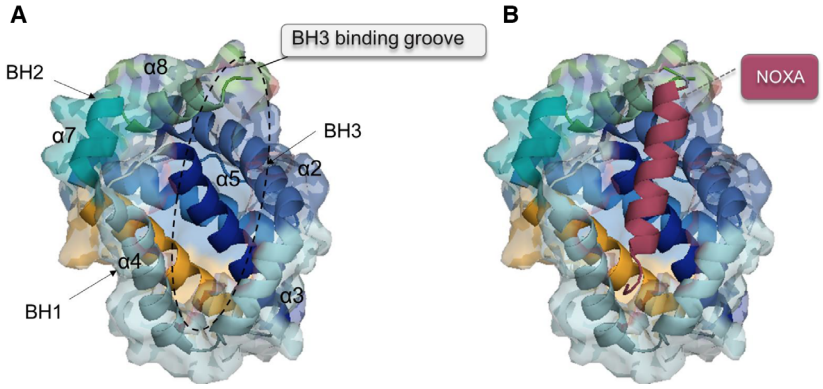


**Fig. 2.** Crystal structure of MCL1. (A) Helical folding of MCL1. Helices (α1–α8) form the helical core where the amphipathic α5 is surrounded by the rest of helices, thereby creating the hydrophobic BH3-binding groove. (B) Structure of MCL1 in complex with the *BH3-only* protein NOXA. Figure was generated using Protein Data Bank (PDB) code 2NLA.

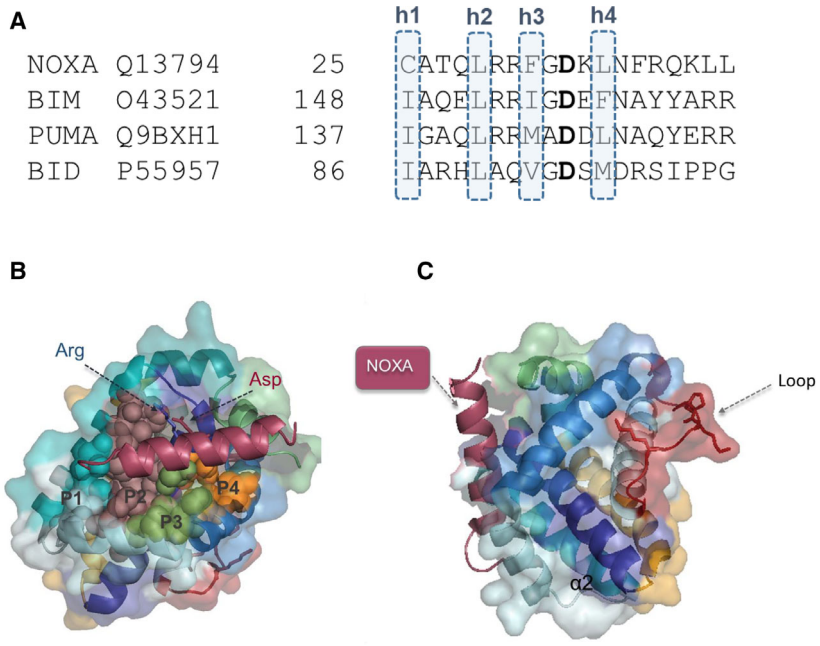


**Fig. 3.** Interaction sites relevant for MCL1 function. (A) Conserved positions of BH3 domains (h1–h4) required to establish interactions with the binding groove. (B) Crystal structure of MCL1 with the P1–P4 pockets of the binding groove highlighted. (C) Crystal structure of MCL1 with the loop relevant for senescence regulation highlighted in red. The figure was generated using PDB code 6YBJ.

The dysregulation of MCL1 transcription in cancer cells can induce resistance to apoptosis. Cytokines (e.g., IL-3, 5, and 6), growth factors [e.g., granulocyte-macrophage colony-stimulating factor (GM-CSF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF)], interferon proteins, and stress stimuli (e.g., ER stress or hypoxia) can all stimulate the transcriptional upregulation of *MCL1* through the direct activity of crucial transcription factors (Fig. 4) [6]. Said factors include signal transducer and activator of transcription (STAT) proteins, cAMP response element-binding protein (CREB), nuclear factor kappa light chain enhancer of activated B cells (NF-κβ), hypoxia-inducible factor 1-alpha (HIF1α), transcription factor binding purine-rich sequence (PU.1), Sp/KLF family of transcription factor 1 (SP1), and estrogen receptor alpha (ERα) [27]. MCL1 transcriptional repression following growth factor withdrawal, ultraviolet light exposure, or flavopiridol (a flavonoid alkaloid cyclin-dependent kinase 9 [CDK9] inhibitor) treatment occurs mainly through the inactivation of the previously mentioned transcription factors or the direct binding of E2F transcription factor 1 (E2F1) to the *MCL1* promoter [28,29].

Post-transcriptional control of *MCL1* occurs at two levels—mRNA splicing and mRNA stability. Several spliceosome components and regulatory RNA-binding proteins (RBPs) modulate MCL1 splicing. As an example, the knockdown of the spliceosome components ubiquitin-like protein 5 (UBL5), pre-mRNA-processing-splicing factor 8 (PRPF8), squamous cell carcinoma antigen recognized by T cells (SART), and the U2 small nuclear ribonucleoprotein (snRNP) splicing factor 3b subunit 1 (SF3B1) induce the generation of the MCL1S variant, which sensitizes MCL1-dependent neuroblastomas to treatment with ABT-737, a small-molecule drug that inhibits BCL2 and BCL-xL [30]. Members of the serine/arginine-rich (SR) protein family of RBPs (SRSF1 and SRSF2) also participate in MCL1 splicing (Fig. 4)—while SRSF1 knockdown increases MCL1S expression, SRSF2 knockdown in renal cancer cells decreases MCL1S expression and inhibits apoptosis [31,32]. While we understand little regarding the epigenetic regulation of MCL1 expression, Khan *et al.* [33] have reported that the acetylation of histones H3 and H4 within exon two favors exon skipping and MCL1S expression. Of note, the splicing factors regulating the formation of MCL1ES remain unidentified.

*MCL1* mRNA has a short half-life (two to three hours average), and RNA sequence elements and the binding of RBPs determine stability (Fig. 4). Several cis-acting elements have been identified in the 3′ untranslated region (UTR) of antiapoptotic BCL2 family members (including CU-rich, AU-rich, and GU-rich elements) that regulate mRNA stability in an RBP-dependent manner. Human antigen R (HuR) binds to AU-rich elements in the *MCL1* 3′ UTR to stabilize mRNA and increase expression in glioma [34]; however, RNA-destabilizing protein Tristetraprolin (TTP) binds to this same motif to destabilize *MCL1* mRNA and decrease expression during


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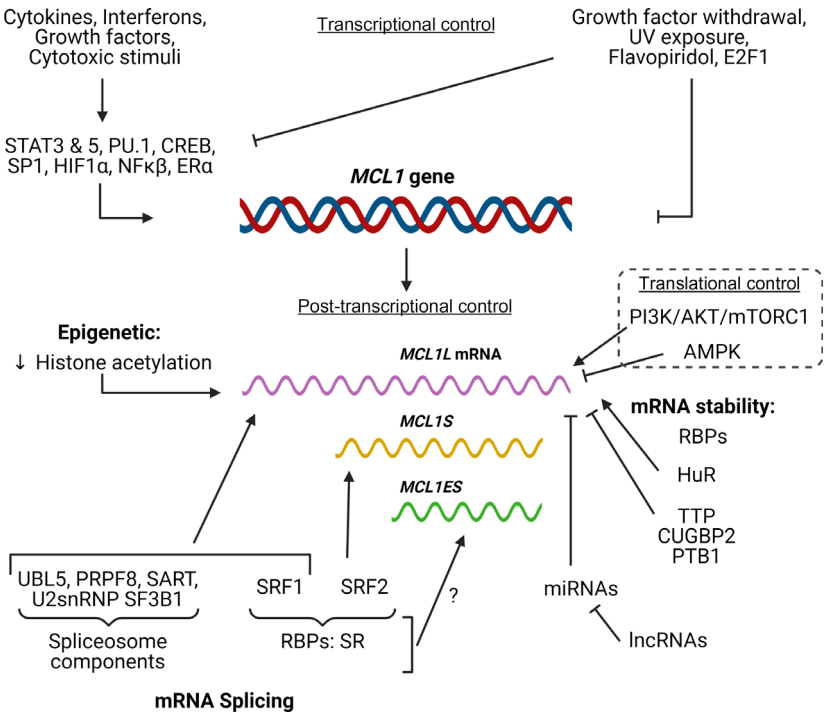


**Fig. 4.** Summary of the main regulators of MCL1 expression at the transcriptional, post-transcriptional, and translational level.

bacterial infection [35]. CUG triplet repeat, RNA-binding protein 2 (CUGBP2) binds to the GU-rich elements in the *MCL1* 3′ UTR to stabilize mRNA and inhibit its translation in colon cancer cells [36]. Finally, polypyrimidine tract-binding protein 1 (PTBP1) binds to CU-rich elements in the *MCL1* 3′ UTR to decrease expression in multiple cancer cell lines [37].

miRNAs, small noncoding RNAs (sncRNAs) of around 22 nucleotides, target the 3′ UTR region of mRNAs via base pairing to induce mRNA degradation or translational repression. There currently exist thirteen validated miRNAs that target *MCL1* (Table 1). While most miRNAs only target *MCL1*, *miR-125b*, *miR-133a*/*b*, and *miR-153* also target other BCL2 family members [38]. Long noncoding RNAs (lncRNAs), which are generally longer than 200 nucleotides, can regulate gene expression through diverse mechanisms, including the prevention of miRNA binding to their target mRNAs; therefore, lncRNAs can modulate *MCL1* mRNA stability. Twelve lncRNAs, including metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), antisense noncoding RNA in the INK4 locus (ANRIL), H19 imprinted maternally expressed transcript (H19) (Table 1), are currently understood to regulate *MCL1* expression [27].

Endoplasmic reticulum stress, ultraviolet light, elevated osmotic pressure, and arsenite treatment also decrease *MCL1* levels through the eukaryotic initiation factor 2 (eIF2)-mediated suppression of mRNA translation [39]. Metabolic sensors such as the mammalian target of rapamycin complex 1 (mTORC1) and AMP-activated protein kinase (AMPK) also modulate the synthesis of MCL1. *MCL1* mRNA is considered a 'weak mRNA' as its 5′ UTR possesses a robust secondary structure. Such mRNAs are translated through a regulated cap-dependent system controlled by the eIF4F protein complex, which, in turn, is regulated by mTORC1 and AMPK. While mTORC1 activity induces MCL1 synthesis [40], AMPK activation and mTORC1 inhibition following a block in glycolysis prompt a decrease in MCL1 synthesis [41].

**Table 1.** Summary of the main identified ncRNAs regulating MCL1.

| miRNAs | | lncRNAs | |
|---|---|---|---|
| Name | References | Name | References |
| miR-29 | [173] | MALAT1 | [174,175] |
| miR-30 | [176] | ANRIL | [177] |
| miR-101 | [178] | circHIPK3 | [179] |
| miR-125b | [180] | H19 | [181] |
| miR-133a | [182] | HULC | [183] |
| miR-133b | [184] | LINC00152 | [185,186] |
| miR-153 | [187] | MYOSLID | [188] |
| miR-181 | [189] | PMS2L2 | [190] |
| miR-193a | [191] | SNHG12 | [192] |
| miR-302b | [193] | | |
| miR-320 | [194] | | |
| miR-512 | [195] | | |

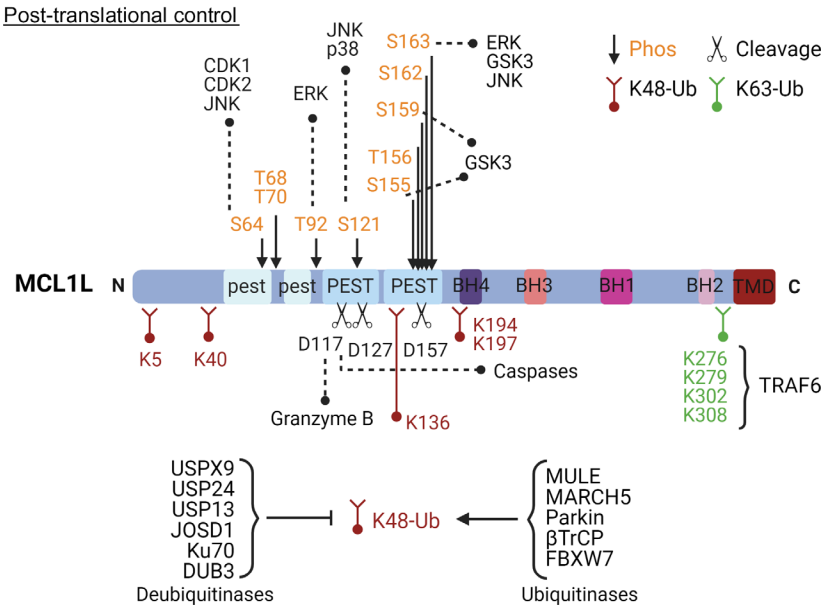


**Fig. 5.** Schematic diagram of MCL1 highlighting the main residues involved in protease cleavage (black), phosphorylation (orange), and ubiquitination regulation (red and green). The main proteins responsible for these post-translational modifications are also named in the figure.

Post-translational modifications such as cleavage, phosphorylation, and ubiquitination regulate the availability of MCL1, which is a short-lived protein (Fig. 5). MCL1 possesses two weak and two strong PEST motifs in the N-terminal region, which function as signals for degradation and induce rapid turnover. Thus, MCL1 becomes degraded by the proteasomal machinery by both ubiquitin-independent and ubiquitin-dependent pathways [42]. The unstructured N-terminal region of MCL1 promotes proteasome recognition and degradation in a ubiquitin-independent manner [43]; therefore, proteins interacting with this region may interfere with proteasome binding and contribute to MCL1 stabilization. While the proapoptotic *BH3-only* family members [13] and translationally controlled tumor protein (TCTP) [44] have been also identified as MCL1 modulators that function by regulating degradation, however, the mechanisms involved remain incompletely understood.

Ubiquitination plays a vital role in the turnover of MCL1—fourteen Lys (K) residues have been identified as putative ubiquitination sites, and several ubiquitin ligases and deubiquitinases can modulate MCL1 stability. The BH3 domain of MULE, a K48 ubiquitin ligase that polyubiquitinates and targets MCL1 for degradation [45], interacts with the MCL1 BH3 groove and competes with the binding of other *BH3-only* proteins. Knockdown of membrane-associated RING-CH protein 5 (MARCH5) reduces degradation of MCL1; however, whether this occurs through a direct ubiquitination-associated mechanism remains unclear. The E3 ubiquitin-protein ligase parkin (Parkin) also directly ubiquitinates MCL1, as do the E3 ligases beta transducing-containing protein (β-TrCP) and F-Box and WD repeat domain containing 7 (FBXW7) polyubiquitinate MCL1, although they require the previous phosphorylation of MCL1 by glycogen synthase kinase 3 (GSK3) to mediate recognition [46]. The K63 ubiquitin ligase tumor necrosis factor receptor-associated factor 6 (TRAF6) also regulates MCL1 by preventing its interaction with the proteasome. Of note, all Lys residues subjected to K63 ubiquitination localize to the C-terminal region of the protein [47]. Several deubiquitinases, including ubiquitin-specific peptidase 9 X-linked (USP9X), ubiquitin carboxyl-terminal hydrolase 13 (USP13), ubiquitin carboxyl-terminal hydrolase 24 (USP24), ubiquitin carboxyl-terminal hydrolase 17 (DUB3), Josephin domain containing 1 (JOSD1), and X-ray repair cross-complementing protein 6 (Ku70), also play roles in controlling MCL1 ubiquitination [27]; however, we require additional studies to fully understand how the interplay between E3 ligases and deubiquitinases regulates MCL1 levels and function.

The phosphorylation of MCL1, the most common post-translational modification, usually implies protein degradation, although the role of all phosphorylated residues has yet to be experimentally confirmed [27,48]. The N-terminal region of MCL1 contains ten putative phosphorylation sites (Fig. 5) that can be modified in a cell context-dependent manner by a range of kinases to produce site-specific outputs. S64 phosphorylation by cyclin-dependent kinase 1 and 2 (CDK1 and 2) and c-Jun N-terminal kinase (JNK) occurs during the G2/M phase of the cell cycle;


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however, these modifications fail to impact MCL1 half-life [49]. Extracellular signal-regulated kinase (ERK)-mediated phosphorylation of T92 stabilizes the interaction of MCL1 with peptidyl-prolyl cis/trans isomerase 1 (PIN1), blocks the association of MCL1 with protein phosphatase 2A (PP2A), and precedes phosphorylation of other residues (such as S121, S159, and T163). Overall, T92 phosphorylation is considered a priming signal for the subsequent phosphorylation and degradation of MCL1 [50]. Phosphorylation of T163 by ERK results in the increased stability and antiapoptotic activity of MCL1; however, phosphorylation of T163 combined with the phosphorylation of T92, S121, and S159 targets MCL1 for ubiquitination and proteasomal degradation.

Finally, MCL1 proteolysis and cleavage also occur during apoptosis. Several groups have identified D127, D157, and D117 as cleavage sites in the N terminus of the protein for executioner caspases and Granzyme B (Fig. 5). Although the function of the resultant fragments remains under investigation, MCL1 cleavage impedes interactions with other members of the BCL2 family and compromises antiapoptotic activity [51–53].

## The survival role of MCL1 in apoptosis

As an antiapoptotic BCL2 family member, MCL1 promotes cell survival by interfering in the cascade of the events that cause MOMP and trigger cell death. Under steady-state conditions, the proapoptotic BAX protein remains in an inactive state in the cytosol, with the C-terminal domain sequestered within a hydrophobic surface groove [54]. Therefore, antiapoptotic proteins perform two complementary prosurvival functions: binding and neutralizing the *BH3-only* 'sensor' proteins and inhibiting BAX and BAK effector proteins. When cells sense proapoptotic stimuli, the upregulation of *BH3-only* proteins produces changes in interaction equilibria among BCL2 family members. The insertion of *BH3-only* proteins into the hydrophobic groove formed by the BH1, BH2, and BH3 domains of antiapoptotic members [12,55–57] decreases the proportion of prosurvival proteins available to inhibit BAX/BAK activity. Moreover, *BH3-only* proteins interact directly with and activate BAX and BAK [58]. During activation, the C-terminal hydrophobic domain of BAX becomes released and interacts with the membrane to promote BAX and BAK proteolipid pore formation to induce MOMP, apoptogenic factor release, apoptosome formation, caspase-3 activation, and apoptosis.

MCL1 prevents cell death by inhibiting the oligomerization of the BAX and BAK effector proteins; thus, MCL1 degradation prompts increased BAK and BAX activity [59]. While studies have established the existence of a direct MCL1–BAK interaction under various conditions, any direct interaction between MCL1 and BAX remains unreported or undetectable [59–61]. Supporting the relevance of MCL1–BAK interactions, Moulding *et al.* [62] reported that apoptosis induced by the specific loss of MCL1 expression occurred alongside an increase in BAK expression; however, BAX expression remained unaltered. Additionally, ABT-737 (a BCL2 and BCL-xL inhibitor) triggers apoptosis in BAK-null HCT116 cells; however, BAX-null HCT116 cells displayed ABT-737 resistance [63]. BAX and BAK are regulated differently. While BAX is mainly cytosolic in healthy cells and needs to go through conformational changes to localize to the mitochondria, as explained above, BAK resides inactive on the outer membrane of mitochondria thanks to a double negative regulation mechanism. BAK BH3 domain binds the hydrophobic groove of MCL1 and also the groove of BCL-xL. Release of BAK from both proteins is required for apoptosis [59]. Thus, inhibition of both mechanisms has to be considered for therapeutic modulation of cell death [64].

MCL1 also interacts with BOK [65,66], another proapoptotic effector of the BCL2 family. Hsu *et al.* [65] described physical interactions between MCL1 and BOK using double hybrid experiments, while more recent studies confirmed this interaction by coimmunoprecipitation [67]. Significantly, MCL1 interacts with BOK in a unique manner from the other BCL2 family members, using transmembrane fragment interactions [68] to afford control over BOK-induced apoptosis [67].

MCL1 also associates with the PUMA, BIM, and NOXA *BH3-only* 'sensor' proteins [13,69]. Analysis using truncated proteins reported the interaction of MCL1 with BID and BMF, although with significantly lower affinity [70,71] (Fig. 6). Of note, binding affinity estimations using soluble proteins fail to consider that interactions generally occur within mitochondrial membranes, thereby suggesting the careful consideration of these results. PUMA, BIM, and NOXA interfere with the binding between MCL1 and BAK to promote apoptosis [59,64,72,73]. NOXA binds to MCL1 with a much higher affinity than other antiapoptotic BCL2 family proteins [70]. Additionally, unlike the rest of the *BH3-only* proteins, NOXA possesses a C-terminal region that interacts with the QRN motif in the MCL1 BH3 domain [13,21]. Therefore, this interaction destabilizes BAK and MCL1 binding and promotes MCL1 degradation. Gomez-Bougie

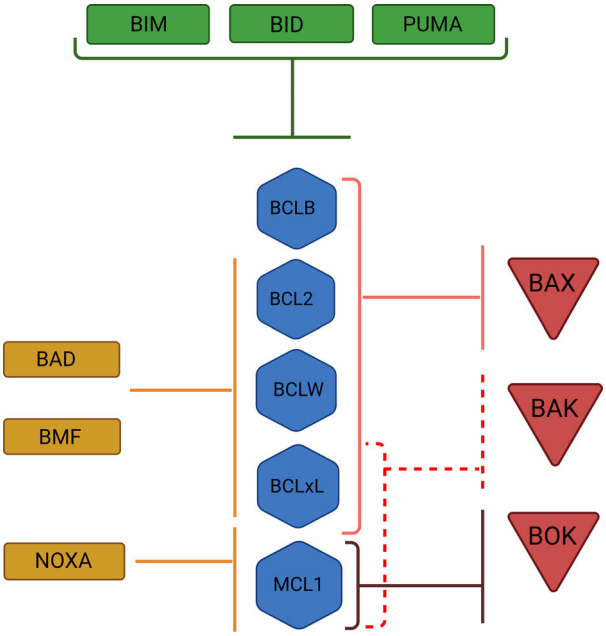


**Fig. 6.** Diagram representing interactions described among the members of the BCL2 family. Red triangles show proapoptotic members; blue hexagons: antiapoptotic group; green rectangles: *BH3-only* activators, and orange rectangles: *BH3-only* sensors.

*et al.* [74] reported that the USP9X deubiquitinase and MULE ubiquitinase participate in this process, although the precise mechanism involved requires further investigation.

Most studies of the interactions between MCL1 and other BCL2 family members have been conducted with protein fragments and a membranous environment. Efforts made with other members of the family, such as BCL-xL, to understand membrane interactions [75] may help to better understand the role of MCL1 in the regulation of MOMP and, in particular, the relevance of its interactions with BOK. This knowledge may be especially relevant, as we now appreciate that TMDs of these proteins, far from having a passive role, intervene in those interactions that modulate apoptosis [67,76,77].

## The nonapoptotic functions of MCL1

Other than apoptosis, MCL1 also plays controlling roles in processes such as embryonic development, mitochondrial homeostasis, mitochondrial bioenergetics, autophagy, cell cycle control, and senescence.

### MCL1 in embryonic development

Rinkenberger *et al.* [78] first revealed the fundamental role of MCL1 in mouse embryonic development by demonstrating preimplantation lethality following MCL1 knockout. MCL1-null blastocysts failed to display any evidence of apoptosis; however, their delayed maturation and lack of cell culture adhesion indicated a defect in trophectoderm development. Furthermore, the survival of neural precursor cells (NPCs) decreased dramatically in MCL1 conditional knockout embryos, suggesting a role for MCL1 in developmental neurogenesis [79]. Finally, Fogarty *et al.* established the dependence of NPCs on MCL1 as neurogenesis commences and then on BCL-xL as neurogenesis proceeds (Table 2). Although this role of MCL1 has been considered independent of its apoptotic function, no strict evidence has been published. Studies showing whether the loss of MCL1 leads to embryonic lethality in the absence of BAX/BAK will be needed for further understanding.

In relation to MCL1 functions during development, studies with knockin and conditional knockout mouse models have also demonstrated the requirement of MCL1 expression for medullary epithelial cell survival, maintenance of thymic architecture, and positive selection in thymocyte differentiation during development [80] (Table 2). The elevated level of MCL1 expression in long-term hematopoietic stem cells (HSCs) and its subsequent decline in HSC-derived progenitor populations suggested that the antiapoptotic function of MCL1 may play a significant role during the earliest stages of hematopoiesis [81]. Interestingly, the conditional knockout of MCL1 in a mouse model resulted in rapid, fatal, multilineage hematopoietic failure [82–85] (Table 2).

### MCL1 in mitochondrial homeostasis and bioenergetics

The expression of MCL1 increases during the generation of induced pluripotent stem cells (iPSCs), with MCL1 localizing to the mitochondrial matrix [86]. As MCL1 interacts with mitochondrial fission and fusion regulators, dynamin-related protein 1 (DRP1), and OPA1 mitochondrial dynamin-like GTPase (OPA1) (see more below), the regulation of mitochondrial dynamics through MCL1 may help to maintain stem cell pluripotency. Mitochondria lacking MCL1 exhibit alterations in the tubular mitochondrial network, increased reactive oxygen species (ROS) production, defective cristae structure, lower respiratory chain efficiency, and decreased ATP levels and oxygen consumption rate [87]. These observations provide further evidence for the role of MCL1 in the regulation of mitochondrial homeostasis and bioenergetics.

The translocation of MCL1 between the OMM and the mitochondrial matrix [87] occurs through the


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**Table 2.** Main identified animal models and their application in the study of MCL1.

| Model type | | Application | | Reference |
|---|---|---|---|---|
| Knockout MCL1 | Absolute $KO^{-/-}$ | Development/ Hematopoiesis | Survival of stem cells | [78] |
| | Conditional $KO^{-/-}$ tissue-specific | Hematopoiesis | Maintenance of lymphocytes | [85] |
| | | | Survival of hematopoietic lineages | [82,196] |
| | | | Megakaryocytic lineage | [83] |
| | | Development | Neurogenesis | [79] |
| | | Pathology | Chronic liver disease and liver tumorigenesis | [121–123,197–200] |
| | | | Breast cancer | [201] |
| | | | Pancreatic β-cell survival | [120] |
| | | | Neutrophil regulator in cerebral stroke | [118] |
| | Conditional $KO^{-/-}$ tissue-specific inducible | Hematopoiesis | Survival of stem cells | [84] |
| | | | Survival plasma cells | [202] |
| | | Pathology | Acute myeloid leukemia | [203] |
| | | | Liver disease | [204] |
| | | | Mitochondrial homeostasis and heart disease | [95,205] |
| | Conditional $KO^{+/-}$ tissue-specific inducible | Hematopoiesis | Activated B-cell survival and B-cell memory | [206,207] |
| | | Pathology | Lymphomas | [208] |
| | | | Neurodegeneration | [209] |
| | | | Neutrophil regulator in cerebral stroke | [118] |
| Knockin MCL1 | KI human MCL1 | Comparative biology | Orthologous proteins | [210,211] |
| | KI humanized MCL1 mice | Pharmacological role | Drug efficacy | [158] |
| | | | Breast cancer | [159] |
| | | Pathology | Allergy and inflammation | [119] |
| | KI degradation resistant | Pharmacological role | Colon cancer | [212] |

activity of a translocase (TIM/TOM) complex from the outer/inner membrane that involves the proteolytic cleavage of the first 50–80 aa, creating a truncated N-terminal form [87–89]. This 'matrix form' of MCL1 promotes oligomerization of the F1 and F0 domains of ATP synthase; however, MCL1 depletion alters ATP synthase oligomerization resulting in a disorganized cristae membrane, reduced ATP levels, lower mitochondrial membrane potential, and elevated ROS production [87]. However, the interaction between MCL1 and ATPase subunits (direct/indirect) remains undefined.

The matrix form of MCL1 also directly interacts with very-long-chain acyl-CoA dehydrogenase (VLCAD), an essential enzyme of the mitochondrial fatty acid β-oxidation pathway, suggesting a functional role for MCL1 in lipid metabolism. The MCL1–VLCAD complex prevents hyperactivity of the fatty acid β-oxidation pathway, maintains normal acetyl-CoA levels, and optimizes substrate consumption. Furthermore, MCL1 interacts with VLCAD in a non-canonical conformation that exposes the MCL1 BH3 helix [90].

As mentioned previously, MCL1 also interacts with mitochondrial proteins related to mitochondrial dynamics and remodeling networks such as DRP1 and OPA1. Thus, MCL1 modulates mitochondrial fission at the OMM and fusion at the IMM [86]. OPA1 locates to the inner mitochondrial membrane (IMM) and regulates mitochondrial fusion and oxidative phosphorylation, while MCL1 stabilizes and maintains OPA1 function. DRP1-mediated mitochondrial fission in response to stress can be protective or detrimental [91,92]. MCL1 exercises antiapoptotic activity within the OMM by inhibiting BAK activation to prevent MOMP and by inducing recruitment of DRP1 to the mitochondria. Thus, the MCL1–DRP1 interaction may allow cells to adapt to stress and induce survival independent of its antiapoptotic role [9,93]. As the MCL1 BH3-binding groove mediates OPA1 and DRP1 interactions, small-molecule inhibitors directed against the canonical MCL1 BH3-binding groove interfere with DRP1–MCL1 and OPA1–MCL1 interactions without influencing fatty acid oxidation [86,90].

Deficiencies in the respiratory chain and ATP production together with increased ROS production could explain the cardiotoxic phenotype observed following MCL1 deletion in cell and animal models [48,94]. While cardiotoxicity represents an important consideration when designing MCL1 inhibitors, the selective targeting of MCL1 (and leaving other proteins of the BCL2 family, e.g., BCL-xL, unaffected) may improve drug tolerability in cardiomyocytes [95] (Table 2).

In agreement with a role in bioenergetics, MCL1 tightly regulates $Ca^{2+}$ flux. MCL1 interacts with the inositol trisphosphate receptor (IP3R) in the ER through its C-terminal region with the same affinity as BCL2 and BCL-xL and increases the release of $Ca^{2+}$ from the ER to the mitochondria at a rate that improves mitochondrial bioenergetics and inhibits apoptosis [96]. Furthermore, overexpression of the proapoptotic MCL1S isoform prompts mitochondrial hyperpolarization, increased mitochondrial $Ca^{2+}$ accumulation, and apoptosis due to DRP1-dependent mitochondrial hyperfusion [9]. Thus, both MCL1 protein content and MCL1L/MCL1S isoform ratio modulate $Ca^{2+}$ and mitochondrial network homeostasis.

MCL1 also binds to voltage-gated anion channel protein (VDAC) in the mitochondria to stimulate $Ca^{2+}$ uptake and, as a consequence, increase ROS generation [97]. Interestingly, $Ca^{2+}$-dependent ROS production promotes lung cancer cell migration without affecting cell proliferation [97]. Furthermore, studies reported that ROS production depends on the MCL1–VDAC interaction, as VDAC-based inhibitor peptides decrease ROS production in cells expressing MCL1; however, MCL1 knockdown failed to induce an effect. These findings are controversial when considering the previously discussed evidence regarding how the MCL1 matrix form impacts ROS production [87]. As there exists evidence that VDAC and IP3R act on the same signaling pathway [98], further investigations will be needed to clarify the connections between IP3R, VDAC, and the OMM- and matrix-forms of MCL1 forms.

In addition to this, Cen *et al.* [99,100] described recently the interaction of MCL1 with microtubule-associated 1A/1B light chain 3A (LC3A) protein to promote mitophagy. The chemical inhibition of MCL1–BAK interaction releases MCL1 allowing MCL1 to interact with LC3A. Interestingly, authors showed that mitophagy could be a potential target for neurodegenerative diseases and in particular, in Alzheimer's disease (AD). In AD, the impaired mitochondria trigger energetic stress, calcium imbalance, and accumulation of amyloid-β deposits in the brains of AD mouse models. Mitophagy is also compromised in AD and contributes to regulation of named mitochondrial alterations. Further studies are needed to understand the whole picture of MCL1 controlling mitochondria homeostasis.

## MCL1 in autophagy

Autophagy, the process of degradation and recycling of intracellular content, intervenes in modulating the equilibria between cell survival or death. The role of MCL1 in autophagy depends on the cellular context. Several studies have described MCL1-driven inhibition of autophagy through the binding of the BECLIN1 BH3 motif to the BH3 binding groove of MCL1 and the inhibition of autophagosome formation [101]. The ER and mitochondria both provide the lipids required for autophagosome formation [102]; therefore, MCL1 may regulate the mitochondrial pathway of autophagosome formation with evidence for this pathway existing in neurons [103]. The MCL1–BECLIN1 interaction may also explain why the oxidative stress-triggered increase in BOK expression leads to induction of autophagy in preeclamptic placentas [104]. Increased BOK protein levels can sequester MCL1, thereby releasing BECLIN1 to promote autophagy.

A second explanation for MCL1-driven autophagy inhibition arises from studies that established how MCL1 downregulation leads to increased BECLIN1 levels and the induction of basal autophagy. MCL1 and BECLIN1 compete to bind to the same region of the USP9X deubiquitinase; therefore, the displacement of BECLIN1 by MCL1 could induce the consequent increase in ubiquitination and proteasomal degradation. The inverse correlation of these proteins supports the development of certain tumors, given that levels of BECLIN1 decrease and MCL1 increase during tumor progression [105]. However, David Vaux work showed that MCL1, among others antiapoptotic proteins, inhibits autophagy pathway by affecting BAX/BAK levels, suggesting an indirect role. In a cellular context where BAX and BAK are not present, inhibition of MCL1 does not increase autophagy rates [106]. In addition, MCL1 exerts a proautophagic function in specific cellular contexts. Thomas *et al.* [48] reported that MCL1 deletion impairs autophagy in cardiomyocytes, with mitochondrial dysfunction, decreased ATP production, and increased ROS production all observed. Thus, the role of MCL1 in autophagy remains controversial. MCL1 may also control apoptosis and autophagy in a context-dependent manner, according to cellular conditions and depending on the relative dominance of autophagic or proapoptotic factors.

## Nuclear roles of MCL1

MCL1 also localizes to the nucleus and plays prominent regulatory roles during various stages of the cell cycle. MCL1 accumulates during the cell cycle and reaches a peak around the late G2 phase [107], where it stimulates checkpoint kinase 1 (CHK1) phosphorylation, a regulator of DNA damage response [108]. As CHK1 phosphorylation mediates G2/M arrest, MCL1 plays a G2/M checkpoint protein role and provides time for DNA damage repair [26,109].


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A study by Jamil *et al.* detected the interaction between a short nuclear form of MCL1 with cyclin-dependent kinase 1 (CDK1), which inhibited kinase activity and cell cycle progression through G2/M phases. Of note, the MCL1–CDK1 association did not depend on interactions of CDK1 with cyclins [110]. Another study established that CDK1 binding to mitotic cyclin B induces the phosphorylation of MCL1 at T92, with phosphorylation and polyubiquitylation promoting MCL1 degradation and inducing apoptosis [107]. Interestingly, if MCL1 levels fall below a critical level as occurs in the mitotic arrest state, cell death occurs via the intrinsic apoptotic pathway [111]. Thus, the inhibition of CDK1 and CHK1 activities makes MCL1 a significant player at the G2/M checkpoint; however, the MCL1S pro-apoptotic isoform counteracts G2/M checkpoint function, accelerates cell cycle progression through mitosis, and promotes DNA damage accumulation [112]. MCL1 also interacts with the S phase cell cycle regulator proliferating cell nuclear antigen (PCNA) to inhibit cell cycle progression [25].

The interaction between MCL1 and cyclin-dependent kinase 4 inhibitor C (P18INK4C), an inhibitor of CDK4/6, promotes G1/S progression by avoiding the inhibitory activity of P18INK4C on CDK4/6. Consequently, CDK4/6 interacts with retinoblastoma (RB1) facilitating S phase entry [113]. MCL1 causes depletion of P18INK4C protein levels; therefore, the accumulation of cells in G2/M after MCL1 overexpression may represent a consequence of increased cell cycle entry promoted by MCL1–P18INK4C interactions. Intriguingly, MCL1 interacts with cyclin-dependent kinase inhibitor 1B (CDKN1B) in the nervous system to control cell cycle progression through the G1 phase to promote the differentiation and survival of NPCs [114].

Widden *et al.* also described the interaction of MCL1 with p73, which plays a role in modulating the expression of target genes associated with the DNA damage response and cell cycle progression [115]. p73 directly interacts with the MCL1 BH3-binding pocket in the nucleus to inhibit the DNA-binding ability of p73. Therefore, MCL1 promotes cell cycle progression by blocking the transcriptional activity of p73 [116].

Overall, MCL1 interacts with various cell cycle proteins to induce divergent outcomes; further research may disentangle the exact mechanisms by which MCL1 modifies cell cycle progression.

### Senescence

Finally, MCL1 inhibits chemotherapy-induced senescence and can enhance tumor growth independent of its apoptotic function. As described before, a domain located outside the classical BH regions mediates this senescence-inhibiting function, which could also be implicated in, for example, the ability of MCL1 to interfere with ROS production [22,23,117]. Overall, the MCL1-mediated inhibition of apoptosis and senescence through distinct molecular domains should be considered when evaluating current developing MCL1 inhibitors.

## MCL1-related pathologies

Given the wide range of cellular functions associated with MCL1, investigations into a range of pathological processes have also established crucial links to MCL1 dysregulation.

The relevance of MCL1 in heart disease has emerged as a significant concern regarding the use of MCL1 inhibitors in the clinic. In cardiomyocyte-based models, MCL1 deletion produces cardiotoxicity, thereby decreasing cell survival and impairing contractile function [94], a phenotype attributed to the roles of MCL1 in the control of mitochondrial homeostasis and autophagy. In agreement with in vitro cell-based findings, mice lacking cardiomyocyte-specific MCL1 expression exhibited signs of heart disease, manifested as a loss of cardiac contractility, defects in mitochondrial structure and mitochondrial respiration, and lower ATP production compared with wild-type [48,95].

MCL1 also plays a prominent role in the outcome of cerebrovascular disorders. For example, MCL1-deficient stroke model mice presented with less harmful lesions in the ischemic area with an almost complete absence of neutrophil recruitment compared with wild-type or heterozygous animals [118]. Therefore, this study provides evidence that small-molecule inhibitors of MCL1 may represent a possible therapeutic avenue for treating ischemic stroke in human patients. Additionally, Felton *et al.* [119] linked the overexpression of the human form of MCL1 in mice with the exacerbation of allergic airway inflammation, with increased cellularity of bronchoalveolar lavage fluid, eosinophil number, total protein, and airway mucus production observed.

Analysis in type I diabetes model has uncovered a pancreas-specific decrease in *MCL1* expression [120]. *MCL1* knockout mice did not display alterations to the development and function of pancreatic islets; however, the loss of *MCL1* expression did affect pancreatic β cells, making them more susceptible to apoptosis mediated by proinflammatory cytokines. β-cells from the pancreas of *MCL1* knockout mice also exhibited hyperglycemia and low pancreatic insulin content

after toxic stimuli. In conclusion, maintaining MCL1 homeostasis may represent an exciting strategy for treating type 1 diabetes patients [120].

Chronic liver disease patients suffer from an elevated risk of developing hepatocellular carcinoma, with epithelial cell apoptosis frequently observed in cirrhotic livers [121]. Hypothetically, the genetic depletion of *MCL1* may increase apoptosis and inhibit tumorigenesis; however, the livers of mice with a hepatocyte-specific knockout of MCL1 displayed increased mild tissue fibrosis, oxidative stress, and inflammatory cytokine release, all of which can support cancer development. The role of MCL1 in cell cycle progression, DNA repair, and ROS production may explain the described phenotype [121–123].

*MCL1* dysregulation occurs in many types of cancers and often correlates with poor prognosis and therapeutic resistance. In fact, genomic data analysis from The Cancer Genome Atlas shows high MCL1 protein expression in at least 22 cancer types [124]. Interestingly, *MCL1* is located in a focal amplification peak region of the chromosome 1qS with other eight genes that shows amplifications in 10.9% of cancers in multiple tissue types. This is considered a common mechanism for cancers, to increase cell survival that could be targeted. In particular, in lung cancer it has been shown that *MCL1* amplification is required for sustained survival and its inhibition delays tumor progression [124,125].

In colon adenoma and carcinoma patients [126], MCL1 protein levels directly correlate with tumor grade/stage and the presence of metastasis [127]. Interestingly, the MCL1 expression pattern correlates with responses to 5-fluorouracil (5-FU)—those patients with perinuclear MCL1 expression responded more frequently to treatment [128]. High MCL1 expression correlates with poor survival for patients with stage III ovarian carcinomas, with diffuse MCL1 expression correlating with advanced clinical stage, high histologic grade, and poor survival [129]. In gastric carcinoma patients, the detection of elevated MCL1 levels by tumor immunohistochemistry correlates with a significantly worse prognosis than patients lacking tumor MCL1 expression [130,131]. In multiple myeloma, *MCL1* mRNA and protein overexpression in patient samples correlated with disease severity and shorter event-free survival times [132]. The mechanisms that multiple myeloma cells employed to survive involved the stabilization of MCL1 by PP2A-mediated dephosphorylation [215]. High MCL1 levels appear at high frequency in non–small-cell lung cancer (NSCLC) cohorts, and retrospective studies have provided evidence of the significantly worse five-year survival rate in those patients with elevated tumor MCL1 expression [133]. Moreover, NSCLC patients with high MCL1 expression display a high cellular proliferation index and lower overall survival rates than patients with low MCL1 expression [134]. MCL1 expression also correlates with protein kinase B (AKT) activity in NSCLC tumors, with interactions between the MCL1 PEST domain and the pleckstrin homology domain of AKT forming at least part of the underlying mechanism. This interaction leaves the AKT kinase site in an active conformation, thereby promoting cell survival [135]. Interestingly, the MCL1 deletion or inhibition of MCL1 activity (with the MCL1 antagonist S63845) caused a significant reduction in tumorigenesis [124]. MCL1 overexpression also occurs in metastatic malignant melanoma [136]; antisense therapies, gene silencing, or inhibitor administration alone or in combination with other chemotherapeutic agents significantly decreased tumor growth [137–140]. In breast cancer patients, MCL1 high expression correlates with poor prognosis, high tumor grade, and poor survival [141–143]. As EGF or ER activation upregulates *MCL1* expression [144–146], targeting MCL1 represents the mechanism of action for many breast cancer-targeted drugs [147–150]. In the aggressive triple-negative breast cancer (TNBC) subtype, inhibiting MCL1 sensitized breast cancer cells to apoptosis by conventional chemotherapy [142,151].

By contrast, the hepatocyte-specific loss of MCL1 increased the frequency of liver tumors [152]. This unexpected response depended on the apoptotic function of MCL1, as elimination of BAK decreased tumorigenicity. The increased frequency of tumors appears to correlate with excessive apoptosis in MCL1-deficient livers, which prompted higher levels of TNF-α production and increased oxidative stress to sustain a protumorigenic liver environment [121]. Induced hepatocyte compensatory liver regeneration as a consequence of excessive apoptosis contributes to liver carcinogenesis [123]; however, liver cancer patients exhibited MCL1 overexpression [153] and studies have demonstrated that specifically inhibiting MCL1 reduces the survival of hepatocellular cancer stem-like cells [154]. Therefore, extensive liver cancer studies with novel specific MCL1 inhibitors may support the design of appropriate pharmacological strategies that will eliminate cancer cells but inhibit detrimental hepatic regeneration mechanisms to improve patient outcomes.

In summary, MCL1 participates in cancer progression, malignancy, and therapeutic resistance in several tumor types. Therefore, the clinical development of specific inhibitors will provide an opportunity


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to understand tumor biology better and, more importantly, provide patients with new therapeutic strategies.

## Therapeutical strategies: MCL1 inhibitors in clinical trials

The critical role of MCL1 in different pathological scenarios has driven the search for pharmacological modulators of MCL1 function. Accordingly, the pharmaceutical industry has focused its attention on the prosurvival effect of MCL1 expression in tumor cells and its involvement in chemotherapy resistance, which has led to the discovery and clinical development of selective MCL1 inhibitors that target the BH3 binding groove and modulate interactions with other BCL2 members.

We now summarize the small molecules designed to neutralize the antiapoptotic activity of MCL1. Table 3 summarizes the small-molecule inhibitors currently under clinical evaluation.

### S64315/MIK665

Structure-guided optimization of S63845, a previously reported small-molecule MCL1 antagonist [155], led to the development of S64315/MIK665, a potent and selective MCL1 inhibitor. Structural studies of S64315/MIK665 complexed with MCL1 revealed that the molecule fills the P2 pocket and the P4-P5 region of MCL1 [156]. Compared with its predecessor, S64315/MIK665 demonstrated improved affinity and cell-based activity. S64315/MIK665 presented low-affinity binding for BCL2 and BCL-xL (58 and 237 μM, respectively) [157] and displaced BAX and BAK proteins (but not their antiapoptotic relatives) from MCL1 [156]. As for other MCL1 inhibitors, S64315/MIK665 increased MCL1 protein accumulation in a dose-dependent manner and induced BAX/BAK-mediated apoptosis in tumor cells. Hematological cancer-derived cell lines displayed high susceptibility ($IC_{50}$ < 100 nM) to S64315/MIK665, while intravenous dosing of S64315/MIK665 for five consecutive days elicited dose-dependent antitumor activity in a multiple myeloma (MM) xenograft model. S64315/MIK665 also induced similar tumor growth inhibition in animal models treated once a week or treated daily for four weeks, highlighting the safety and tolerability of this drug.

Given that S63845 displayed a higher affinity to human MCL1 than mouse MCL1, Brennan *et al.* [158] evaluated efficacy and tolerability in a mouse model expressing human MCL1. Engineered mice displayed greater sensitivity to S63845 treatment (as evidenced by its ability to inhibit tumorigenesis) than wild-type mice but did prompt the transient reduction in B cells in the blood, spleen, and bone marrow. In addition, the study also noted a nonsignificant reduction in neutrophil levels in the bone marrow [158].

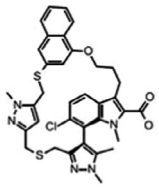
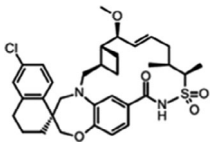
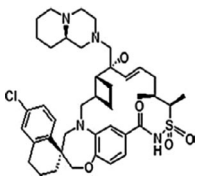
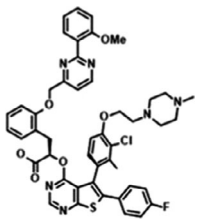
Based on its favorable preclinical evaluation, intravenous S64315/MIK665 has entered clinical trials to treat relapsed and/or refractory MM, lymphoma, acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS) (NCT02979366 and NCT02992483). In addition, S64315/MIK665 combined with Venetoclax is also under evaluation for AML treatment (NCT03672695).

### AMG-176 and AMG-397

Amgen optimized a series of spiromacrocyclic molecules guided by a small-molecule conformational restriction strategy that led to the development of AMG-176 [159]. AMG-176 (and the analog AM-8621) have a picomolar affinity for human MCL1. AM-8621 has been used as a molecular tool to characterize the molecular mechanism of AMG-176 in cellular assays. AM-8621 disrupted MCL1 interactions with BAK and BIM and induced both MCL1 stabilization and BAX/BAK-dependent apoptosis. Hematologic cancer cell lines displayed greater sensitivity to AM-8621 than solid tumor lines, with the most significant sensitivity displayed by MM, AML, B-cell lymphoma, and subsets of acute lymphocytic leukemia and Burkitt lymphoma cell lines. Breast cancer cells displayed the most significant sensitivity among solid tumor cell lines. In OPM-2 mouse xenografts (a model of MM), oral administration of AMG-176 activated BAK, cleaved caspase-3 and poly (ADP-ribose) polymerase (PARP), and induced apoptosis and tumor regression.

This study also reported a synergistic effect of AMG-176 with Venetoclax in an AML orthotopic model, where twice-weekly AMG-176 and daily Venetoclax treatments prompted tumor inhibition and regression. Mice carrying human MCL1 were also used to evaluate AMG-176 efficacy and tolerability. The dosing regimen of AMG-176 required for tumor growth inhibition resulted in reduced numbers of B cells, monocytes, and neutrophils in blood and bone marrow; however, AMG-176 failed to induce systemic toxicity based on body weight change. In the orthotopic AML model, combined MCL1 and BCL2 inhibition by AMG-176 and Venetoclax were well tolerated, and no overt toxicity was determined; however, the study did note a decrease in peripheral blood B cells and monocytes.

**Table 3.** Summary of MCL1 inhibitors under clinical trials.

| Compound, chemical structure, and institution | Potency (TR-FRET) | Clinical trials[a] | Study | Administration | Indication | References |
|---|---|---|---|---|---|---|
|  AZD5991 AstraZeneca | MCL1 $K_i$ = 0.2 nM<br>BCL2 $K_i$ = 6.8 μM<br>BCL-xL $K_i$ = 18 μM<br>BCLW $K_i$ = 25 μM<br>BFL1 $K_i$ = 12 μM | NCT03218683 | Phase I, monotherapy, and combination | IV | Refractory Hematological malignancies | [213] |
|  AMG-176 Amgen | MCL1 $K_i$ = 0.06 nM<br>BCL2 $K_i$ = 0.95 μM<br>BCL-xL $K_i$ = 0.7 μM | NCT02675452 | Phase I, monotherapy | IV | Relapsed or refractory MM/AML | [159] |
| | | NCT03797261 | Phase I, monotherapy with venetoclax | IV | AML/ Lymphoma | |
|  AMG-397 Amgen | Not disclosed | NCT03465540 | Phase I, monotherapy | Oral | MM/AML/NHL | [214] |
|  S64315/MIK665 Servier/Novartis | MCL1 $K_i$ = 1.2 nM | NCT02979366 | Phase I, monotherapy | IV | AML/MDS | [156] |
| | | NCT02992483 | Phase I, monotherapy | IV | MM/Lymphoma | |
| | | NCT03672695 | Phase I, combination with venetoclax | IV | AML/MDS | |
| | | NCT04629443 | Phase II, combination with azacitidine | IV | AML | |
| | | NCT04702425 | Phase I, combination with VOB560 | IV | NHL/AML/MM | |
| Structure not disclosed ABBV-467 AbbVie | Not disclosed | NCT04178902 | Phase I, monotherapy | IV | MM | Not disclosed |
| Structure not disclosed PRT1419 Prelude Therapeutics | Not disclosed | NCT04543305 | Phase I, monotherapy | Oral | Refractory Hematological malignancies | Not disclosed |
| | | NCT04837677 | Phase I, monotherapy | IV | Relapsed or refractory solid tumors | |

[a]From www.ClinicalTrials.gov.

Clinical evaluation of AMG-176 has been initiated in a phase I clinical trial (NCT02675452) in patients with relapsed or refractory MM and AML, and preliminary results have been disclosed [160]. Although the maximum tolerated dose was not reached for relapsed MM patients, predominant side effects included hematological (anemia and neutropenia) and gastrointestinal (nausea and diarrhea) problems.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Nevertheless, disease status remained stable in 11 out of 26 patients. Another phase I clinical trial is also currently evaluating AMG-176 in combination with Venetoclax in patients with AML and non-Hodgkin lymphoma (NCT03797261).

From the same molecular family as AMG-176, AMG-397 (the first oral MCL1 inhibitor used in the clinic) has been evaluated for the treatment of patients with MM, AML, and non-Hodgkin lymphoma (NCT03465540); however, the FDA halted these trials due to cardiac toxicity (ASHClinicalNews. FDA places trials of MCL1 inhibitor on clinical hold (2019). www.ashclinicalnews.org/news/latest-and-greatest/fda-places-trials-MCL1-inhibitor-clinical-hold/). As described previously, MCL1 may be essential for normal mitochondrial activity in cardiomyocytes [95]. The side effects that may relate to AMG-397 on-target activity may limit the therapeutic scope of AMG-397 (and that of other MCL1 inhibitors) [86].

### AZD5991

AstraZeneca researchers applied structure-based drug design to a series of previously reported indole-2-carboxylic acids (WO2008130970A1, WO2008131000A2, and Ref. [161]) to develop AZD5991, a selective and potent macrocyclic MCL1 inhibitor. AZD5991 selectively binds to MCL1 over other antiapoptotic proteins due to its specific interactions with and the flexibility of the MCL1 BH3-binding groove. Furthermore, as reported for other selective MCL1 inhibitors, AZD5991 stabilized MCL1 in a concentration-dependent manner, disrupted MCL1–BAK interactions, and induced apoptosis in a BAK-dependent manner.

AZD5991 preferentially induced cell death in hematological cell lines and subsets of breast cancer and NSCLC lines. AZD5991 also promoted apoptosis in MM primary cells and mouse models. Results suggested the overall tolerability of a single intravenous infusion of AZD5991 (i.e., acceptable body weight changes) and demonstrated complete tumor regression at the highest doses. MCL1 inhibition with AZD5991 also exhibited a dose-dependent antitumor activity in mouse and rat AML models.

The combination of AZD5991 with the proteasome inhibitor bortezomib provided for synergistic antitumor activity in a MM mouse model. In addition, AZD5991 combined with Venetoclax also induced enhanced tumor growth inhibition in subcutaneous MM and AML xenografts.

AZD5991 also demonstrated potent antitumor activity and increased survival in MCL1-dependent T-cell lymphoma patient-derived xenografts in mice when combined with CHOP chemotherapy (cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone) [162].

The antitumor activity of AZD5991 has also been demonstrated in solid tumor models. AZD5991 restored sensitivity to regorafenib, a multi-kinase inhibitor for colorectal cancer treatment, during in vitro assays with colorectal carcinoma cell lines [163], while AZD5991 combined with BRAF inhibitors (BRAFi) or MEK inhibitors (MEKi) inhibited the clonogenic survival of melanoma cells. A synergistic effect of between BRAFi/MEKi with AZD5991 was also observed in melanoma animal models, including melanoma xenografts, BRAF-mutant patient-derived xenografts, and BRAFi- and/or MEKi-resistant melanomas. In addition, combination therapy induced tumor regression or total growth inhibition and overcame BRAFi- and MEKi-acquired resistance [138].

AZD5991 was selected as a clinical candidate and is currently under evaluation in a phase I clinical trial as a monotherapy for relapsed/refractory chronic lymphocytic leukemia, AML/myelodysplastic syndromes, and MM patients. In addition, a combination therapy with Venetoclax is also being evaluated in patients with relapsed/refractory AML/MDS (NCT03218683).

Two undisclosed MCL1 inhibitors (PRT1419 and ABBV-467) have also entered clinical trials. Prelude Therapeutics employed a structure-based design to identify PRT1419, a selective and potent oral MCL1 inhibitor. Once-weekly dosing of PRT1419 prompted tumor regression in mouse models of MM and AML, and diffuse large B-cell lymphoma in preclinical studies. Moreover, mouse models display enhanced antitumor activity in combination with Venetoclax in a model of AML. PRT1419 is currently in a phase I clinical trial to treat relapsed/refractory hematologic malignancies (NCT04543305). In addition, AbbVie has also started a phase I clinical trial with ABBV-467 as monotherapy for MM patients (NCT04178902).

## MCL1 therapeutics under development

The Fesik group recently reported on the development of two MCL1 inhibitors: VU661013, which has a picomolar affinity for MCL1 with no detectable binding for BCL-xL and BCL2, and Compound **42** [164]. VU661013 demonstrated potent cytotoxic activity in AML cell lines, Venetoclax-resistant AML cells, and AML patient-derived xenograft models. Furthermore, the combination of Venetoclax with VU661013 further enhanced antitumor activity in murine models of AML [164]. Meanwhile, Compound **42** potently

inhibited MCL1 activity and demonstrated in vivo efficacy in xenograft models derived from hematologic and TNBC cell lines [165].

Alternative approaches have also been developed to target the antiapoptotic function of MCL1. For example, complex 14, a copper (II) complex containing β-carboline ligands, disrupted MCL1 binding to BAX and BAK, induced BAX/BAK-dependent apoptosis, and inhibited tumor growth in NSCLC xenografts [166].

The downregulation of *MCL1* gene expression also represents a promising therapeutic strategy, with flavonoids the most well-known regulators of MCL1 transcription. Certain flavonoids inhibit CDKs, which ultimately leads to decreased levels of short-lived proteins such as MCL1. As CDK9 inhibitors, Voruciclib and AZD4573 have proven antitumor activity through indirect MCL1 suppression in hematologic cancer models [167,168].

Other chemical compounds achieve MCL1 inhibition by blocking mRNA translation. For instance, Norcantharidin upregulated miR-320d, a negative regulator of MCL1 expression, and induced apoptosis in prostate cancer cells [169]. In addition, simultaneous silencing of MCL1 and the efflux pump P-glycoprotein by two specific short interfering RNA (siRNAs) in doxorubicin-resistant breast cancer cells efficiently reduced protein levels and induced significant levels of apoptosis [170].

Proteolysis-targeting chimeras (PROTACs) represent a powerful technology that can increase the proteasomal degradation of MCL1. PROTACs are engineered bifunctional molecules comprising two components: an MCL1-binding ligand and an effector ligand that recruits an E3 ubiquitin ligase to trigger proteasomal degradation. A first example employed the A1210477 MCL1 inhibitor as a target ligand and 4-hydroxythalidomide as an effector ligand to recruit to the E3 ligase cereblon (CRBN) [171], while a second example linked another MCL1 inhibitor to the cereblon–ligand pomalidomide. Both PROTACs induced MCL1 degradation at nanomolar concentrations and induced apoptosis [171].

Finally, cell-penetrating 'Alphabodies', single-chain polypeptides featuring antiparallel triple-helix coiled-coil fold, have been developed to target MCL1. The beta-helix of the CMPX-321A and CMPX-383B Alphabodies bind to the MCL1 BH3 groove to induce selective high-affinity MCL1 inhibition. As a result, CMPX-321A displayed cell-penetrating capacity and induced BAK-mediated cell death in MCL1-dependent cancer cell lines, while CMPX-383B possessed an extended serum half-life and exhibited a potent antitumor activity that reduced tumor burden in MM xenograft mice [172].

While most of the described strategies targeting MCL1 modulate its antiapoptotic function, there remains the potential to compromise the nonapoptotic functions of MCL1, which remain poorly understood. This risk makes it essential to describe the specific interaction sites involved in MCL1's various roles, design selective MCL1 inhibitors against these interaction sites, and inhibit a specific function of MCL1 without affecting the others, thus preventing potential toxicities related to the unspecific targeting. Deciphering the interacting domains that MCL1 utilizes to execute its prosurvival and nonapoptotic roles represents an important future direction and would allow the development of safe and effective MCL1 inhibitors and the successful clinical translation of anti-MCL1 therapies.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

All authors wrote, edited, and revised the manuscript.

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