

Perspective

Therapeutic Potential of Micheliolide and ACT001 in Age-Related Diseases: Molecular Mechanisms and Clinical Prospects

Hui Xu^{1,2}, Xin Cong^{2*}, Lian-Jun Lin^{1*}

¹Department of Geriatrics, Peking University First Hospital, Beijing 100034, China. ²Department of Physiology and Pathophysiology, Peking University School of Basic Medical Sciences, State Key Laboratory of Vascular Homeostasis and Remodeling, Beijing 100191, China.

[Received December 21, 2025; Revised April 3, 2026; Accepted April 20, 2026]

ABSTRACT: Aging is the predominant risk factor for several chronic disorders; therefore, elucidating the molecular and cellular mechanisms underlying age-related pathologies is essential for therapeutic advancement. Micheliolide (MCL), a naturally occurring guaianolide sesquiterpene lactone derived from *Michelia champaca* and *Michelia compressa*, has attracted increasing attention for its pharmacological potential. Its derivative, ACT001, enables the sustained release of MCL into the plasma *in vivo*, thereby enhancing oral bioavailability and improving therapeutic efficacy. ACT001 has recently received orphan drug designation for glioblastoma treatment, highlighting its clinical feasibility and safety profile. Increasing evidence indicates that MCL regulates age-related diseases by modulating inflammation, cell cycle, mitochondrial dysfunction, oxidative stress, and autophagy. By targeting key signaling pathways, including NF- κ B, STAT3, NRF2, AMPK, and NLRP3, MCL/ACT001 demonstrated its protective effect against age-related diseases. This paper summarizes the current mechanistic insight and disease-specific evidence regarding MCL/ACT001 and further evaluates their therapeutic repositioning potential for age-related diseases, including cardiovascular and cerebrovascular diseases, fibrotic conditions, immune disorders, metabolic diseases, and tumors. Additionally, we discussed key translational challenges.

Keywords: Micheliolide, ACT001, Sesquiterpenes, Inflammation, Oxidative stress, Age-related disease

1. Introduction

Aging is a complex, multifaceted biological process driven in part by the recognized hallmarks of aging, including cellular senescence, mitochondrial dysfunction, altered intercellular communication, telomere attrition, epigenetic alterations, stem cell exhaustion, deregulated nutrient sensing, chronic inflammation, loss of proteostasis, genomic instability, impaired macroautophagy, and dysbiosis [1, 2]. Cellular senescence refers to an irreversible state of cell cycle arrest and is implicated in diverse physiological processes and numerous age-related disorders, including cancer,

osteoporosis, fibrosis, atherosclerosis, Alzheimer's Disease (AD), and metabolic diseases. Notably, cell senescence has beneficial effects contributing to embryonic development, tumor suppression, fibrosis regression, and wound healing. However, prolonged cellular senescence can lead to harmful sequelae, including chronic inflammation, mitochondrial dysfunction, metabolic dysregulation, immune deficiencies, stem cell depletion, and other harmful effects that lead to homeostasis imbalances in various tissues, resulting in age-related pathologies and increased susceptibility to death [3]. By 2050, the global population aged 60 and above is projected to increase to 22%, reaching a staggering 2.1 billion [4]. Advanced age is one

*Correspondence should be addressed to: Dr. Xin Cong, Peking University School of Basic Medical Sciences, State Key Laboratory of Vascular Homeostasis and Remodeling, Beijing 100191, China. Email: congxin@bjmu.edu.cn; Lian-Jun Lin, Peking University First Hospital, Beijing, China. Email: 06474@pkufh.com

Copyright: © 2026 Xu H. et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

of the most critical risk determinants that render individuals susceptible to the onset and development of chronic pathologies. Over 90% of adults aged 65 years have at least one chronic disease, and > 70% have two or more comorbidities [5]. Age-related diseases impose a substantial burden on global public healthcare systems and economies worldwide. These realities highlight the critical need for developing innovative and effective therapeutic strategies aimed at preventing, delaying, or mitigating the wide spectrum of diseases associated with aging.

Micheliolide (MCL), a naturally occurring guaianolide sesquiterpene lactone, is isolated from *Michelia compressa* and *Michelia champaca* plants (Fig. 1A) [6]. Additionally, it can be synthesized from

parthenolide through chemical reactions (Fig. 1B) [7]. MCL was developed as a more stable analog of parthenolide, a compound with well-documented potent anti-tumor and anti-inflammatory activities, but limited by poor stability [8, 9]. Its derivative, DMAMCL, is synthesized through the formation of a dimethylamino Michael adduct of MCL. ACT001 represents the fumarate salt form of DMAMCL and is currently undergoing clinical evaluation (Fig. 1C). Upon administration *in vivo*, ACT001 acts as a prodrug that gradually releases MCL into the circulation, thereby enhancing oral bioavailability and therapeutic efficacy. Notably, ACT001 has received orphan drug designation for glioblastoma (GBM) treatment, highlighting its clinical feasibility and safety profile.

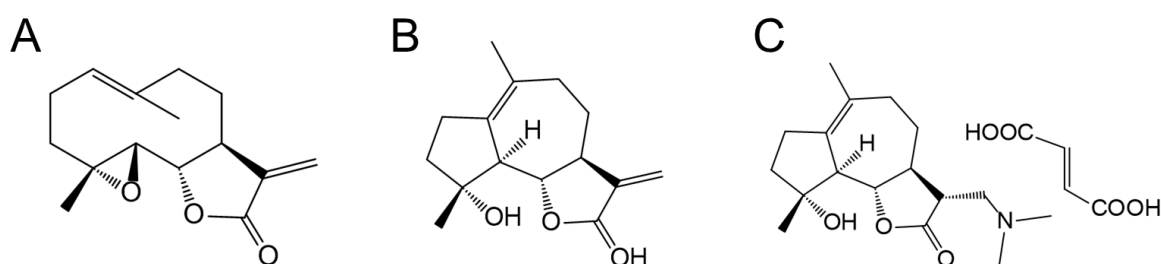


Figure 1. Chemical structures of the three compounds. (A) MCL structure. **(B)** Parthenolide structure. **(C)** ACT001 structure.

MCL and its derivative ACT001 exhibit diverse pharmacological activities, particularly in the modulation of inflammation, oxidative stress, and cell death [9-11]. The α -methylene- γ -lactone moiety in MCL is a core structural determinant of its biological bioactivity [8, 12]. Through alkylation of cysteine38 on the p65 subunit, MCL inhibits p65/nuclear factor- κ B (NF- κ B) binding to DNA, manifesting its antagonistic activity against NF- κ B [12]. Additionally, MCL activates nuclear factor erythroid 2-related factor 2 (NRF2) signaling, promoting antioxidant responses and enhancing cellular resistance to oxidative stress [11, 13]. *In vivo* studies further confirmed that MCL covalently binds to Cys424 of pyruvate kinase M2 (PKM2), facilitating its tetramerization. The interaction enhances PKM2 activity and significantly suppresses tumor growth [14]. Moreover, MCL modulates cell apoptosis [7, 15], paraptosis [16], ferroptosis [11, 16], pyroptosis [17], and immunogenic cell death [18] by targeting metabolic enzymes and redox pathways. For instance, MCL promoted tumor regression by the inhibition of thioredoxin reductase (TrxR) and immunogenic cell death induction [18]. Beyond its anti-inflammatory and anticancer effects, MCL has been implicated in autophagy regulation. Preclinical *in vivo* studies indicate that MCL alleviated renal, peritoneal, and pulmonary fibrosis through autophagy activation and modulation of

associated signaling pathways [19, 20]. Collectively, MCL/ACT001 demonstrates broad mechanistic potential across a broad spectrum of age-related diseases. However, for many indications, including hepatic steatosis [19], intestinal inflammation and colitis-associated cancer [9], renal fibrosis [21], rheumatoid arthritis [22], atherosclerosis [11], and AD [23], current evidence remains largely confined to preclinical investigation.

Emerging evidence highlights the growing relevance of MCL and ACT001 in age-related pathologies. However, their integrated systematic molecular and cellular mechanisms remain incompletely defined. This paper primarily summarizes current preclinical and early clinical evidence on MCL/ACT001 in age-related diseases and various pathological processes by highlighting its essential functions and underlying molecular and cellular mechanisms. Moreover, we evaluated the therapeutic potential of MCL and ACT001 in a broad spectrum of age-related pathologies, including cardiovascular and cerebrovascular diseases, fibrotic disorders, immune-mediated conditions, metabolic dysfunction, and cancers. Therefore, we proposed MCL/ACT001 as a mechanistically rational candidate for age-related pathologies and emphasized that this repositioning remains investigational and requires rigorous preclinical and clinical validation.

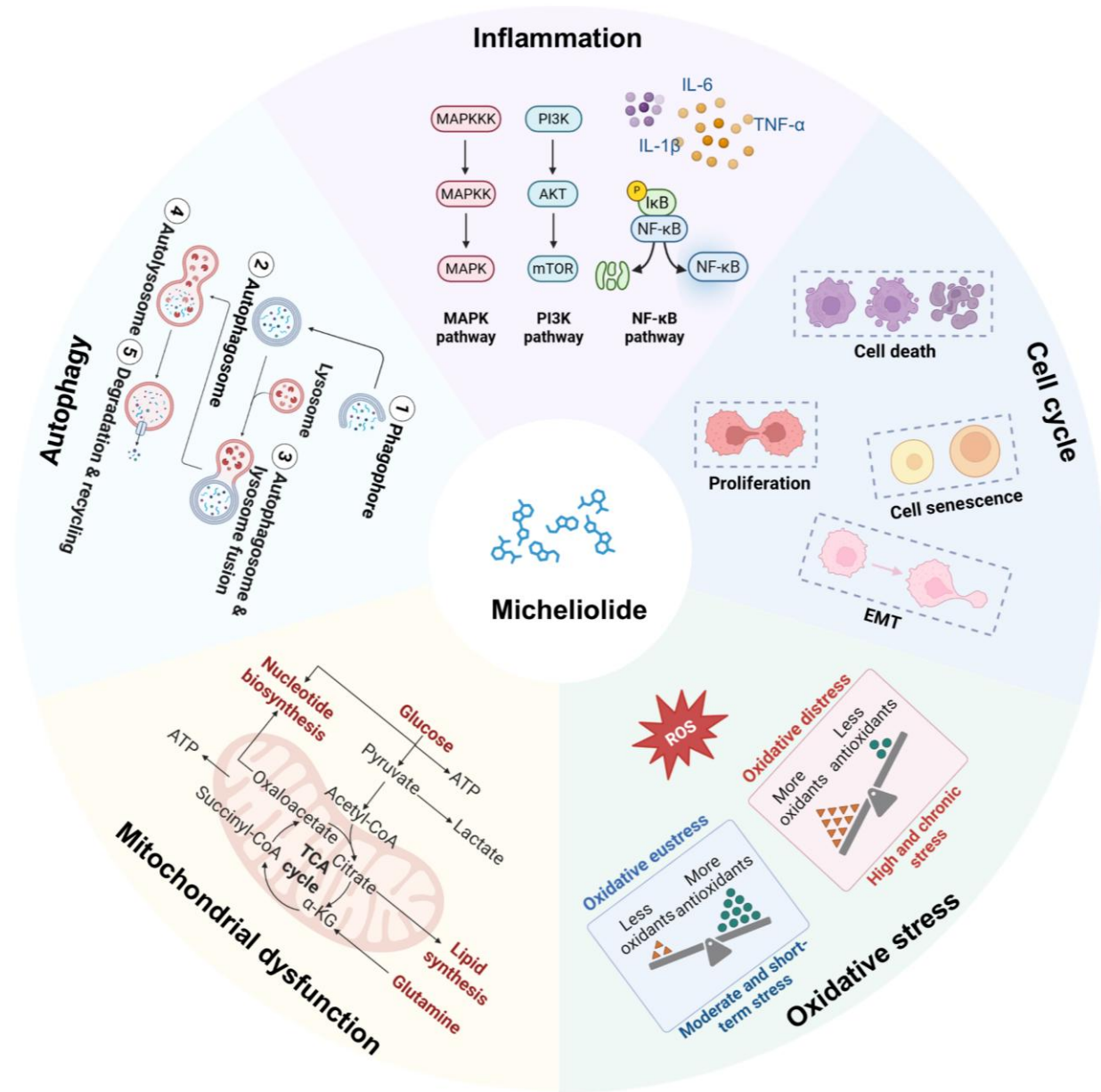


Figure 2. Potential regulatory mechanisms of MCL in age-related pathologies. MCL has emerged as a promising candidate for mitigating age-related pathologies through multiple mechanisms, including inflammation, oxidative stress, mitochondrial dysfunction, autophagy, and cell cycle. MCL synergistically exerts broad-spectrum anti-inflammatory effects through multiple signaling pathways, including NF- κ B, PI3K/Akt, and MAPKs. The cellular lifecycle, including cell proliferation, differentiation, senescence, and cell death, is a precisely coordinated dynamic equilibrium process essential for individual development, tissue homeostasis, and health. MCL is involved in the regulation of cell proliferation, EMT, senescence, and cell death. MCL has significant interactions with oxidative stress. Additionally, MCL exhibits antioxidant properties to modulate cellular redox balance and exhibits therapeutic effects. Conversely, MCL exerts its anticancer effect by promoting oxidative stress. There are accumulating findings to support an integral role for MCL in regulating mitochondrial dysfunction. *In vitro* studies have revealed that MCL upregulated LONP1 and ClpP expression, indicating that MCL was implicated in the activation of UPR_{mt}. Additionally, MCL enhances mitochondrial membrane potential. MCL induces mitochondrial dysfunction in GBM and leukemia cells. Increasing evidence has revealed that MCL is essential in autophagy by modulating multiple signaling pathways, including the MEK4/MAPK signaling pathway, ROS/MEK/ERK signaling pathway, Akt/mTOR, AMPK/mTOR pathway, and NRF2/PINK1/Parkin axis, thereby exerting therapeutic effects in various age-related diseases. The figure was created using BioRender.

2. Mechanisms of MCL/ACT001

Aging is an inevitable biological process that affects all living organisms; however, its progression can be modulated by multiple factors. Although aging cannot be prevented, its trajectory may be altered through biological and genetic interventions, pharmacological strategies, lifestyle modifications, and environmental factors [24]. Among these strategies, MCL/ACT001 has emerged as a promising candidate for the treatment of age-related pathologies. Its mechanisms involve inflammation, mitochondrial dysfunction, oxidative stress, autophagy, cell proliferation, senescence, and death (Fig. 2).

2.1 Inflammation

Human aging is characterized by a chronic low-grade, “sterile” inflammatory state, known as “inflammaging” [25], which plays a critical role in the pathogenesis and progression of multiple age-related diseases, including atherosclerosis [26], chronic kidney diseases [27], and metabolic syndrome [28]. Consequently, anti-inflammatory strategies are increasingly recognized as potential anti-aging treatments [29]. MCL has attracted significant attention owing to its potent anti-inflammatory effects. On the one hand, MCL exerts anti-inflammatory effects by inhibiting I κ B α (Ser32/36) phosphorylation and suppressing the toll-like receptor 4 (TLR4)/MyD88/NF- κ B axis, reducing the expression of pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-1 β , tumor necrosis factor (TNF- α), monocyte chemoattractant protein-1, and IFN- γ , and enhancing IL-10 levels [13, 30, 31]. Additionally, *in vitro* studies confirmed that MCL exhibited a significantly inhibitory effect on LPS-induced nuclear localization of p-p65 [23, 32]. On the other hand, MCL suppressed activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling. MCL decreased phosphorylated-Akt levels [13, 23, 33] and enhanced PI3K/Akt signaling inhibitor p-PTEN levels [34]. Besides, MCL suppressed p70S6K phosphorylation at Thr389 [31]. In LPS-induced BV2 cell models, MCL treatment inhibits the mitogen-activated protein kinases (MAPKs) signaling pathway and subsequently suppresses NF- κ B activity and pro-inflammatory cytokine expression [13]. In addition, MCL treatment significantly inhibits M1-type macrophage polarization and inflammatory factor production by inhibiting PFKFB3-driven glycolysis [35].

Collectively, MCL synergistically exerts a broad-spectrum anti-inflammatory effect through the coordinated modulation of multiple signaling pathways, including NF- κ B, PI3K/Akt, and MAPK signaling pathways. These properties position MCL as a promising therapeutic candidate for treating inflammatory disorders.

Given the well-established association between inflammation and aging, MCL may play a role in mitigating inflammaging, the chronic and low-grade inflammatory state associated with aging. However, further studies are required to evaluate its therapeutic potential in age-related inflammatory diseases.

2.2 Cellular lifecycle

The cellular lifecycle, including cell proliferation, differentiation, senescence, and cell death, is a precisely coordinated dynamic equilibrium essential for organismal development, tissue homeostasis, and overall health. With aging, external environmental stressors and the accumulation of internal damage, such as telomere shortening, oxidative stress, and DNA damage, lead to a gradual disruption of these processes [1]. This imbalance contributes to the development of various age-related diseases. Consequently, maintaining the integrity of these processes is a key strategy for combating age-related diseases. Cumulative studies revealed that MCL was implicated in regulating the cellular lifecycle.

2.2.1 MCL and cell proliferation

Cell proliferation refers to the process by which cells increase in number through mitosis or meiosis [36]. Dysregulated proliferation is a hallmark of cancer [37]. *In vitro* studies have demonstrated that MCL treatment attenuates aberrant tumor cell proliferation by inducing cell cycle arrest at the G1 phase. Besides, ACT001 effectively suppresses tumor growth in rodent models [14, 15, 38] and inhibits colony formation and GBM cell proliferation. Mechanistically, MCL covalently binds with the monomeric PKM2 at Cys424, promoting its tetramerization and enhancing its pyruvate kinase activity of PKM2. Non-targeted metabolomics analyses revealed that ACT001 treatment reduces several metabolites implicated in glycolysis, including glucose-6-phosphate and lactate. These findings suggest that ACT001 inhibits cell proliferation through metabolic PKM2 activation [14, 39]. Collectively, MCL and ACT001 exhibited promising application potential in tumor treatment.

2.2.2 MCL and epithelial-mesenchymal transition (EMT)

EMT is a biological program in which epithelial cells lose their polarity and cell-cell adhesion and acquire mesenchymal phenotypes [40]. Accumulating evidence indicates that MCL and ACT001 exert substantial antifibrotic effects by inhibiting EMT and its associated signaling pathways. On the one hand, MCL competitively suppressed the interaction between IL-11 and IL-11R α 1,

thereby inhibiting STAT3 and ERK/metadherin (Mtdh) pathways and ameliorating IL-11-induced renal tubular EMT [21]. On the other hand, MCL/ACT001 treatment suppressed TGF- β 1-induced EMT through inhibition of the Mtdh/BMP/MAPK signaling axis [12, 41]. Additionally, MCL modulates key EMT-inducing transcription factors, including ZEB1, ZEB2, Twist, Slug, and Snail, thereby regulating the expression of epithelial

and mesenchymal cell markers and reducing extracellular matrix (ECM) deposition [42]. Besides, MCL inhibits EMT by suppressing Smad2/3 phosphorylation. Mechanistically, MCL suppressed BRG1 binding to the acetylated lysine 14 of histone H3 by combining with the asparagine (N1540) of BRG1, thereby repressing the TGF- β 1/Smad2/3 signaling axis [43].

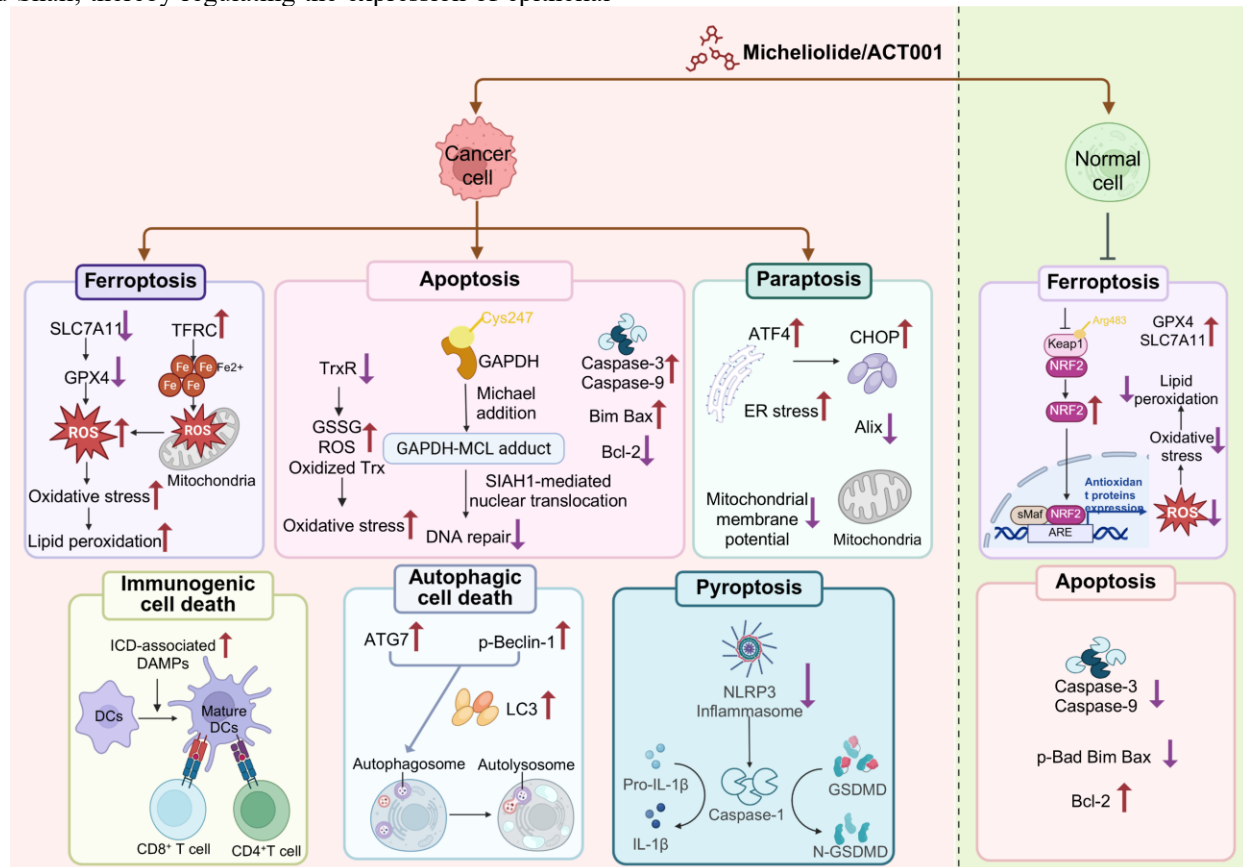


Figure 3. Potential regulatory mechanisms of MCL in cell death. Cancer is a complex genetic disorder characterized by the gradual imbalance of cell apoptosis and proliferation. The impact of MCL on cell death depends on multiple factors, including cell type, drug concentration, duration of action, and the pathological state of the cells. MCL exhibits a dual relationship with apoptosis. On the one hand, MCL can induce apoptosis through various mechanisms. MCL covalently binds to the Sec498 of TrxR, inhibiting the biological function of TrxR and promoting oxidative stress-mediated apoptosis of HeLa cells. Besides, MCL promotes the nuclear translocation of GAPDH by binding to Cys247 and induces GBM cell death. On the other hand, in certain cell types, MCL may exert anti-apoptotic effects by suppressing the expression of apoptotic proteins such as Bax, caspase-3, and caspase-9 and increasing the level of Bcl-2. Moreover, MCL suppressed macrophage ferroptosis through activating the NRF2 pathway; the related mechanism is by binding to the Arg483 site of KEAP1 competitively. Conversely, MCL promoted ferroptosis in tumor cells through decreasing SLC7A11 expression, increasing the levels of iron transport protein TFRC, and increasing intracellular iron levels. Furthermore, MCL facilitates paraptosis, indicated by decreased Alix and increased ATF4 and CHOP levels. MCL induced autophagic cell death by increasing the levels of autophagy markers, including ATG7, p-Beclin-1, and Beclin-1. In addition, MCL elicited ROS-mediated ERS-driven ICD through inducing ICD-associated damage-associated molecular patterns, including CRT exposure, ATP secretion, and HMGB1 release. Additionally, ACT001 suppressed NLRP3 inflammasome-mediated pyroptosis and diminished pro-inflammatory cytokine release. The figure was created using BioRender.

2.2.3 MCL and cell differentiation and senescence

Cell differentiation is the process by which less specialized cells develop into distinct cell types with specific morphological and functional characteristics.

MCL regulates osteoclast differentiation by suppressing RANKL-induced p38 MAPK signaling activation [44]. Cellular senescence is an irreversible state of cell cycle arrest accompanied by characteristic morphological changes and the secretion of pro-inflammatory factors

[45]. MCL inhibits the secretion of key components of the senescence-associated secretory phenotype, including IL-6 [11].

2.2.4 MCL and cell death

Cell death is the irreversible cessation of cellular biological functions and is essential for maintaining tissue homeostasis and proper immune responses in multicellular organisms. It can occur through mechanisms including apoptosis, pyroptosis, and necroptosis, or as a consequence of metabolic dysregulation, such as ferroptosis [46]. The effects of MCL on cell death depend on multiple factors, including cell type, drug concentration, duration of action, and the pathological state of the cells (Fig. 3).

Apoptosis is a type of programmed cell death characterized by caspase activation, cell shrinkage, plasma membrane blebbing, phosphatidylserine externalization, chromatin condensation, and nuclear fragmentation. MCL exhibits a dual role in apoptosis regulation. On the one hand, MCL can induce apoptosis through multiple mechanisms. Zhang et al. discovered that MCL covalently bound to the Sec498 of TrxR, enhancing oxidative stress-mediated apoptosis of HeLa cells [7]. In addition, MCL selectively and covalently targets the conserved Cys247 residue of GAPDH, without affecting oligomerization or GAPDH enzymatic activity, promoting the GAPDH/Siah1 complex nuclear translocation, thereby facilitating cell death [47]. Furthermore, MCL induces apoptosis by activating P53 through the elevated phosphorylation of p53Ser15 and increased Bim and Bax expression [47]. Additionally, MCL markedly inhibited cell viability and triggered apoptosis through cytochrome c/caspase-dependent apoptotic cascade activation. MCL administration triggers cytochrome c translocation from mitochondria to the cytosol in U251MG glioma cells [48]. MCL activates caspase-3 in a time- and concentration-dependent manner, thereby implicating its role in apoptosis induction [7]. Moreover, MCL enhanced cleaved caspase-3 and cleaved caspase-9 and Bax expression and decreased Bcl-2 levels [48]. MCL is involved in reactive oxygen species (ROS)-associated mitochondrial apoptotic signaling. As illustrated in breast cancer cells, MCL-mediated Drp1 upregulation triggers mitochondrial fragmentation, ROS accumulation, and cytochrome c efflux, consequently promoting apoptosis [49]. Conversely, in certain cell types, MCL exerts anti-apoptotic effects through PI3K/Akt activation and suppresses the expression of apoptotic proteins [34]. Lei et al. reported that MCL inhibited apoptosis and enhanced the LPS-treated HK2 cell viability [50]. Animal experiments have verified that MCL suppressed the transcription of pro-apoptotic genes

Bax, caspase-3, and caspase-9 and enhanced anti-apoptotic Bcl-2 expression in diabetic rats [30].

Pyroptosis is an inflammasome-dependent type of programmed cell death mediated by inflammasome activation and executed through gasdermin proteins [51]. This process is mediated by persistent cellular swelling culminating in cell membrane rupture, which facilitates the extracellular release of intracellular constituents and triggers potent inflammatory signaling. Excessive pyroptosis can lead to inappropriate and sustained inflammatory responses, tissue damage, and organ dysfunction [52]. MCL/ACT001 attenuates pyroptosis by inhibiting NLRP3 inflammasome activation [17, 53], thereby suppressing caspase-1 activation and mitigating cleaved caspase-1 and GSDMD-N expression.

Ferroptosis is an iron-dependent type of regulated cell death driven by lipid peroxidation [54, 55]. It predominantly implicates two fundamental cellular events, dysregulation of oxidative stress equilibrium and lipid peroxidation-mediated membrane damage [56]. It is implicated in various biological processes, including tumorigenesis and cardiovascular disease. On the one hand, MCL exerts its anti-ferroptotic effects, in part, by orchestrating iron homeostasis, reducing lipid peroxidation, and reinforcing endogenous antioxidant capacity. Specifically, MCL/ACT001 upregulates the abundance of glutathione (GSH), glutathione peroxidase 4 (GPX4), and the cystine/glutamate transporter SLC7A11, thereby limiting oxidative damage, preserving mitochondrial function, and restraining lipid peroxide accumulation [11]. In addition, MCL disrupts the KEAP1/NRF2 complex, promoting NRF2 nuclear translocation. Molecular docking studies suggest that Arg483 on KEAP1 may serve as a binding site for MCL. NRF2 inhibition and the KEAP1-R483S mutation eliminate the anti-ferroptotic property of MCL in macrophages [11]. Conversely, MCL promoted ferroptosis in tumor cells by decreasing SLC7A11 expression, increasing the levels of iron transport protein TFRC, and elevating intracellular iron levels [16].

MCL is a dual-function modulator of cell death, with its promoting or inhibitory effects determined by cellular and pathological conditions. MCL exhibits selective cytotoxicity toward tumor cells characterized by metabolic reprogramming, redox imbalance, constitutive activation of pro-survival signaling pathways, including PI3K/Akt/mTOR, STAT3, NF- κ B, and aberrant expression of critical molecular targets, including GAPDH, IKK β , and Drp1. These effects induce programmed cell death. Conversely, at concentrations generally below 10 μ M, MCL exerts cytoprotective effects in normal cells. Under stress conditions, MCL activates the NRF2-mediated antioxidant defense system. This activation promotes NRF2 nuclear translocation and

its binding to antioxidant response elements, resulting in upregulation of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and heme oxygenase-1. The enhanced ROS accumulation mitigates oxidative damage to cellular membranes, DNA, and mitochondria, thereby inhibiting normal cell death. This functional duality underpins the diverse biological effects and highlights its therapeutic potential.

2.3 Oxidative stress

Oxidative stress denotes a disruption of redox homeostasis wherein pro-oxidant forces overwhelm the antioxidant defense system [57]. Mitochondria serve as the primary intracellular source of reactive oxygen species (ROS) and represent a central hub in cellular stress responses. Oxidative stress is a major driver of macromolecular damage and plays a critical role in the aging process. It is progressively acknowledged as a causative contributor to a spectrum of age-associated pathologies. Emerging evidence indicates that MCL interacts strongly with oxidative stress pathways, modulates cellular redox balance, and exerts therapeutic effects. MCL inhibits mitochondrial ROS production [35]. Transcriptome sequencing revealed that MCL was implicated in regulating the GSH pathway, including upregulation of GSH-related genes.

MCL enhanced catalase, glutathione S-transferase, SOD, and GPX enzymatic activities; elevated the GSH/GSSG ratio; and attenuated lipid peroxidation as evidenced by reduced MDA levels [30, 35, 58, 59]. Furthermore, MCL decreased NOX1 and NOX4 expression in diabetic rats [30]. Emerging evidence demonstrated that the antioxidant properties of MCL are conferred through NRF2 pathway activation [30, 34, 59]. Furthermore, MCL directly binds to KEAP1, thereby facilitating NRF2 nuclear translocation [11]. Conversely, MCL/ACT001 exerts its anticancer effect by facilitating oxidative stress [60]. ACT001 decreases cyclin B1/CDC2 expression and triggers G2/M phase arrest by enhancing ROS production in U118 MG cells [61]. The effect of MCL/ACT001 on redox balance, whether cytoprotective or cytotoxic, depends on cell type, oxidative baseline, and drug concentration, requiring careful dose optimization and patient stratification in future trials.

2.4 Mitochondrial dysfunction

Mitochondrial dysfunction is characterized by reduced mitochondrial respiratory capacity, depolarized membrane potential, and excessive ROS generation. It represents a key driver of organismal senescence and contributes to diverse age-associated pathologies. It triggers oxidative stress, disrupts metabolic homeostasis,

activates inflammatory responses, and promotes cellular senescence, thereby contributing to progressive tissue functional decline and serving as a central element of cellular damage accumulation during aging [62]. Elucidating and ameliorating mitochondrial dysfunction is essential for enhancing mitochondrial integrity, maintaining cellular homeostasis, and prolonging health span. Mitochondrial-targeted interventions are a highly promising therapeutic strategy for ameliorating age-associated pathologies [63]. There are accumulating findings to support an integral role for MCL in regulating mitochondrial dysfunction.

In vitro studies revealed that MCL was implicated in the mitochondrial unfolded protein response (UPR^m) activation through LONP1 and ClpP regulation. Additionally, MCL enhances mitochondrial membrane potential [35]. Conversely, in GBM and leukemia cells, MCL elicited mitochondrial impairment. On the one hand, the GSH depletion induced by MCL impairs the tricarboxylic acid (TCA) cycle in malignant cells. MCL treatment substantially inhibits the activities of key metabolic enzymes within the TCA cycle, including fumaric acid hydratase, isocitrate dehydrogenase 2, oxoglutarate dehydrogenase, and citrate synthase. MCL markedly reduced the levels of metabolic intermediates, including succinate, fumarate, and malate, in the TCA cycle. On the other hand, MCL is involved in mitochondrial respiratory chain dysfunction. MCL improves the mitochondrial oxidative phosphorylation process and activates AMPK signaling. In addition, MCL increases intracellular and mitochondrial ROS levels, decreases mitochondrial membrane potential, and alters mitochondrial morphology [60, 64]. Another study revealed that MCL dose-dependently enhanced Drp1 expression in breast cancer cells. Confocal microscopy examination revealed a transformation of mitochondria from elongated to rounded and shortened shapes subsequent to treatment, indicative of mitochondrial fission [49]. Overall, MCL preserves mitochondrial function by maintaining membrane potential, limiting excessive ROS synthesis, and preventing activation of the mitochondrial apoptotic cascade, including cytochrome c release and caspase activation. Conversely, its anticancer activity relies on the induction of mitochondrial dysfunction. These observations highlight the therapeutic potential of MCL against mitochondrial-related pathologies.

2.5 Autophagy

Autophagy is an evolutionarily conserved lysosomal degradation pathway through which cells remove dysfunctional or superfluous components to maintain intracellular homeostasis. Autophagic activity declines

with age, leading to the accumulation of dysfunctional organelles and aggregated proteins, which precipitate diverse pathophysiological states, including malignancy, autoimmune disorders, renal injury, cardiomyopathy, diabetes mellitus, and neurodegenerative conditions [65, 66]. Autophagy is tightly associated with cellular senescence and aging. The treatments for extending longevity, including mTOR signaling inhibition and calorie restriction, activate autophagy [67]. Emerging evidence indicates that MCL and ACT001 act as important regulators of autophagy through multiple pathways, including intracellular signaling pathways, including the MEK4/MAPK signaling pathway [68], ROS/MEK/ERK signaling pathway [64], Akt/mTOR [69], AMPK/mTOR pathway [19, 70], and NRF2/PINK1/Parkin axis [50], exerting therapeutic effects in various age-related diseases. MCL/ACT001 administration upregulated autophagy-related gene 7 (ATG7), p-Beclin-1, Beclin-1, and LC3-II expressions [16, 19, 20, 53]. Besides, MCL promoted mitophagy by restraining NLRP3 inflammasome activation through the regulation of the NRF2/PINK1/Parkin axis [50]. ACT001 induced autophagic activity through the

regulation of Akt/mTOR and ROS/MAPK pathways [69]. Conversely, MCL alleviated hepatic stellate cells autophagy through modulation of the TrxR1/2-mediated ROS/MEK/ERK axis [16]. Collectively, these findings suggest that MCL/ACT001 may exert the effects of alleviating age-related diseases through autophagy and mitophagy.

3. Role of MCL/ACT001 in age-related cardio-cerebral diseases

Age-related diseases represent a major global health challenge that significantly affects the well-being and quality of life of older populations. With population aging, the escalating prevalence and incidence of these conditions have imposed substantial burdens on healthcare systems. Owing to its pleiotropic mechanisms of action, MCL/ACT001 holds promise as a therapeutic candidate for a broad spectrum of age-associated diseases, including cardiovascular diseases, neurodegenerative disorders, fibrosis, metabolic diseases, and tumors (Table 1, Fig. 4).

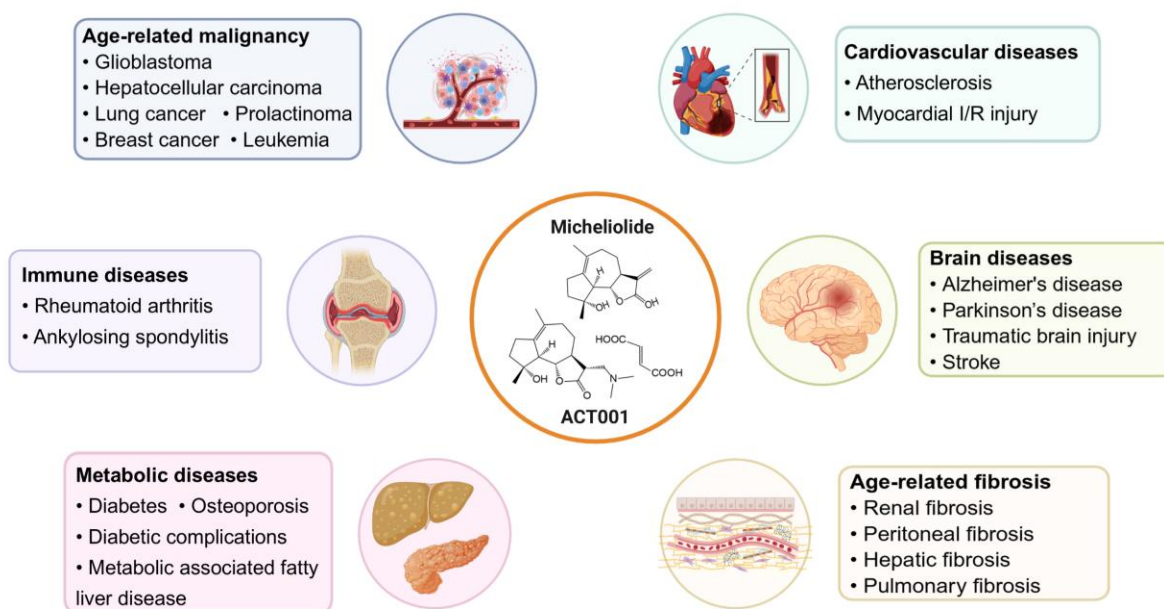


Figure 4. Roles of MCL/ACT001 in age-related diseases. The figure was created using BioRender.

3.1 Atherosclerosis

Advanced age represents a major risk factor for atherosclerotic cardiovascular disease [71]. It is characterized by lipid accumulation and plaque formation within the arterial intima, leading to chronic, systemic, and inflammatory disease, and is the primary cause of

cardiovascular disease development globally [72]. Throughout atherosclerosis, progressive lipid accumulation and necrotic core expansion compromise plaque stability, predisposing to plaque rupture and thrombotic events. Despite advances in lipid-lowering and anti-inflammatory therapies, a substantial subset of patients continues to experience myocardial infarction

[73]. Consequently, identifying novel therapeutic targets and pharmacological agents for atherosclerosis is imperative.

Table 1. Summary of preclinical studies on MCL/ACT001 mechanism in age-related diseases.

Disease Group	Diseases	Drug	<i>In vivo</i> dose concentration	<i>In vitro</i> dose concentration	Mechanisms	Ref.
Cardiovascular	Atherosclerosis	MCL	100 mg/kg/day in ApoE ^{-/-} mice	10 uM in RAW264.7 cell	Alleviated atherosclerosis through inhibiting macrophage ferroptosis via KEAP1/NRF2 axis	[11]
	Myocardial ischemia/reperfusion injury	MCL	100 mg/kg/day in Sprague-Dawley rats	/	Alleviated myocardial ischemia/reperfusion injury via KEAP1/NRF2 pathway	[77]
	Ischemic stroke	MCL	A single dose of 10 mg/kg in C57BL/6 mice	10 uM in Bone marrow-derived macrophages	Reduced lesion volumes and improved neurological function by interfering PKM2-P65 interaction and inhibiting inflammasome activation	[83]
	Alzheimer's disease	MCL	10 and 20 mg/kg/day in C57BL/6J mice	12 uM in BV2 cell	Improved cognitive impairment by inhibiting neuroinflammation via NF-κB and PI3K/Akt pathways	[23]
Neurological	Parkinson's disease	ACT001	20 mg/kg/day in Balb/c mice	/	Reduced motor dysfunction and dopaminergic neurodegeneration through increasing Bcl-2 and the pAkt/Akt ratio and decreasing Bax and Caspase3 activation in the substantia nigra and striatum	[88]
		ACT001	20 mg/kg/day in Balb/c mice	/	Alleviated movement impairment and cognitive deficit by suppressing NLRP3-mediated neuroinflammation	[89]
	Traumatic brain injury	ACT001	100 mg/kg/day in C57BL/6 mice	10 μM in BV2 cell and 1-2 μM in primary microglia cell	Attenuated motor function defects through modulating Akt/NF-κB/NLRP3 pathway	[93]
	Neuropathic pain	ACT001	50 mg/kg/day in Sprague-Dawley rats	100 μM in BV2	Attenuated peripheral nerve injury through inhibition of TLR4 signaling via targeting co-receptor MD2	[95]
Fibrotic	Renal fibrosis	MCL ACT001	25 mg/kg/day ACT001 in C57BL/6 mice	1.25, 2.5, 5, or 10 μM MCL in mouse tubular epithelial cell	Improved renal function and structure by regulating Mtdh/BMP/MAPK pathway	[12]
		MCL ACT001	25 mg/kg/day ACT001 in C57BL/6 mice	2.5, 5, and 10 μM MCL in mouse tubular epithelial cell	Alleviated renal fibrogenesis through disrupting the IL-11/IL-11Rα1 interface and consequently blocking STAT3 and ERK1/2/Mtdh pathways	[21]
	Peritoneal fibrosis	MCL ACT001	12.5, 25, and 50 mg/kg/day ACT001 in C57BL/6 mice	1.25, 2.5, 5 μM MCL in HMrSV5 cell	Ameliorated peritoneal fibrosis by activating autophagy	[20]
		MCL ACT001	25 mg/kg/day ACT001 in C57BL/6 mice	1.25, 2.5, 5 μM MCL primary rat peritoneal mesothelial cell and HMrSV5 cell	Inhibited the fibrotic responses through impeding BRG1 from recognizing and attaching to histone H3 lysine 14 acetylation via bound to the asparagine (N1540) of BRG1	[43]
	Hepatic fibrosis	MCL	10 and 20 mg/kg/day in C57BL/6 mice	4, 8, 12 μM in LX-2 cell	Ameliorated hepatic fibrosis via the TrxR1/2-mediated ROS/MEK/ERK pathway	[64]

Metabolic	Pulmonary fibrosis	MCL	60 mg/kg/day in C57BL/6 mice	/	Mitigated CCL4-induced liver injury and fibrosis by activation of SIRT1	[110]
		ACT001	/	0-10 μ M in primary lung fibroblasts	Reduced fibroblast activity in idiopathic pulmonary fibrosis by inhibition of NF- κ B	[114]
		ACT001	50 and 100 mg/kg/day in Sprague-Dawley rats	/	Attenuated radiation-triggered EMT through suppressing ROS generation and downregulating TGF- β 1 expression, Ameliorated insulin resistance by repressing hepatic gluconeogenesis through down-regulating glucose-6-phosphatase and FoxO1 expression	[17]
	Diabetes	ACT001	10 mg/kg/day in C57BL/6 mice	/	Attenuated obesity-associated kidney disease through modulation of the GPR43 and AMPK signaling pathways	[119]
	Diabetic complications	MCL	20 and 40 mg/kg/day in Sprague-Dawley rats	/	Mitigated diabetic nephropathy via NRF2/Keap1 activation and TLR4/MyD88/NF- κ B inhibition	[30]
		MCL	12.5, 25, and 50 mg/kg/day in db/db mice	2.5, 5, and 10 μ M in AML12 and LO2 cells	Alleviated hepatic steatosis via PPAR γ -mediated NF- κ B and AMPK/mTOR pathways	[19]
		ACT001	200 mg/kg/day in C57BL/6 mice	/	Attenuated obesity-associated kidney disease through modulation of the GPR43 and AMPK signaling pathways	[120]
	Metabolic associated fatty liver disease	ACT001	200 mg/kg/day in C57BL/6 mice	/	Reduced hepatocyte injury and dyslipidemia by regulating the gut-microbiota/bile-acid/FXR axis	[125]
	Non-alcoholic steatohepatitis	ACT001	100, 200, and 400 mg/kg/day in C57BL/6 mice	/	Alleviated liver steatosis, inflammation and fibrosis by inhibiting oxidative stress and NLRP3 inflammasome activation	[127]
	Osteoporosis	MCL	30 mg/kg/day in C57BL/6 mice	5, 10, and 20 μ M in osteoclasts	Protected against estrogen deficiency-induced bone loss via inhibiting osteoclast bone resorption	[44]
	Rheumatoid arthritis	MCL	30 mg/kg/day in DBA/1 mice	/	Reduced paw swelling and inhibited joint cartilage degeneration	[22]
	Ankylosing spondylitis	MCL	20 and 50 mg/kg/day in Balb/c mice	/	Alleviated disease progression by repressing NLRP3-driven pyroptosis, restoring Th1/Th2 equilibrium and interrupting the NF- κ B signaling cascades	[135]
		ACT001	/	7.5, 15, and 30 μ M in U118MG, U251, SF126, SHG44, and GL261 cell lines	Inhibited human glioma cell proliferation by directly binding to IKK β and driving ROS-mediated G2/M phase arrest and cell apoptosis	[61]
		ACT001	100 and 400 mg/kg/day in C57BL/6 mice	20, 40, and 80 μ M in U251MG, TJ905, SNB19, LN18, TJ179, GL261, and HMO6 cell lines	Reduced PD-L1 expression through suppressing the phosphorylation of STAT3 in glioblastoma	[140]
Malignant	Glioblastoma	ACT001	/	10-400 μ M in U118MG cell	Suppressed GBM proliferation, invasion and migration by modulating PAI-1/PI3K/Akt signaling	[141]
		MCL ACT001	400 mg/kg/day ACT001 in Balb/c mice	5-40 μ M in U118MG, U251MG, and U87MG cell lines	Induced glioblastoma cell death via covalently bind to Cys247 of GAPDH, initiating non-	[47]

				glycolytic related functions in GAPDH	
		ACT001	100 mg/kg/day in NUDE mice	5 and 10 μ M in GSC 1123 and R39 cells	Suppressed glioma-like stem cell proliferation and glioma spheres formation by targeting AEBP1/PI3K/Akt signaling [143]
		ACT001	/	5 μ M in U118MG	Prevented the progression of gliomas via the MDK/c-Myc complex [144]
Hepatocellular carcinoma		MCL	20 mg/kg/day in NUDE mice	10, 30, 60, 90 μ M in Huh7, HepG2, QGY-7703, Bel-7404, Hep3B, and PLC/PRF/5 cell lines	Suppressed liver cancer cell growth through promoting apoptosis and disrupting F-actin cytoskeleton [146]
		MCL	25 mg/kg/day in C57BL/6 mice	10, 20, and 30 μ M HepG2, Hepa 1-6, LO2, Huh7 and Huh7R cells	Inhibited tumor growth by eliciting ROS-mediated ERS driven immunogenic cell death [18]
		ACT001	100 mg/kg/day in NUDE mice	5, 10, 20, and 50 μ M in A549 and NCI-H820	Attenuated stem-like properties of lung cancer by targeting and inducing Olig2 ubiquitination degradation [149]
		ACT001	200 mg/kg/day in NUDE mice	10-1000 μ M for NCI-H1703, EBC-1, NCI-H226, NCI-H1975, PC9, LTEP-A2, and HCC827 cells	Repressed the proliferation and G1/S transition of NSCLC cells through upregulating NKTR expression [150]
Lung cancer		ACT001	400 mg/kg/day in C57BL/6 mice	12 μ M for H1299 and 12.7 μ M for H1975	Inhibited tumor progression via suppressing the phosphorylation of STAT1 and STAT3 [151]
		MCL	/	5, 10, and 20 μ M for H1299 and Calu-1 cells	Enhanced radiosensitive of p53-deficient non-small cell lung cancer through promoting HIF-1 α Degradation [152]
		ACT001	200 mg/kg/day in NUDE mice	5, 10, 20, and 40 μ M for NCI-H1688 and NCI-H446 cells	Suppressed small cell lung cancer progression via the p-PGK1/p-PDHK axis [154]
		MCL ACT001	400 mg/kg/day ACT001 in NUDE mice	5, 10, and 20 Mm MCL for MCF7 and T47D	Inhibited cell proliferation by restraining ROS/AKT/ASAHI pathway by stabilizing NRF2 [38]
Breast cancer		ACT001	/	10 and 15 μ M for T47D, 2.5 and 5 μ M for MCF7 and ZR7530	Prevented and reversed tamoxifen resistance via inhibition of NF- κ B activation [156]
		MCL	400 mg/kg/day in NUDE mice	5, 10, 20, and 40 μ M for MCF-7 cell	Ameliorated KLF4-driven cisplatin resistance by downregulating glutathione biosynthesis [157]
		ACT001	100 mg/kg/day in NUDE mice and 200 mg/kg/day in Fischer 344 rats	10, 20, and 50 μ M for GH3 and MMQ cells	Inhibited pituitary tumor growth through inducing autophagic cell death via MEK4/MAPK pathway [68]
		ACT001	100 mg/kg/day in NUDE mice	20 μ M for GH3 and MMQ cells	Reversed resistance of prolactinomas through targeting AMPK-mediated EGR1 and mTOR pathways [70]
		ACT001	1, 3, and 10 μ g/mL in Zebrafish	0-70 μ M for HL-60, KG-1a, K562, HL-60/ADR, KG-1a/ADR, K562/ADR, SW1990, and SH-SY5Y cells	Suppressed leukemia via activation pyruvate kinase M2 [14]
Hematologic malignancy		MCL ACT001	400 mg/kg/day ACT001 in NUDE mice	0-20 μ M MCL for U118MG, U251MG, HL60, and KG1A cells	Promoted cell apoptosis by targeting redox and metabolic circuit [60]

Gastroenteric tumor	ACT001	400 mg/kg/day in NSG mice	10, 20, and 30 μ M for U2932 and SU-DHL2 cells	Inhibited primary central nervous system lymphoma tumor growth through increasing the anti-tumor effect of T cells [163]
	MCL	/	10, 20, and 40 μ M for MIA PaCa-2 and HT-29 cells	Suppressed pancreatic ductal adenocarcinoma growth by modulating the EGFR-Akt-Bim and MAPK axes [16]
	MCL ACT001	50 mg/kg/day ACT001 in NUDE mice	0-40 μ M MCL for AGS and N87 cells	Repressed gastric cancer cell proliferation and promoted apoptosis via regulation of IL6/STAT3 pathway [165]

Macrophage death plays a central role in plaque progression and necrotic core formation, contributing to lesion instability [74]. Therefore, targeting programmed macrophage death in atherosclerosis and pharmacological interventions against it may be potential strategies for stabilizing vulnerable plaques and preventing atherosclerosis progression. MCL administration significantly attenuated atherosclerotic plaque formation, diminished plaque burden and lipid accumulation, and enhanced collagen deposition within the lesion [11, 75]. Compared to high-fat diet controls, the MCL intervention group demonstrated significantly increased GPX4 and xCT levels in macrophages localized to the atherosclerotic root. On the one hand, MCL administration attenuated inflammatory responses, as evidenced by decreased TNF- α , IL-1 β , and IL-18 levels and elevated IL-4 and IL-10 concentrations. On the other hand, MCL markedly decreased iron deposition in atherosclerotic plaques and serum iron levels, with significant reductions in MDA and 4-HNE levels and elevated SOD and GSH levels in the aorta, suppressing ferroptosis in macrophages and alleviating oxidative damage. Furthermore, dyslipidemia, defined primarily by increased circulating levels of atherogenic lipids or lipoproteins, is a critical contributor to cellular senescence and atherogenesis. Additionally, MCL treatment improves the lipid profile in atherosclerotic mice, with decreased serum TC, TG, and LDL-C levels and elevated HDL-C levels. Moreover, the aging process is closely associated with an increase in IL-6 levels in the murine aorta. This increase in IL-6 exacerbates inflammation and interacts with impaired mitochondrial function in the vasculature, establishing a vicious cycle that hastens atherogenesis [71]. This positive feedback loop is detrimental to vascular health, as it perpetuates inflammation and mitochondrial dysfunction, which are key drivers of atherosclerotic plaque formation and progression. Interestingly, MCL treatment effectively attenuates this pathological process by significantly reducing IL-6 levels, thereby presenting a promising therapeutic strategy for limiting atherosclerotic progression.

3.2 Myocardial ischemia/reperfusion (I/R) injury

Ischemic heart disease remains a leading cause of global morbidity and mortality. Percutaneous coronary intervention is the primary therapeutic strategy for acute myocardial infarction. Although recent optimizations in revascularization techniques have significantly decreased mortality among patients with acute myocardial infarction, the reperfusion process can induce additional cardiomyocyte death and irreversible myocardial damage, a phenomenon known as I/R injury [76]. Emerging evidence suggests that MCL exerts cardioprotective effects against myocardial I/R injury. Liu et al. reported that MCL treatment significantly attenuated I/R-induced cardiac injury in rats, as evidenced by reduced myocardial necrosis, myofiber disruption, and cardiomyocyte structural deformation, accompanied by suppression of inflammation and oxidative stress. Furthermore, MCL normalized serum levels of cardiac biomarkers, including CK-MB, cTnI, and BNP. Mechanistically, MCL suppressed cardiomyocyte apoptosis by regulating the apoptotic pathway, specifically by enhancing Bcl-2 and suppressing cleaved caspase-3 and Bax levels [77].

3.3 Stroke

Age is the most significant non-modifiable risk factor for stroke. With increasing global life expectancy, age-associated brain disorders, including stroke and dementia, have become leading causes of mortality and long-term disability, posing a substantial burden to public health [78, 79]. Integrative analysis of the aging process implicates immunosenescence as a key mechanistic driver of age-related cerebral injury and diseases [80].

Stroke is the major cause of chronic disability and mortality globally. In China, the number of adults affected by stroke reached approximately 17.8 million in 2020, whereas in the United States, the prevalence is projected to increase from 2.7% in 2020 to 3.8% by 2050 [79, 81]. The global projections suggest the annual economic toll of stroke already exceeds 890 billion dollars and could nearly double by 2050 [82]. The abrupt loss of cerebral

blood flow in acute ischemic stroke induces neuronal death and a subsequent post-injury neuroinflammation that exacerbates the initial tissue injury. This neuroinflammatory cascade is now recognized as a central driver of ischemic brain injury, with macrophages acting as key determinants of the destructive milieu. Accordingly, targeting post-stroke neuroinflammation has emerged as a promising therapeutic avenue. MCL exerts anti-inflammatory effects through modulation of macrophage function. Notably, MCL can cross the blood–brain barrier and preferentially accumulates in neural tissue, supporting its potential utility in cerebral ischemia. Wang et al. demonstrated that post-stroke MCL administration markedly reduces infarct volume and improves neurological outcomes in mice at 3 and 14 days after injury [83]. Additionally, MCL administration enhances recovery of neurological functions after ischemic stroke. Rotarod assessment revealed that MCL-treated mice exhibited better persistence. Cognitive performance probed with the novel-object recognition paradigm indicated that MCL-treated animals devoted significantly more time to investigating the unfamiliar object than their vehicle-treated littermates. Likewise, in the Morris water maze, they located the hidden platform with shorter escape latencies [83].

3.4 AD

AD is a progressive neurodegenerative disorder of the central nervous system, characterized by irreversible cognitive deterioration and behavioral impairment. Epidemiological data indicate that over 55 million individuals worldwide live with AD or related dementia. In the absence of curative or preventive interventions, this figure is projected to increase to 78 million by 2030 and 139 million by 2050 [84]. AD accounts for approximately 60%–70% of dementia cases in individuals aged ≥ 65 years, imposing substantial social, psychological, physical, and economic burdens on patients, caregivers, and health systems. Given global population aging, the incidence of AD is expected to increase markedly, highlighting the need for efficacious treatments and supportive care frameworks. Increasing evidence implicates chronic neuroinflammatory processes as fundamental to AD pathogenesis, wherein microglia, the innate immune sentinels of the central nervous system, play a crucial role. Microglia-mediated inflammatory response forms a central axis linking amyloid- β deposition, tau pathology, and neuronal demise, thereby driving disease progression [85].

Among the major signaling pathways implicated in this process, NF- κ B and PI3K/Akt act as critical regulators of glial activation and pro-inflammatory cytokine release in the AD brain [86]. MCL alleviated neuroinflammation by

repressing NF- κ B and PI3K/Akt pathways and improved cognitive impairment in the AD mouse model (Fig. 5) [23]. On the one hand, AD mice exhibited impairments in fleeting and enduring memory, whereas MCL treatment significantly ameliorated these cognitive deficits across temporal domains. On the other hand, spatial disorientation, another main clinical symptom of AD, was similarly ameliorated. In the Morris water maze, AD mice exhibited prolonged escape latency during the positioning navigation stage compared with MCL-treated counterparts. During the probe trial, treated AD mice stayed in the target quadrant, and the frequency of crossing the platform area was substantially lower than that of MCL-treated mice. In addition, cortical and hippocampal dentate gyrus and CA1 regions of MCL-treated mice carried significantly lighter A β plaque burdens compared with AD mice. Moreover, MCL was significantly implicated in the inhibition of central and peripheral inflammation [23]. Administration of MCL significantly suppressed glial cell activation and proliferation in AD mice. Additionally, a significant decrease was observed in the levels of various chemokines, including CXCL1, G-CSF, GM-CSF, IL-1 α , IL-4, MIP-1 α , and MIP-1 β [23]. Peripherally, MCL treatment exposure markedly reduced circulating inflammatory cells, including white blood cells, neutrophils, monocytes, and lymphocytes.

3.5 Parkinson's disease (PD)

PD is the second most common neurodegenerative disorder in the aging population, with its global prevalence projected to approximately double over the next 30 years [87]. Neuroinflammation is implicated in PD progression. Accumulating evidence positions ACT001 as a promising neuroprotectant for PD. L-DOPA remains the main drug for PD treatment. However, long-term administration is associated with significant side effects. ACT001 synergizes with low-dose L-DOPA (5 mg/kg), achieving comparable motor improvement to high-dose L-DOPA (8 mg/kg) in the MPTP-induced PD mouse model and attenuating dyskinesia risk. Mechanistically, ACT001 attenuated α -synuclein, astrocyte activation, and IL-1 β overexpression in the substantia nigra and striatum of PD mice. Additionally, ACT001 enhanced Bcl-2 expression and the p-Akt/Akt ratio and decreased Bax level and caspase-3 activity, thereby promoting dopaminergic neuron survival through inhibition of neuroinflammation and apoptosis [88]. Moreover, Liu et al. reported that ACT001 treatment reduced nigral dopaminergic degeneration and striatal dopamine depletion and ameliorated motor deficits and circadian rhythm disruption by inhibiting MPTP-induced

microglial activation, inflammation, oxidative stress, and NLRP3 activation [89].

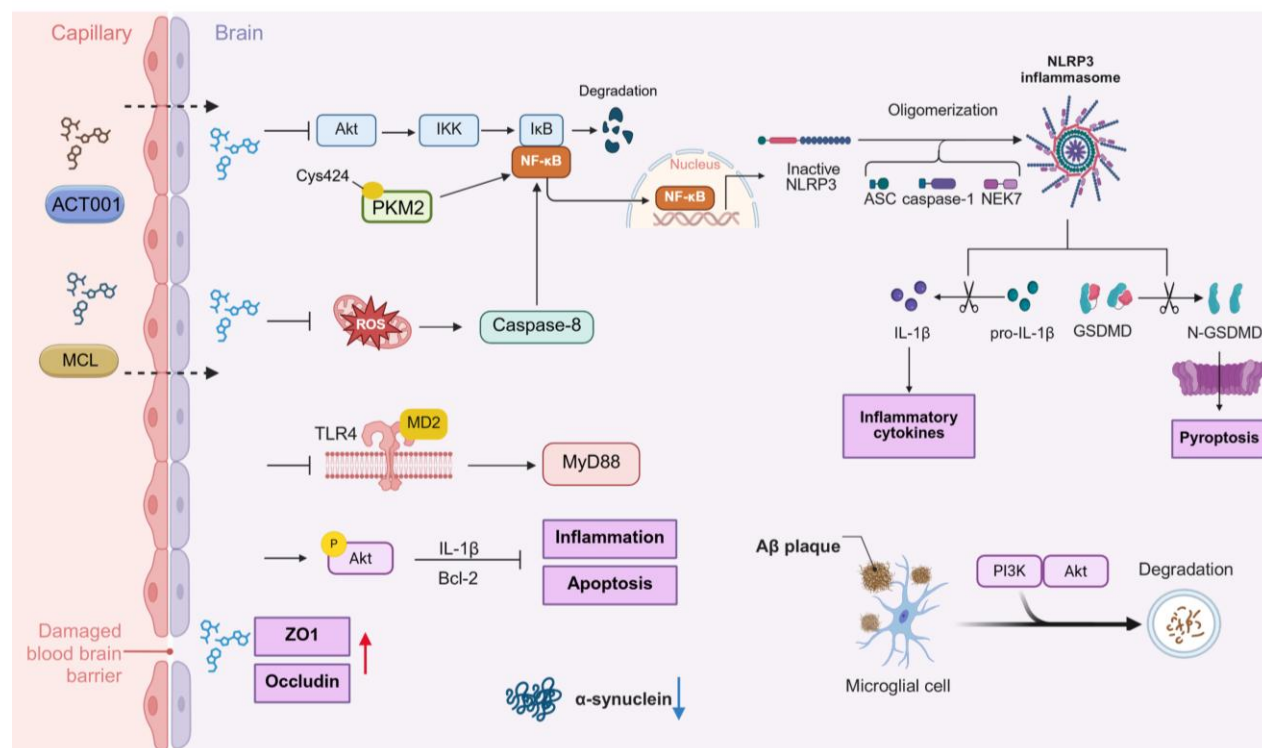


Figure 5. Potential mechanisms of MCL/ACT001 in age-related brain diseases. Given its diverse mechanisms of action, MCL/ACT001 holds promise as a therapeutic candidate for age-related brain conditions. ACT001 reduced microglia-mediated neuroinflammation after traumatic brain injury by inhibiting the AKT/NF- κ B/NLRP3 pathway. Additionally, MCL interfered with the PKM2-P65 interaction and inhibited inflammasome activation in macrophages after ischemic stroke. Moreover, ACT001 inhibited the activation of the NLRP3 inflammasome in MPTP-induced PD mice and suppressed the loss of dopaminergic neurons. Besides, ACT001 inhibited TLR4 signaling through targeting co-receptor MD2 and attenuated neuropathic pain. Additionally, ACT001 abolished MPTP-induced α -synuclein overexpression, astrocyte activation, and IL-1 β production in the substantia nigra and striatum of the mouse brain. In addition, ACT001 improved MPTP-induced PD by repressing apoptosis and inflammation. The figure was created using BioRender.

3.6 Other brain diseases

Experimental autoimmune encephalomyelitis is a well-established murine model of central nervous system inflammation that simulates the symptoms of multiple sclerosis and exhibits microglia with strong aging-associated transcriptional characteristics [90]. In this model, ACT001 disrupted the pathogenic feed-forward crosstalk between Th17 cells and microglia by suppressing microglial STING/NF- κ B pathway signaling activation, thereby attenuating autoimmune inflammation and ameliorating experimental autoimmune encephalomyelitis outcomes [91].

Traumatic brain injury is a neurological condition caused by external force, resulting in structural damage and/or physiological dysfunction of the brain, and represents a major cause of global mortality and long-term morbidity [92]. TBI is commonly associated with

intracranial hemorrhage, cerebral edema, and neuronal injury, all of which contribute to persistent neurological impairment. Increasing evidence suggests that ACT001 has significant therapeutic potential in the management of neurological disorders. Specifically, ACT001 attenuates trauma-induced microglial cell activation, preserves blood-brain barrier integrity after brain injury, and restores tight junction protein expression, including ZO1 and occludin, thereby improving neurological and motor function defects. *In vitro* studies revealed that ACT001 mitigated angiolysis induced by activated microglia and facilitated partial restoration of angiogenic capacity in bEnd.3 cells. Functionally, these effects are associated with the Akt/NF- κ B/NLRP3 pathway [93].

Neuropathic pain, defined as pain arising from a lesion or disease of the somatosensory system, affects approximately 3% to 17% of the general population and remains clinically challenging because of its

heterogeneous etiology, absence of objective diagnostic markers, and frequent resistance to conventional analgesics [94]. ACT001 targeted MD2 to interfere with the TLR4/MD2/MyD88 axis, thereby inhibiting downstream NF- κ B and MAPK signaling cascades and attenuating pro-inflammatory cytokine production. Besides, ACT001 alleviated nerve injury-induced sensory

abnormalities and downregulated Iba-1 and GFAP in the spinal dorsal horn [95]. Collectively, current evidence for MCL/ACT001 in age-related cardio-cerebral diseases consists primarily of *in vitro* cell studies and animal models, with no published human trials to date.

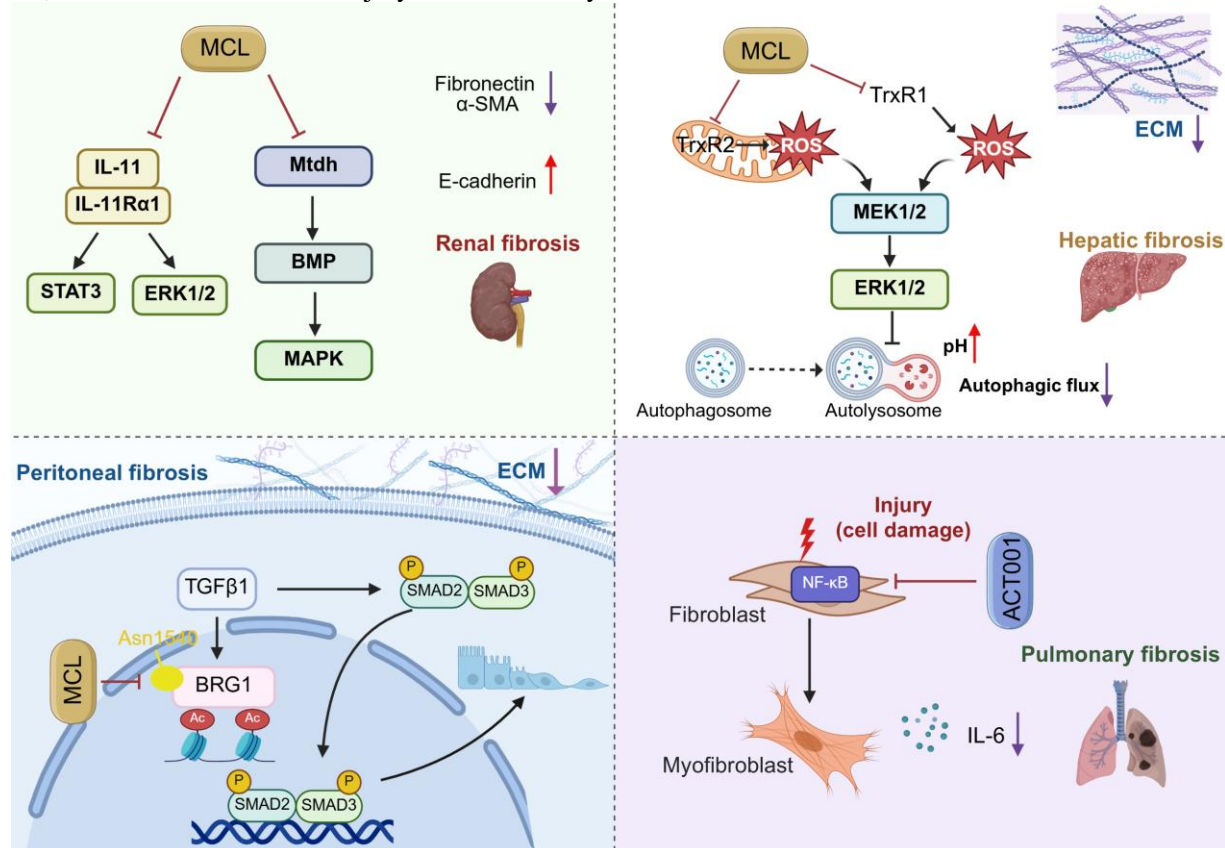


Figure 6. Potential mechanisms of MCL/ACT001 in age-related fibrosis. Renal fibrosis is a pathologic process that leads to irreversible renal failure without effective treatment. MCL competitively inhibited the binding of IL-11 with IL-11R α 1, suppressing the activation of STAT3 and ERK1/2-Mtdh pathways, and inhibiting renal tubular EMT and interstitial fibrosis induced by IL-11. Besides, MCL ameliorated renal fibrosis by suppressing the Mtdh/BMP/MAPK pathway. Hepatic fibrosis is a major global health issue without an optimal drug treatment. MCL ameliorated hepatic fibrosis through suppressing autophagy via the TrxR1/2-mediated ROS/MEK/ERK pathway. Progressive peritoneal fibrosis is a global public health concern affecting patients undergoing peritoneal dialysis. MCL is bound to the asparagine (N1540) of BRG1, blocking the recognition and binding of BRG1 to the acetylation of histone H3 at lysine 14, repressing fibrotic responses and the TGF- β 1-Smad2/3 signaling pathway. Idiopathic pulmonary fibrosis is a progressive fibrotic lung disease of unknown etiology and poor prognosis. ACT001 can inhibit NF- κ B, reducing the activity of fibroblasts in idiopathic pulmonary fibrosis. The figure was created using BioRender.

4. Role of MCL/ACT001 in age-related fibrosis

Fibrosis is a pervasive, age-associated pathological process and a pathogenic factor in the functional failure of multiple aging-related organs and chronic disease [96, 97]. The progressive accumulation of senescent cells plays a central role in tissue degeneration, chronic inflammation, and subsequent fibrotic disease across diverse organs [98]. Pathological fibrosis is characterized by excessive ECM deposition and is generally regarded as an aberrant and imbalanced tissue repair process [99].

Once the tempo of matrix production exceeds the counter-rhythm of physiological degradation, parenchyma is replaced by a stiff, avascular scar that distorts architecture and culminates in organ failure [100]. Epidemiologically, the global cumulative incidence of fibrosis disease is estimated at approximately 4968 cases per 100000 individuals annually, constituting approximately 45% of mortalities in developed countries [101]. Targeting the fibrogenic cascade represents a generic, high-value therapeutic strategy that transcends individual organ specialties. Recently, preclinical studies indicate that

MCL/ACT001 can attenuate ECM accumulation in the kidney [21], peritoneum [20], liver [64], and lung [35]. Here, we synthesize current evidence regarding the role of MCL/ACT001 in age-related fibrosis and clarify its underlying mechanisms (Fig. 6).

4.1 Renal fibrosis

The aging kidney is defined by the buildup of senescent cells and chronic low-grade inflammation, which fuel renal fibrosis and result in functional decline [102]. Renal fibrosis is the convergent pathway where chronic kidney disease progresses to end-stage renal failure. Within the renal tubular interstitium, scar tissue slowly crowds out the interstitium, glomerulosclerosis, tubular atrophy, capillaries vanish, and chronic interstitial inflammation [103]. One in every ten adults has chronic kidney disease, a condition that silently causes 1.2 million deaths annually [104]. Renal tubular epithelial cells (RTECs), the primary constituents of the renal parenchyma, are increasingly considered the primary initiators of fibrotic processes [105]. During renal fibrosis pathogenesis, RTECs undergo EMT, a process defined by phenotypic shifts including the downregulation of adhesion molecules such as E-cadherin and the upregulation of mesenchymal markers including α -SMA and vimentin.

In unilateral ureteric obstruction (UVO) and ischemia–reperfusion injury renal fibrosis models, daily administration of ACT001 markedly reduced serum creatinine and urea nitrogen levels, attenuated renal collagen deposition, downregulated α -SMA and fibronectin expression, and restored E-cadherin expression, indicating its improvement of renal function and structure [12]. *In vitro*, MCL dose-dependently downregulated the increase of α -SMA and fibronectin induced by TGF- β 1 and reversed it by E-cadherin reduction, confirming its direct intervention effect on RTECs EMT. Mechanistically, MCL directly inhibited the expression of the profibrotic mediator Mtdh, dismantling its downstream BMP/MAPK axis, thereby suppressing renal tubular EMT. This effect can be abrogated by Mtdh overexpression, indicating that Mtdh is a crucial molecular target of MCL in the kidney. Specifically, Mtdh overexpression decreased BMP7 and BMPRIa expression and elevated p-Smad1/5/9 levels [12, 41]. IL-11 is an essential profibrotic effector operating downstream of TGF- β 1 [106, 107]. Li et al. reported that IL-11 and IL-11R α 1 were markedly elevated in UVO-induced renal fibrosis [21]. Virtual molecular docking studies demonstrated that MCL stabilizes within the hydrophobic cavity of IL-11 by forming hydrogen bonds with active site residues ARG77, PRO132, and ASP134 through its hydroxyl and carbonyl groups. This interaction significantly disrupted the binding stability

between IL-11 and IL-11R α 1. Consistently, ACT001 significantly reduced IL-11 and IL-11R α 1 expression and mitigated the UVO-induced renal fibrosis. Mechanistically, MCL competitively occupies the IL-11 ligand-binding interface, preventing IL-11 from engaging IL-11R α 1 and thereby inhibiting STAT3 and ERK1/2/Mtdh signaling and alleviating IL-11-induced EMT and renal fibrogenesis [21]. These results refine the understanding of molecular mechanisms driving renal fibrosis and unveil a readily translatable therapeutic strategy for this intractable condition.

4.2 Peritoneal fibrosis

Peritoneal dialysis is a widely used renal replacement therapy for approximately 3.8 million patients with end-stage kidney disease globally [108]. Peritoneal fibrosis (PF) is a common complication of long-term peritoneal dialysis, leading to peritoneal damage and loss of peritoneal ultrafiltration. The hallmark features of PF include mesothelial-mesenchymal transition, chronic inflammatory response, and aberrant angiogenesis. Accordingly, there is a critical need for effective novel therapeutic strategies to prevent and mitigate peritoneal dialysis-related PF. Emerging studies demonstrated that MCL/ACT001 can alleviate PF. On the one hand, ACT001 treatment prevented PF in the animal peritoneal dialysis model. Prophylactic administration of ACT001 blunted the anticipated increase in α -SMA, fibronectin, and collagen I and restored E-cadherin, thereby reducing the PM thickness in the anterior abdominal wall of mice treated with peritoneal dialysis fluid. On the other hand, delayed ACT001 treatment, initiated after fibrotic lesions were already established, prevented PF progression [20]. MCL inhibited ECM deposition and reversed EMT by activating autophagy. Genetic silencing of ATG7 abolished the protective effect, indicating that autophagy activation is an obligate step in MCL-mediated antifibrotic effects. Another study identified an additional mechanism of action. MCL alleviated the PF caused by BRG1 overexpression and inhibited the TGF β 1-Smad2/3 signaling pathway [43]. Mechanistically, MCL targeted the asparagine (N1540) of BRG1, thereby preventing its interaction with the H3K14ac site on histone H3, inhibiting the fibrotic responses. Mutation of N1540 to alanine abolishes MCL binding and negates its inhibitory effects on BRG1-driven ECM production.

4.3 Hepatic fibrosis

Hepatic fibrosis is a common pathological hallmark of chronic liver injury. Globally, approximately 844 million individuals are affected by chronic liver disease, with an estimated 2 million deaths annually [109]. As most

patients present with established liver fibrosis/hyperplasia at the time of diagnosis, there is a critical need for therapeutic agents capable of halting or reversing fibrotic progression. Accumulating evidence suggests that MCL/ACT001 exerts protective effects against the progression of hepatic fibrosis. In the experimental model, ACT001 significantly reduced inflammatory infiltrates and hepatocyte degeneration, restored hepatic lobular architecture, and decreased collagen fiber deposition in the liver fibrosis model. Additionally, MCL improved liver function, as indicated by reduced serum levels of alanine aminotransferase, aspartate aminotransferase, and hydroxyproline, and downregulation of collagen type I alpha 1 and fibronectin expression in liver tissue [64]. Activated hepatic stellate cells (HSCs) are the principal mediators of hepatic fibrogenesis. MCL targets HSCs through multiple mechanisms. First, it induces G2/M phase cell cycle arrest by reducing CDK1 and c-Myc levels, thereby inhibiting HSC proliferation. Second, MCL downregulated ECM components and promoted collagen degradation by elevating MMP-1, -3, and -14 mRNA, inhibiting HSC activation [64]. Besides, MCL disrupts mitochondrial function, as evidenced by decreased mitochondrial membrane potential and structural alterations, which in turn activate MEK/ERK signaling and promote autophagy. Furthermore, MCL has been shown to attenuate carbon tetrachloride (CCl₄)-induced liver injury and fibrosis through activation of SIRT1 signaling [110].

4.4 Pulmonary fibrosis

Pulmonary fibrosis includes a group of chronic interstitial lung disorders characterized by the progressive ECM remodeling and replacement of functional parenchyma, leading to alveolar destruction, reduced lung compliance, impaired gas exchange, and respiratory failure and death [111]. Idiopathic pulmonary fibrosis, the common form, affects approximately 3 million individuals globally, with an increasing incidence with advancing age [112]. Fibroblasts are dysregulated in IPF. NF- κ B signaling mediates fibroblast-to-myofibroblast transition (FMT), associated with fibroblast accumulation in IPF tissue, and drives ECM deposition [113, 114]. Therefore, NF- κ B inhibition is a rational strategy to attenuate pulmonary fibrosis. Pharmacological blockade of NF- κ B with ACT001 in primary IPF fibroblasts suppresses cell proliferation and viability, reduces fibronectin deposition, and inhibits TGF- β 1-driven FMT and IL-6 production [114]. Similar, although less pronounced, effects were observed in age-matched normal fibroblasts. Relative to normal fibroblasts, IPF fibroblasts exhibit increased sensitivity to ACT001, requiring three-fold lower concentrations to inhibit TGF β 1-driven FMT and ten-fold

lower doses to inhibit FBS-induced NF- κ B phosphorylation. These differential responses suggest a therapeutic window in which ACT001 preferentially targets pathological fibroblasts and spares normal cells, thereby enhancing its therapeutic index for IPF and minimizing off-target effects. In addition to IPF, ACT001 has demonstrated efficacy in radiation-induced pulmonary fibrosis (RIPF), a severe complication of thoracic radiotherapy characterized by progressive fibrotic remodeling of irradiated lung tissue [115]. The pathogenesis remains poorly understood, and effective clinical interventions are lacking. Consequently, RIPF carries a dismal prognosis. A recent study demonstrated that ACT001 attenuated radiation-triggered EMT and ECM accumulation, limiting fibrotic remodeling. Mechanistically, ACT001 suppressed ROS generation and subsequently downregulated TGF- β 1 expression, highlighting ACT001 as a potential preventive and therapeutic agent for RIPF [17].

5. Role of MCL/ACT001 in age-related metabolic diseases

Advancing age is the principal risk factor for metabolic disorders, including type 2 diabetes mellitus (T2DM) and metabolic dysfunction-associated fatty liver disease (MAFLD). Aging is accompanied by a preferential redistribution of adipose tissue to the visceral compartment, a shift closely associated with insulin resistance and the pathogenesis of T2DM, fatty liver disease, and metabolic complications [116]. Accumulating evidence indicates that MCL/ACT001 effectively lowers hyperglycemia, ameliorates metabolic-related hepatic and renal dysfunction, and exerts robust anti-inflammatory and anti-oxidative actions, highlighting its potential to attenuate age-related metabolic disturbances (Fig. 7).

5.1 Diabetes

The prevalence of T2DM increases progressively with age, peaking in adults aged 75–79 years [117]. In 2021, approximately 529 million people were living with diabetes globally, with a particularly high burden among those aged 65 years [118]. Safety concerns associated with conventional antidiabetic therapies have intensified interest in identifying safer and more effective alternatives. Emerging evidence indicates that ACT001 exerts beneficial metabolic effects by reducing blood glucose, insulin levels, and body weight and improving glycemic dysregulation and insulin resistance conditions in high-fat-diet-induced T2DM mice. ACT001 reduced hepatic gluconeogenesis by down-regulating glucose-6-phosphatase (G6Pase) and FoxO1 expression [119].

Additionally, lipogenic drivers fatty acid-binding protein 4 (FABP4) and PPAR γ were significantly reduced, whereas SREBP1c was increased, suggesting a metabolic shift away from lipid storage. Histologically, ACT001 ameliorated high-fat-diet-induced hepatic injury; hepatocyte nuclear crowding and large lipid droplets were

significantly reduced; adipocyte size normalized; and liver integrity improved. Moreover, ACT001 improved circadian rhythm through upregulation of core clock genes, including *CRY1*, *CRY2*, *PER1*, and *PER2*, stabilizing whole-body metabolic homeostasis [119].

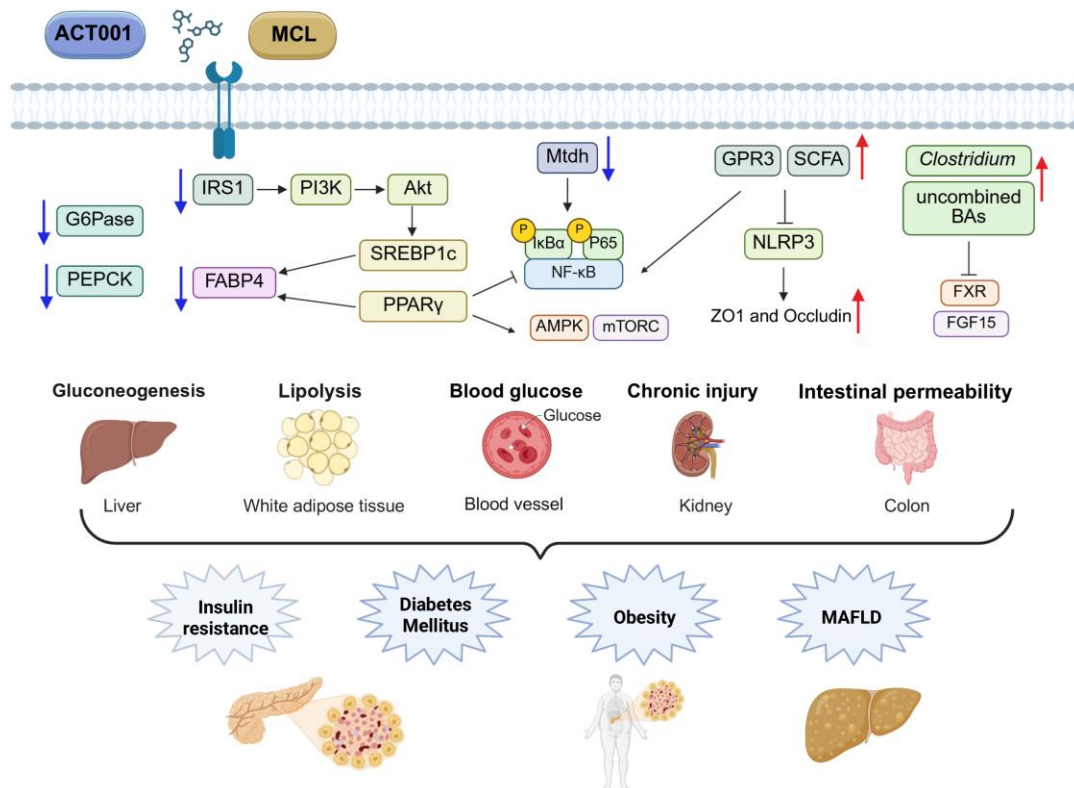


Figure 7. Potential mechanisms of MCL/ACT001 in age-related metabolic diseases. MCL effectively lowers hyperglycemia, ameliorates metabolic-related hepatic and renal dysfunction, and exerts robust anti-inflammatory and anti-oxidative actions, positioning it as a promising therapeutic option for mitigating age-related metabolic dysfunction. ACT001 markedly reduces blood glucose levels in diabetic mice, primarily by downregulating the expressions of phosphoenolpyruvate carboxykinase 2 (PEPCK) and glucose-6-phosphatase (G6Pase). Besides, ACT001 decreased the level of FABP4. Moreover, MCL ameliorated diabetic kidney disease through inhibiting Mtdh-mediated renal inflammation in type 2 diabetic db/db mice. Additionally, MCL alleviated hepatic steatosis in db/db mice through suppressing inflammation and promoting autophagy via PPAR γ -mediated NF- κ B and AMPK/mTOR signaling. Furthermore, ACT001 exerted a multifactorial therapeutic effect on MAFLD, potentially linked to the regulation of the gut microbiota-bile acids (BAs)-farnesoid X receptor (FXR)-fibroblast growth factor 15 (FGF15) pathway. The figure was created using BioRender.

Beyond glycemic regulation, MCL markedly attenuates diabetic microvascular complications. MCL treatment significantly restores serum electrolyte balance and lipid profiles of T2DM rats, improves kidney function metrics, and reduces NGAL and KIM-1 levels. Mechanistically, MCL bolstered antioxidant defenses by activating the KEAP1/NRF2 axis and inhibiting NOX1 and NOX4 expression and concurrently exerting anti-inflammatory actions through the inhibition of the TLR4/MyD88/NF- κ B cascade, resulting in diminished TNF- α , IL-6, IL-1 β , and IFN- γ levels and elevated IL-10

[30]. Additionally, ACT001 conferred renoprotection by transcriptionally repressing Mtdh and enhancing its ubiquitin-proteasomal degradation, reducing proteinuria, histological injury, and renal inflammation [10]. Furthermore, MCL decreased body and liver weight, food consumption, circulating lipids, transaminases, and hepatic lipid accumulation and alleviated steatosis in db/db mice. These effects were mediated by PPAR- γ upregulation, which suppressed NF- κ B-mediated inflammation and activation of AMPK/mTOR-dependent autophagy, thereby alleviating hepatic steatosis [19].

Additionally, ACT001 ameliorated colitis and enhanced the intestinal mucosal barrier through the modulation of the GPR43 and AMPK signaling pathways, decreased renal lipid deposition, suppressed oxidative stress and inflammation, and attenuated obesity-related kidney disorder [120].

5.2 MAFLD

The term “non-alcoholic fatty liver disease” has been replaced by “MAFLD” following international consensus recommendations [121]. MAFLD affects approximately 38% of the global population and represents a primary contributor to the global rates of chronic liver disease-related sickness and mortalities [122]. Advancing age is a major risk factor. With the demographic shift, MAFLD in older adults will become a dominant public-health challenge, imposing heavy clinical and economic burdens [123]. Accordingly, it is imperative to determine feasible and cost-effective drug treatment targets for MAFLD. Preclinical evidence suggests that ACT001 has significant therapeutic potential for MAFLD treatment. ACT001 markedly ameliorates high-fat-diet-induced hepatocyte injury and dyslipidemia. Histologically, it reduced hepatic collagen deposition, decreased lipid vacuoles, and downregulated serum alanine aminotransferase, aspartate transaminase, total cholesterol, and triglycerides. ACT001 alleviated MAFLD by regulating linoleic acid and glutathione metabolic pathways, implicating lipid metabolism and oxidative stress as key mechanistic nodes [124]. Niu et al. demonstrated that ACT001 ameliorated MAFLD through the gut-microbiota/bile-acid/FXR axis, restoring intestinal-barrier integrity [125]. 16S rDNA sequencing revealed partial rebalancing of dysbiosis, with increased relative *Clostridium* and *Erysipelotrichaceae_Clostridium* abundance.

Additionally, ACT001 promoted the generation of unconjugated bile acids and the accumulation of specific bile acid species, including 23-Nor-deoxycholic acid and lithocholic acid-3-sulfate, in the ileum. These changes downregulated the FXR/fibroblast growth factor 15 signaling pathway, contributing to reduced hepatic lipid-lowering and improved metabolic homeostasis.

Non-alcoholic steatohepatitis (NASH) is an advanced stage of non-alcoholic fatty liver disease defined by hepatic steatosis exceeding 5% plus lobular inflammation and hepatocyte injury, irrespective of the presence or absence of fibrosis [126]. ACT001 markedly reduced liver steatosis, inflammation, and fibrosis and suppressed NLRP3 activation and oxidative stress; however, its precise molecular basis remained unclear. Isotope labeling and relative and absolute quantification technique analysis identified 381 differentially expressed proteins (162 up-, 219 down-regulated) between NASH

and ACT001-treated mice [127]. Functional enrichment analysis revealed that these proteins are primarily involved in pathways related to transcriptional regulation, JAK/STAT and PI3K/Akt axes, EGFR-tyrosine-kinase inhibition, oxidative phosphorylation, autophagy, NAFLD pathways, diabetic cardiomyopathy, thermogenesis, and ROS-driven carcinogenesis. Among these, COL6A2, APOA1, MAVS, PPIB, and CES2A were identified as novel putative targets through which ACT001 exerts its anti-NASH efficacy.

5.3 Osteoporosis

Aging is a major determinant of bone deterioration [128]. Osteocyte function is hindered by the accumulation of senescent cells and chronic low-grade inflammatory responses, leading to progressive bone mineral loss, micro-architectural degradation, and increased fracture risk. Osteoporosis is the decrease in bone mineral density and the gradual bone tissue degeneration, which increases the incidence of fractures and osteoporosis [129]. The lifetime fracture risk associated with osteoporosis is as high as 40%, and the 12-month additional mortality rate can reach up to 20%. Recent studies indicate that MCL exerts protective effects against estrogen deficiency-induced bone loss in mice by suppressing osteoclastogenesis and osteoclast absorption through the P38 MAPK signaling pathway [44]. Micro-CT revealed that MCL treatment increased bone mineral density, bone volume/bone surface area, trabecular number, and trabecular thickness and reduced trabecular separation. Consistently, serum CTX-1 and histomorphometric parameters N.Oc/BS and Oc.S/BS were markedly decreased, suggesting suppressed osteoclastic resorption without adverse effects on bone formation indices. Moreover, MCL suppressed the transcription of genes associated with osteoclastogenesis, notably Trap, Cathepsin K, NFATc1, C-fos, and DC-STAMP [44].

6. Role of MCL/ACT001 in age-related immune diseases

6.1 Rheumatoid arthritis (RA)

RA is a systemic autoimmune disease that affects approximately 0.25%–1% of the global population and is associated with a reduction in life expectancy of 1–10 years [130, 131]. Beyond polyarticular pain, RA frequently complicates cardiovascular and pulmonary systems and accelerates severe disability. Accelerated biological aging has been established as a risk factor for age-related morbidities, increasing RA risk [132]. Preclinical studies indicate that MCL can alleviate RA. MCL treatment markedly decreased paw swelling in RA

mice and inhibited joint cartilage degeneration [22]. In addition, MCL upregulated the serum levels of complement component 5a, macrophage colony-stimulating factor, and tissue inhibitor of metalloproteinase-1 in arthritis mice. Moreover, Liu et al. developed a dual-functional nanoplateform (Janus-CPS), consisting of CeO₂-Pt nanoenzyme components and the periodic mesoporous organosilica subunits, enabling efficient MCL loading and delivery into the mesopores of the periodic mesoporous organosilica subunits, achieving synergistic anti-inflammatory and anti-oxidative action that significantly enhances RA therapeutic efficacy, positioning MCL as a promising oral or nano-delivered candidate for mitigating RA-related joint damage [133].

6.2 Ankylosing spondylitis (AS)

AS is a chronic inflammatory disease characterized by chronic inflammation and aberrant new bone formation. Increasing evidence implicates oxidative stress and mitochondrial dysfunction as key contributors to disease development [134]. MCL is involved in attenuating AS by blocking NLRP3-driven pyroptosis, restoring Th1/Th2 equilibrium, and interrupting the NF- κ B signaling cascades [135]. In murine models of AS, MCL treatment significantly reduced serum concentrations of IL-1 β , IL-6, IL-18, TNF- α , and IFN- γ and simultaneously suppressed NLRP3 and caspase-1 expression. Mechanistically, MCL blocked NLRP3 activation and inhibited the NF- κ B pathway by reducing p-p65 levels and elevating p-I κ B α .

7. Role of MCL/ACT001 in age-related cancers

Aging and cancer are closely associated processes [136]. Carcinomas and most malignancies, including GBM, lymphomas, melanomas, leukemias, and sarcomas, generally do not exhibit symptoms until after the age of 50, with incidence increasing steadily to 85 years [137]. Senescent-cell accumulation drives systemic degeneration, promotes age-related comorbidities, and facilitates tumor progression [138]. Conversely, lifestyle measures that delay biological aging have been associated with reduced cancer risk [139]. Natural products are life-saving therapeutics. Accumulating data indicate that MCL/ACT001 suppressed the onset of several cancers, including GBM, hepatocellular carcinoma (HCC), lung cancer, and breast cancer, by inducing apoptosis, inhibiting cellular proliferation, blocking tumor growth and metastasis, reducing drug resistance, mitigating chemotherapy/radiation side effects, and potentiating conventional anticancer regimens.

7.1 GBM

GBM is nearly uniformly fatal, with a median survival of 14.6–16.8 months and a 2-year rate of 13%–26%. Despite surgery and radiochemotherapy, virtually all patients relapse. Although ACT001 readily crosses the blood–brain barrier and has demonstrated clinical efficacy against GBM, its precise molecular and cellular mechanism remains incompletely defined. *In vitro* studies demonstrated that ACT001 suppressed GBM cells proliferation. Mechanistically, ACT001 directly binds to IKK β , suppressing its phosphorylation and thereby inhibiting its canonical NF- κ B, increasing ROS levels, and driving ROS-mediated G2/M phase arrest and cell apoptosis [61]. Additionally, ACT001 interacts with STAT3, inhibiting its phosphorylation and PD-L1 expression, which reduces M2 macrophage infiltration and boosts T cell responses [140]. Cellular thermal-shift assays have identified PAI-1 as a direct target of ACT001. Through modulation of the PAI-1/PI3K/Akt signaling axis, ACT001 suppresses GBM proliferation, invasion, and migration [141]. GAPDH is elevated in GBM and functions as a critical driver of disease development. GAPDH knockdown diminished MCL-mediated GBM cell growth suppression. MCL is covalently bound to Cys247 of GAPDH, initiating non-glycolytic-related functions in GAPDH to enhance glioma cell death. Notably, MCL left GAPDH enzymatic function unaffected and facilitated the nuclear import of the GAPDH/Siah1 complex [47]. Glioma-like stem cells, a subpopulation of GBM cells involved in tumor initiation, radiochemotherapy resistance, and recurrence, are targeted by ACT001 [142]. Moreover, ACT001 suppressed the proliferation of glioma-like stem cells and the formation of glioma spheres by targeting AEBP1/PI3K/Akt signaling [143]. Temozolomide (TMZ) is the standard-of-care chemotherapeutic for gliomas, but acquired resistance invariably limits its efficacy. ACT001 therapy significantly enhanced TMZ-resistant glioma cells' sensitivity to TMZ, enhancing cell death and inhibiting cell proliferation. MDK is a potential biomarker for GBM, and targeting MDK may be beneficial for glioma treatment. Mechanistically, ACT001 enhanced the c-Myc degradation by targeting the MDK/c-Myc complex and controlled the Wnt/ β -catenin signaling through MDK, preventing glioma progression [144].

Furthermore, combined treatment may be more effective than single treatment. Studies demonstrated that GBM cannot be completely eradicated solely by ACT001 and MCL. Therefore, combining ACT001 with other therapies may be valuable. SHP099 is an efficient and orally available SHP-2 inhibitor that can inhibit multiple cancer progression. Hou et al. reported that combined SHP099 and ACT001 treatment markedly reduced p-

AKT levels and glioma-like stem cell proliferation compared with monotherapy [143]. Moreover, this combination regimen diminished AEBP1, Ki-67, and hNestin expression and tumor burden and prolonged survival compared with ACT001 monotherapy.

7.2 HCC

Globally, primary liver cancer is the eighth most common neoplasm and the third leading cause of cancer-related deaths, with HCC accounting for approximately 80% of cases [145]. Although curative hepatectomy followed by systemic or loco-regional chemotherapy remains the mainstay treatment, recurrence is frequently due to the emergence of drug resistance and tumor escape mechanisms. Identification of effective anticancer targets coupled with the discovery of their potential modulators represents a compelling strategy for advancing future oncology therapeutics. Experimental studies have demonstrated that MCL significantly suppresses HCC cell proliferation, invasion, and cancer stemness [146]. After 4 h of treatment with MCL, mitochondrial ROS are markedly elevated, followed by intense apoptosis at 8 h. Morphologically, untreated Huh7 cells exhibit stretched filamentous F-actin cables and evenly distributed mitochondria. MCL treatment induced F-actin fragmentation and disorganization, accompanied by mitochondrial aggregation. These data revealed that MCL disrupted the F-actin cytoskeleton, triggered abnormal mitochondrial aggregation, provoked mitochondrial dysfunction, and consequently induced apoptosis. Pretreatment with the NAC antioxidant markedly attenuated MCL-induced apoptosis [146]. Immunogenic cell death (ICD), a distinctive, immunity-activating type of apoptosis, provides a means to eliminate residual disease and generate durable anti-tumor memory [147]. ICD facilitates the clinical efficacy of chemotherapy drugs by enhancing long-term cancer immune responses. ICD inducers are recognized as promising and potential strategies in HCC. Additionally, MCL triggered ICD-related damage-related molecular patterns, including HMGB1 release, ATP secretion, CRT expression, and increased HSP90 levels. MCL caused tumor regression, promoted dendritic cell maturation, and enhanced CD4⁺ and CD8⁺ T cell responses, reshaping the immune microenvironment and inhibiting tumor growth without damaging heart, liver, spleen, or lung tissue. Additionally, tumor angiogenesis is significantly associated with the tumor immune microenvironment. MCL dose-dependently suppresses angiogenesis in zebrafish models. The extent of MCL-induced ICD effects is closely dependent on ROS-mediated ERS. Post-MCL treatment, the endoplasmic-reticulum lumen swelling or expansion and calcium-binding protein, p-eIF2 α , ATF4, Grp78, and

CHOP increase in a dose- and time-dependent manner [18]. Scavenging ROS abolished MCL-induced ERS, whereas TrxR knockdown synergistically amplifies it. Therefore, MCL functions as a redox- immunogenic cell death-inducing agent with promising therapeutic potential for HCC.

7.3 Lung cancer

Lung cancer remains the leading cause of cancer-related morbidity and mortality globally, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of cases [148]. Lung cancer stem cells represent a distinct subpopulation responsible for tumor initiation, recurrence, metastasis, and poor prognosis, presenting a formidable therapeutic obstacle. Consequently, the selective eradication of lung cancer stem cells represents a compelling therapeutic strategy. ACT001 directly binds to Olig2, enhancing its ubiquitin-dependent degradation and suppressing CD133 promoter activity, thereby reducing stem-like properties of lung cancer [149]. Besides, ACT001 inhibits NSCLC cell proliferation and G1/S phase transition through upregulation of NKTR [150]. Moreover, ACT001 markedly attenuated PD-L1 expression by inhibiting p-STAT1 and p-STAT3, enhancing T cell activation and promoting apoptosis [151]. Furthermore, MCL promotes ubiquitin-dependent degradation of HIF-1 α , inhibiting HIF-1 α and its downstream target vascular endothelial growth factor, making p53-deficient NSCLC cells more sensitive to radiotherapy [152]. MCL and ACT001 may serve as an effective radiosensitizer to augment the efficacy of radiation therapy in NSCLC.

Small-cell lung cancer (SCLC) remains a treatment-resistant malignancy, with a bleak 5-year overall survival rate of 12%–30% [153]. Tumor cells alter their metabolism toward aerobic glycolysis, leading to the accumulation of tumor metabolites, including lactic acid, and establishing the tumor's immune microenvironment. Consequently, targeting this metabolic pathway presents a compelling strategy to rejuvenate anti-tumor immunity. Studies demonstrated that ACT001 inhibits lactic acid production by targeting phosphoglycerate kinase 1 (PGK1), thereby affecting its downstream p-PGK1/p-PDHK axis, diminishing M2 macrophage infiltration, and augmenting M1 macrophage functionality [154]. Mechanistically, ACT001 covalently binds to Cys316 of PGK1, thereby inhibiting PGK1 activity and suppressing mitochondrial pyruvate metabolism. These findings indicate that MCL/ACT001 may possess potential therapeutic value and good application prospects in lung cancer.

7.4 Breast cancer

Breast cancer remains the most common malignancy in females and the leading cause of cancer-related mortality, accounting for over 15% of global cancer deaths. Approximately 70% of cases are estrogen-receptor-positive breast cancer. Endocrine therapy serves as a primary intervention for estrogen receptor-positive breast cancers, with tamoxifen being the most commonly prescribed endocrine agent. Approximately 50% of the patients demonstrate clinical tamoxifen resistance [155]. Under tamoxifen stimulation, the resistant breast cancer cells MCF7 and T47D exhibited a high oxidative state and hyperactive amidohydrolase 1. MCL activated the cellular antioxidant system through the NRF2/KEAP1 pathway and suppressed amidohydrolase 1 via the ROS/AKT axis, thereby inhibiting cell proliferation [38]. In addition, ACT001 inhibited and reversed tamoxifen resistance by directly inhibiting NF- κ B activation [156]. Besides, MCL ameliorated KLF4-driven cisplatin resistance by downregulating glutathione biosynthesis [157]. Triple-negative breast cancer, accounting for approximately 15% of all breast cancer cases, is notably aggressive [158]. Liu et al. demonstrated that ACT001 upregulated pro-apoptotic Bax, inducing apoptosis in 4T1 TNBC cells, and simultaneously inhibited p-NF- κ B and I κ B- α , resulting in decreased GM-CSF secretion, fewer myeloid-derived suppressor cells, and diminished angiogenesis [159]. Collectively, MCL/ACT001 targets oxidative, inflammatory, and drug resistance pathways in various breast cancer subtypes, providing rational combination strategies to circumvent chemoendocrine resistance and enhance breast cancer outcomes.

7.5 Other cancers

Prolactinoma is the predominant subtype of pituitary adenoma. Although 75%–90% of patients with prolactinomas experience clinical benefit from dopamine-agonist therapy, a distinct subset exhibits negligible or no response to these agents, highlighting the need for alternative therapeutic strategies. Cai et al. reported that ACT001 potently suppressed proliferation and tumor growth by activating MEK4-mediated p38/JNK phosphorylation, inducing autophagic cell death with hallmark features of LC3-II accumulation and autophagosome formation [68]. Moreover, ACT001 mitigated dopamine-agonist resistance by increasing intracellular ROS and enhancing the AMP/ATP ratio, consequently activating AMPK signaling. Specifically, ACT001 countered cabergoline resistance in GH3 cells through mTOR inhibition and autophagy induction, whereas bromocriptine resistance in MMQ cells is mitigated through EGR1-mediated apoptosis [70].

Acute myeloid leukemia (AML) is a clonal malignancy of the hematopoietic system that disrupts the normal hematopoietic process and culminates in bone marrow failure and mortality. Approximately 4.1 new cases are reported annually for every 100,000 people, increasing significantly after age 65 [160]. ACT001 decreased the leukemic-stem-cell fraction (CD34⁺CD38⁻) in primary AML samples and demonstrated potent therapeutic efficacy [161]. In addition, MCL selectively activated PKM2 by covalently binding Cys424, promoting irreversible tetramer formation, blocking lysine433 acetylation, and hindering nuclear translocation of PKM2, thus markedly inhibiting leukemic-cell proliferation and leukemogenesis [14]. Furthermore, Guo et al. reported that the oxidative stress caused by MCL-induced GSH depletion culminates in mitochondrial damage and leukemia cell apoptosis [60]. Primary central nervous system lymphoma is a highly aggressive subtype of non-Hodgkin lymphoma that predominantly affects older adults [162]. ACT001 markedly suppressed tumor growth by downregulating PD-1/PD-L1 signaling [163].

Pancreatic ductal adenocarcinoma (PDAC) remains a major oncological challenge, resulting in over 400,000 lives annually, with a 5-year survival rate below 10% and increasing. MCL/ACT001 dose-dependently suppressed PDAC growth, inhibiting migration, enhancing apoptosis, and increasing intracellular ROS levels [164]. Mechanistically, MCL demonstrated anti-tumor efficacy by modulating the EGFR-Akt-Bim axis and activating the MAPK pathway to induce ferroptosis, paraptosis, and autophagy in colonic and pancreatic cancer cells [16]. On the one hand, MCL is implicated in regulating ferroptosis by decreasing SLC7A11, increasing TFRC, and elevating intracellular iron levels. On the other hand, MCL facilitated paraptosis by diminishing Alix and increasing ATF4 and CHOP expression. Additionally, MCL induced autophagy, as indicated by increased levels of Beclin-1, p-Beclin-1, and ATG7 [16]. Moreover, MCL inhibits gastric cancer cell proliferation and facilitates apoptosis through the IL6/STAT3 axis, suppressing tumor growth [165].

Collectively, by inhibiting NF κ B, ACT001 reduces the DNA repair capacity of cancer cells following chemoradiotherapy, resulting in synergistic radiosensitization. Moreover, by inhibiting STAT3 activation, ACT001 synergizes with immune checkpoint inhibitors. Both signaling pathways participate in various inflammation-related chronic pathological processes and in the onset of and resistance to primary and metastatic cancers. Beyond regulating T cell activity and the tissue microenvironment by inhibiting NF- κ B and STAT3 signaling, ACT001 stimulates the NRF2 signaling pathway to diminish ROS levels, thereby protecting normal tissues, including lymphocytes.

8. Therapeutic potential of MCL/ACT001 for age-related diseases

8.1 Pharmacokinetics and stability of MCL/ACT001

MCL, a naturally occurring guaianolide sesquiterpene lactone, is derived from *Michelia compressa* and *Michelia champaca* plants. Additionally, it can be synthesized from parthenolide. Its derivative, DMAMCL, is produced as a dimethylamino Michael adduct of MCL. ACT001 denotes the fumarate salt variants of DMAMCL and is an investigational compound presently undergoing clinical evaluation. Compared to MCL, DMAMCL and ACT001 demonstrate superior stability, increased efficacy, and diminished cytotoxicity toward normal cells. Furthermore, ACT001 and DMAMCL gradually and continuously release MCL *in vivo* for up to 8 h. This sustained-release profile preserves therapeutically relevant plasma concentrations and facilitates efficient blood–brain-barrier penetration [161]. Notably, DMAMCL demonstrates markedly reduced activity *in vitro* [161]. These compounds possess an α -methyl- γ -lactone pharmacophore, with the exocyclic methyl lactone moiety functioning as a Michael acceptor. This group participates in hetero-Michael addition with intracellular thiols (glutathione) and, as previously established, allows the compounds to covalently target proteins possessing accessible cysteine residues [166, 167].

Toxicological studies in BALB/c mice demonstrated excellent oral safety. Single gavage doses up to 500 mg/kg or 1000 mg/kg daily for 7 consecutive days resulted in no histopathological alterations in lung, heart, kidney, liver, and spleen. The maximum tolerated dose of DMAMCL administered by intravenous injection was approximately 200 mg/kg. The significant differences in maximum tolerated dose between oral and intravenous routes can be attributed to divergent pharmacokinetic profiles of DMAMCL. Intravenous administration resulted in distinct peak-and-trough plasma concentration fluctuations, whereas oral administration enabled sustained absorption and gradual release [161]. Accordingly, oral DMAMCL/ACT001 exhibits convenient administration and high tolerability, supporting its clinical development across systemic and central nervous system indications [167].

8.2 Early-stage therapeutic potential and clinical development status of ACT001

In the previous sections, we have systematically examined the current preclinical evidence. We summarized the early-stage therapeutic potential and clinical development status of ACT001, with clinical evidence primarily

presented in Table 2, which provides a comprehensive overview of ACT001 clinical trials. ACT001 is a promising therapeutic candidate for GBM, having obtained orphan drug designation from the United States FDA in 2017 and the EMA in 2018. Furthermore, in 2022, the FDA expanded this designation to encompass diffuse midline glioma. As one of the few clinically accessible immunomodulators that readily crosses the blood–brain barrier, its development focuses on brain tumors, fibrosis, and neuroinflammatory disorders.

In the Phase I clinical trial (ACTRN 12618001934235), ACT001 exhibited a favorable safety profile and significant anti-tumor activity in patients with diffuse midline gliomas [168]. Subsequent interim findings from a Phase II trial demonstrated that combining ACT001 with radiotherapy was well tolerated. Notably, preliminary evidence of therapeutic efficacy was observed in approximately 50% of efficacy-evaluable patients with diffuse intrinsic pontine glioma [169]. In patients with malignant glioma receiving doses of 400 mg twice daily or lower, the drug exhibited favorable bioavailability and preliminary signs of anti-tumor activity [170, 171]. Furthermore, a Phase II trial examining the combination of ACT001 and TMZ in recurrent GBM revealed that ACT001 treatment restored TMZ sensitivity in these patients [172]. Moreover, a Phase Ib/IIa trial (ChiCTR2000037739) evaluated the efficacy and safety of ACT001 combined with whole-brain radiation therapy in 45 patients with solid tumors and brain metastases. The study cohort comprised 38 patients with lung cancer, comprising 14 cases of NSCLC and 22 cases of SCLC; 7 patients with breast cancer, and 2 patients with other malignancies. The results revealed that ACT001 seems to ameliorate the adverse events associated with whole-brain radiation therapy and reduce the intracranial tumor metastasis load [173]. Furthermore, for patients with progressive fibrosing interstitial lung disease (fILD), including IPF, the current treatment options are limited, and specific patient groups cannot tolerate the approved treatment methods. A Phase II study (ACTRN12619001643167) evaluating the tolerability and safety of ACT001 in the treatment of adult patients with fILD and IPF included 44 cases (35 cases with IPF and 9 with fILD). All patients had received at least one administration. The study population had a mean age of 70.0 years, with male patients comprising 75.0% of the cohort. An interim analysis in week 52 involving 40 efficacy patients (4 fILD and 7 IPF in Arm 1 and 3 fILD and 26 IPF in Arm 2) revealed that the average baseline FVC% pred was 75.3%. Overall, ACT001 treatment reduced the deterioration rate of pulmonary function test indicators within 52 weeks. In multiple subgroups of patients with IPF and fILD, approximately 50% of the patients exhibited stabilization of their condition [174].

This 52-week exposure significantly exceeded the 12–28-week durations of global IPF phase II studies, providing a more robust assessment of efficacy and safety and markedly reducing the risk of further clinical trials. These

data robustly advocate for ACT001 progression into extensive, controlled trials to confirm its therapeutic potential in pulmonary fibrosis.

Table 2. Clinical trials of ACT001.

Registration number	Condition	Administration dosage	Phase
ChiCTR-OIC-17013604	Glioblastoma	Not reported	I
ChiCTR2000031254	Recurrent neuromyelitis optica spectrum disorder-related optic neuritis	200 mg/day, 400 mg/day, 800 mg/day, 1200 mg/day	I/IIa
ChiCTR2000031532	Solid tumors and brain tumors	376 mg/m ² /day, 470mg/m ² /day, 590mg/m ² /day	I/IIa
ChiCTR2000035315	Recurrent glioblastoma	400 mg BID	II
ChiCTR2000037739	Brain metastases from solid tumors	200mg BID, 400 mg BID	Ib/IIa
ChiCTR2300067517	Myelofibrosis	Not reported	II
ChiCTR2300069129	Lung cancer patients with brain metastasis	400 mg BID	IIb/III
ChiCTR2300074598	Diffuse intrinsic pontine gliomas	Not reported	II
ChiCTR2500108747	Brain metastasis of small cell lung cancer	400 mg BID	III
ACTRN12619001643167	Progressing fibrosing interstitial lung disease and idiopathic pulmonary fibrosis	400 mg/day	II
ACTRN12621001172897	Solid tumors	400 mg/day	I
ACTRN12621000093886	Brain metastases from solid tumors	400 mg BID	II
ACTRN12618001934235	Brain and solid tumors	118 mg/m ² BID, 425mg/m ² BID, 530 mg/m ² BID, 700 mg/ m ² BID, 875 mg/m ² BID	I
ACTRN12616000228482	Solid tumors	Daily doses of 200 mg, 400 mg, 800 mg and 1200 mg	I
NCT06838676	Diffuse intrinsic pontine gliomas and H3K27-altered high grade glioma	875 mg/m ² BID	II
NCT05053880	Recurrent glioblastoma multiforme	200mg BID, 400mg BID, and 800mg BID	Ib/IIa
NCT06894225	Recurrent glioblastoma	400 mg BID	II

In age-related cancers, ACT001 demonstrates pro-oxidant, pro-apoptotic, and pro-ferroptotic properties that impede tumor growth. Conversely, in age-related metabolic disorders, cardiovascular diseases, and neurodegenerative diseases, MCL/ACT001 demonstrates antioxidant and cytoprotective properties that mitigate disease progression. This dichotomy presents a major practical concern as the therapeutic window may be limited and highly context-dependent, especially in older individuals with baseline frailty, redox imbalance, or compromised organ function. Nevertheless, during preclinical and early-stage clinical studies, multiple reports on MCL/ACT001 have demonstrated radiosensitizing effects in various animal models of cancers, including lung cancer, glioma, and osteosarcoma, and exhibit protective effects on normal tissues by mitigating radiation-induced inflammation and subsequent fibrosis. Beyond regulating T cell activity and the tissue microenvironment by inhibiting NF-κB and STAT3 signaling, ACT001 activates the NRF2 signaling pathway to diminish ROS levels, thereby protecting normal tissues, including lymphocytes. For instance, in a

phase 2b clinical trial of ACT001 combined with whole-brain radiotherapy for brain metastases from solid tumors, ACT001 mitigated the suppressive effect of chemoradiotherapy on lymphocytes. Furthermore, the protective effect of ACT001 on normal cells is reflected in a clinical trial combining ACT001 with TMZ for recurrent GBM treatment [172]. The incidence rates of grade 3 lymphopenia and grade 4 thrombocytopenia were significantly lower than those observed when TMZ was used alone. Moreover, two out of six patients achieved a survival period of ≥ 4 years, and the 3-year long-term survival rate was also significantly higher than the reported 1/437 [175]. Although the anticancer activity of MCL/ACT001 relies on its pro-oxidant and pro-apoptotic actions in malignant cells, its protective effects on normal tissues, including reducing radiation-induced fibrosis or chemotherapy-induced cytopenias, depend on antioxidant and anti-inflammatory mechanisms. This dichotomy increases the possibility that the same agent may have different effects depending on cell type, disease stage, and baseline physiological status. Therefore, MCL/ACT001 may possess extensive application prospects.

9. Conclusions and future perspectives

As the global population ages, the increasing prevalence of age-related pathologies has become a significant public health crisis. The unique ability of MCL/ACT001 to regulate inflammation, oxidative stress, autophagy, mitochondrial function, and neuroprotection makes these compounds highly promising candidates for therapeutic intervention against age-related disease. The primary mechanism of action of MCL/ACT001 lies in its potent and specific inhibition of NF- κ B and STAT3. For instance, by selectively inhibiting IKK β kinase activity, the abnormal NF- κ B activation can be effectively suppressed, resulting in significant attenuation of inflammatory responses that propel the aging process and various age-associated pathologies. This intervention markedly downregulated pro-inflammatory factors and thereby disrupted the paracrine damage of the tissue microenvironment caused by senescent cells, indirectly slowing tissue degeneration and functional decline. A substantial body of preclinical evidence has convincingly demonstrated the potential clinical efficacy of MCL/ACT001 in addressing several age-related conditions. In AD and PD mouse models, ACT001 markedly reduced neuroinflammation and enhanced cognitive and motor functions. Its superior blood-brain barrier permeability constitutes a key benefit for central nervous system-targeted applications. Moreover, ACT001 was beneficial in enhancing insulin resistance and MAFLD, consistent with the fundamental role of inflammation in metabolic disorders. ACT001, formulated as a water-soluble prodrug of MCL, demonstrates substantially improved bioavailability. A concluded phase I/IIa trial in patients with GBM has preliminarily established favorable human tolerability and safety, laying the groundwork for clinical expansion into aging-related diseases. Although MCL/ACT001 exhibits great potential in age-related disease, translation of MCL/ACT001 into an effective anti-aging or disease-modifying intervention requires systematic clarification of its overall regulatory effect on the complex network of aging pathways, including NF- κ B, NRF2, autophagy, mitochondrial dysfunction, and epigenetic regulation. Furthermore, investigating and validating blood or imaging biomarkers that can sensitively reflect ACT001 efficacy in clinical trials, including specific combinations of senescence-associated secretory phenotype factors, inflammation-related metabolites, and neuroimaging biomarkers, is essential for evaluating the targeted effect and early efficacy in humans. Combining strong preclinical efficacy and initial human safety data positions MCL/ACT001 for rapid advancement into mechanistic and proof-of-concept clinical studies for age-related diseases.

Despite significant advancements in clarifying the mechanistic foundations and therapeutic potential of MCL and ACT001, several critical questions remain unresolved and require further investigation. First, investigating the optimal dosage regimen. In the Phase I dose-escalation study, ACT001 was administered at increasing doses of 100, 200, 400, 600, 900, and 1,200 mg twice daily. The treatment was well tolerated during the study, with no dose-limiting toxicities or ACT001-related severe adverse events observed [176]. ACT001 exhibited a plasma half-life of approximately 3–4 h, with no evidence of drug accumulation after multiple administrations. The available safety data revealed that the primary adverse events associated with ACT001 were Grade 1–2 gastrointestinal disturbances, including vomiting and abdominal distension, weight loss, and reduced lymphocyte counts. A significant translational challenge stemming from the dual actions of MCL/ACT001, pro-oxidant, pro-apoptotic, and pro-ferroptotic in malignant cells versus antioxidant and cytoprotective in normal tissues, is that the therapeutic window may be limited and highly context-dependent. This issue is particularly relevant for older adults, who frequently present with baseline oxidative stress, mitochondrial dysfunction, and reduced organ reserve.

In such individuals, unintended drug accumulation in non-target tissues or administration at supra-pharmacological doses may induce harmful pro-oxidant effects, potentially exacerbating age-related frailty or causing off-target toxicity. Currently, most preclinical studies have been conducted in young animals, which fail to accurately reflect the clinical realities of geriatric patients with multiple comorbidities. Therefore, we recommend that subsequent preclinical evaluations of MCL/ACT001 explicitly include aged animal models (18–24-month-old mice) exhibiting relevant age-associated diseases, including metabolic syndrome, chronic kidney disease, or cardiac dysfunction. Such studies should include dose-finding arms, pharmacokinetic analyses in aged tissues, and monitoring of redox biomarkers to establish a safe and effective dosing window. Moreover, in older adults, physiological changes including elevated gastric pH, decreased gastrointestinal motility, reduced hepatic blood flow, and diminished glomerular filtration rate may lead to increased peak plasma concentrations and prolonged elimination half-lives of ACT001. Leveraging the established experience with low-dose aspirin in cardiovascular disease prevention, future studies should examine the efficacy of low-dose ACT001 in mitigating cognitive decline or enhancing vascular function. Besides, standardized dosing regimen studies and comprehensive pharmacokinetic analyses are required to establish the optimal therapeutic window for MCL and ACT001 in

specific age-related indications. Second, evaluating combination therapies. How can MCL/ACT001 be optimally integrated with established standard-of-care therapies, including metformin, rapamycin analogs, and statins, with particular attention to improve therapeutic outcomes of concomitant agents or ameliorate their associated adverse effects?

A systematic preclinical assessment of combination matrices, succeeded by carefully designed clinical trials, will be essential to establish actionable therapeutic protocols. Third, creating targeted drug delivery systems to augment site-specific accumulation and further enhance safety profiles. Although ACT001 demonstrated favorable blood-brain barrier penetration, how can we achieve selective enrichment in other tissues, including bone, myocardium, and renal parenchyma, and minimize systemic exposure? Future efforts should prioritize the development of targeted delivery platforms, including liposomes, polymeric nanoparticles, and extracellular vesicles. By effectively encapsulating ACT001 within these functional vectors, active or passive targeted delivery can be achieved, facilitating precise enrichment at pathological sites. This targeted biodistribution profile would maximize therapeutic efficacy at the pathological site and minimize collateral exposure to healthy organs, thereby decreasing the incidence and severity of dose-limiting toxicities and related adverse events. Additionally, do inflammatory signatures, including high-sensitivity C-reactive protein, IL-6, and senescence-associated secretory phenotype factor profiles, or frailty indices effectively stratify patient subpopulations most likely to respond to MCL/ACT001 therapy? Creating and validating predictive biomarker algorithms could markedly improve clinical trial efficiency by identifying responders early, especially by tracking long-term modulation of key inflammatory markers. Addressing these outstanding questions will be crucial in realizing the comprehensive therapeutic potential of MCL/ACT001 as a multifaceted intervention against age-related diseases and for establishing evidence-based frameworks for their clinical application.

Acknowledgments

This study was supported by and National Natural Science Foundation of China (82570507 and 62575026) and National High Level Hospital Clinical Research Funding (Scientific Research Fund of Peking University First Hospital) [2024CX14].

Author contributions

Hui Xu collected the literature and wrote the manuscript. Xing Cong and Lian-Jun Lin conceived the idea and

supervised the manuscript. All the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

- [1] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2023). Hallmarks of aging: An expanding universe. *Cell*, 186:243-278.
- [2] Zhang L, Pitcher LE, Yousefzadeh MJ, Niedernhofer LJ, Robbins PD, Zhu Y (2022). Cellular senescence: a key therapeutic target in aging and diseases. *J Clin Invest*, 132.
- [3] Huang W, Hickson LJ, Eirin A, Kirkland JL, Lerman LO (2022). Cellular senescence: the good, the bad and the unknown. *Nat Rev Nephrol*, 18:611-627.
- [4] Chen Z, Han F, Du Y, Shi H, Zhou W (2023). Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther*, 8:70.
- [5] Dorronsoro A, Santiago FE, Grassi D, Zhang T, Lai RC, McGowan SJ, et al. (2021). Mesenchymal stem cell-derived extracellular vesicles reduce senescence and extend health span in mouse models of aging. *Aging Cell*, 20:e13337.
- [6] Li Q, Chen Y, Chen Y, Hua Z, Gong B, Liu Z, et al. (2024). Novel small molecule DMAMCL induces differentiation in rhabdomyosarcoma by downregulating of DLL1. *Biomed Pharmacother*, 174:116562.
- [7] Zhang J, Chen Y, Fang J (2022). Targeting thioredoxin reductase by micheliolide contributes to radiosensitizing and inducing apoptosis of HeLa cells. *Free Radic Biol Med*, 186:99-109.
- [8] Zeng B, Cheng Y, Zheng K, Liu S, Shen L, Hu J, et al. (2021). Design, synthesis and in vivo anticancer activity of novel parthenolide and micheliolide derivatives as NF- κ B and STAT3 inhibitors. *Bioorg Chem*, 111:104973.
- [9] Viennois E, Xiao B, Ayyadurai S, Wang L, Wang PG, Zhang Q, et al. (2014). Micheliolide, a new sesquiterpene lactone that inhibits intestinal inflammation and colitis-associated cancer. *Lab Invest*, 94:950-965.
- [10] Liu W, Chen X, Wang Y, Chen Y, Chen S, Gong W, et al. (2019). Micheliolide ameliorates diabetic kidney disease by inhibiting Mtdh-mediated renal inflammation in type 2 diabetic db/db mice. *Pharmacol Res*, 150:104506.
- [11] Luo X, Wang Y, Zhu X, Chen Y, Xu B, Bai X, et al. (2024). MCL attenuates atherosclerosis by suppressing macrophage ferroptosis via targeting KEAP1/NRF2 interaction. *Redox Biol*, 69:102987.
- [12] Peng F, Li H, Li S, Wang Y, Liu W, Gong W, et al. (2019). Micheliolide ameliorates renal fibrosis by suppressing the Mtdh/BMP/MAPK pathway. *Lab Invest*, 99:1092-1106.

- [13] Sun Z, Li G, Tong T, Chen J (2017). Micheliolide suppresses LPS-induced neuroinflammatory responses. *PLoS One*, 12:e0186592.
- [14] Li J, Li S, Guo J, Li Q, Long J, Ma C, et al. (2018). Natural Product Micheliolide (MCL) Irreversibly Activates Pyruvate Kinase M2 and Suppresses Leukemia. *J Med Chem*, 61:4155-4164.
- [15] An Y, Guo W, Li L, Xu C, Yang D, Wang S, et al. (2015). Micheliolide derivative DMAMCL inhibits glioma cell growth in vitro and in vivo. *PLoS One*, 10:e0116202.
- [16] Yang MH, Baek SH, Jung YY, Um JY, Ahn KS (2024). Activation of autophagy, paraptosis, and ferroptosis by micheliolide through modulation of the MAPK signaling pathway in pancreatic and colon tumor cells. *Pathol Res Pract*, 263:155654.
- [17] Luo H, Liu X, Liu H, Wang Y, Xu K, Li J, et al. (2023). ACT001 Ameliorates ionizing radiation-induced lung injury by inhibiting NLRP3 inflammasome pathway. *Biomed Pharmacother*, 163:114808.
- [18] Xu Z, Xu J, Sun S, Lin W, Li Y, Lu Q, et al. (2022). Mecheliolide elicits ROS-mediated ERS driven immunogenic cell death in hepatocellular carcinoma. *Redox Biol*, 54:102351.
- [19] Zhong J, Gong W, Chen J, Qing Y, Wu S, Li H, et al. (2018). Micheliolide alleviates hepatic steatosis in db/db mice by inhibiting inflammation and promoting autophagy via PPAR- γ -mediated NF- κ B and AMPK/mTOR signaling. *Int Immunopharmacol*, 59:197-208.
- [20] Li S, Peng F, Gong W, Wu J, Wang Y, Xu Z, et al. (2019). Dimethylaminomicheliolide ameliorates peritoneal fibrosis through the activation of autophagy. *J Mol Med (Berl)*, 97:659-674.
- [21] Li Y, Luo C, Zeng Y, Zheng Z, Tao D, Liu Q, et al. (2023). Renal Fibrosis Is Alleviated through Targeted Inhibition of IL-11-Induced Renal Tubular Epithelial-to-Mesenchymal Transition. *Am J Pathol*, 193:1936-1952.
- [22] Xu H, Wang J, Wang C, Chang G, Lin Y, Zhang H, et al. (2015). Therapeutic effects of micheliolide on a murine model of rheumatoid arthritis. *Mol Med Rep*, 11:489-493.
- [23] Yang G, Hu Y, Qin X, Sun J, Miao Z, Wang L, et al. (2023). Micheliolide attenuates neuroinflammation to improve cognitive impairment of Alzheimer's disease by inhibiting NF- κ B and PI3K/Akt signaling pathways. *Heliyon*, 9:e17848.
- [24] Li Y, Tian X, Luo J, Bao T, Wang S, Wu X (2024). Molecular mechanisms of aging and anti-aging strategies. *Cell Commun Signal*, 22:285.
- [25] Di Giosia P, Stamerra CA, Giorgini P, Jamialahamdi T, Butler AE, Sahebkar A (2022). The role of nutrition in inflammaging. *Ageing Res Rev*, 77:101596.
- [26] Kong P, Cui ZY, Huang XF, Zhang DD, Guo RJ, Han M (2022). Inflammation and atherosclerosis: signaling pathways and therapeutic intervention. *Signal Transduct Target Ther*, 7:131.
- [27] Rayego-Mateos S, Rodrigues-Diez RR, Fernandez-Fernandez B, Mora-Fernández C, Marchant V, Donate-Correa J, et al. (2023). Targeting inflammation to treat diabetic kidney disease: the road to 2030. *Kidney Int*, 103:282-296.
- [28] Hotamisligil GS (2006). Inflammation and metabolic disorders. *Nature*, 444:860-867.
- [29] Andonian BJ, Hippensteel JA, Abuabara K, Boyle EM, Colbert JF, Devinney MJ, et al. (2025). Inflammation and aging-related disease: A transdisciplinary inflammaging framework. *Geroscience*, 47:515-542.
- [30] Zhang C, Sun B, Wang L, Korla PK, Liu C (2025). Micheliolide mitigates diabetic nephropathy by modulating oxidative stress and inflammation in rats. *Phytomedicine*, 145:157025.
- [31] Qin X, Jiang X, Jiang X, Wang Y, Miao Z, He W, et al. (2016). Micheliolide inhibits LPS-induced inflammatory response and protects mice from LPS challenge. *Sci Rep*, 6:23240.
- [32] Lei X, Li S, Luo C, Wang Y, Liu Y, Xu Z, et al. (2020). Micheliolide Attenuates Lipopolysaccharide-Induced Inflammation by Modulating the mROS/NF- κ B/NLRP3 Axis in Renal Tubular Epithelial Cells. *Mediators Inflamm*, 2020:3934769.
- [33] Zhang Q, Jiang X, He W, Wei K, Sun J, Qin X, et al. (2017). MCL Plays an Anti-Inflammatory Role in Mycobacterium tuberculosis-Induced Immune Response by Inhibiting NF- κ B and NLRP3 Inflammasome Activation. *Mediators Inflamm*, 2017:2432904.
- [34] Kalantary-Charvadeh A, Sanajou D, Hemmati-Dinarvand M, Marandi Y, Khojastehfard M, Hajipour H, et al. (2019). Micheliolide Protects Against Doxorubicin-Induced Cardiotoxicity in Mice by Regulating PI3K/Akt/NF- κ B Signaling Pathway. *Cardiovasc Toxicol*, 19:297-305.
- [35] Li W, Li Y, Xiao L, Xie Z, Peng J, Huang W, et al. (2025). Micheliolide attenuates sepsis-induced acute lung injury by suppressing mitochondrial oxidative stress and PFKFB3-driven glycolysis. *J Transl Med*, 23:181.
- [36] Höllring K, Nuić L, Rogić L, Kaliman S, Gehrler S, Wollnik C, et al. (2024). Capturing the mechanosensitivity of cell proliferation in models of epithelium. *Proc Natl Acad Sci U S A*, 121:e2308126121.
- [37] Fuentes-Antrás J, Bedard PL, Cescon DW (2024). Seize the engine: Emerging cell cycle targets in breast cancer. *Clin Transl Med*, 14:e1544.
- [38] Han X, Zhang Y, Li Y, Lin Z, Fu Z, Wang C, et al. (2024). MCL restrained ROS/AKT/ASAHI pathway to therapy tamoxifen resistance breast cancer by stabilizing NRF2. *Cell Prolif*, 57:e13700.
- [39] Guo J, Xue Q, Liu K, Ge W, Liu W, Wang J, et al. (2019). Dimethylaminomicheliolide (DMAMCL) Suppresses the Proliferation of Glioblastoma Cells via Targeting Pyruvate Kinase 2 (PKM2) and Rewiring Aerobic Glycolysis. *Front Oncol*, 9:993.
- [40] Youssef KK, Nieto MA (2024). Epithelial-mesenchymal transition in tissue repair and degeneration. *Nat Rev Mol Cell Biol*, 25:720-739.
- [41] Peng F, Li H, Li S, Wang Y, Liu W, Gong W, et al. (2020). Correction: Micheliolide ameliorates renal

- fibrosis by suppressing the Mtdh/BMP/MAPK pathway. *Lab Invest*, 100:786-787.
- [42] Massagué J, Sheppard D (2023). TGF- β signaling in health and disease. *Cell*, 186:4007-4037.
- [43] Li S, Luo C, Chen S, Zhuang Y, Ji Y, Zeng Y, et al. (2023). Brahma-related gene 1 acts as a profibrotic mediator and targeting it by micheliolide ameliorates peritoneal fibrosis. *J Transl Med*, 21:639.
- [44] Gan Z, Huang J, Xu M, Yuan X, Shang X, Chen X, et al. (2023). Micheliolide prevents estrogen deficiency-induced bone loss via inhibiting osteoclast bone resorption. *Aging (Albany NY)*, 15:10732-10745.
- [45] Wang B, Han J, Elisseeff JH, Demaria M (2024). The senescence-associated secretory phenotype and its physiological and pathological implications. *Nat Rev Mol Cell Biol*, 25:958-978.
- [46] Newton K, Strasser A, Kayagaki N, Dixit VM (2024). Cell death. *Cell*, 187:235-256.
- [47] Guo JS, Wang JY, Chen SH, Deng YP, Gao QY, Liu ZX, et al. (2025). The natural product micheliolide promotes the nuclear translocation of GAPDH via binding to Cys247 and induces glioblastoma cell death in combination with temozolomide. *Biochem Pharmacol*, 233:116759.
- [48] Feng D, Liu M, Liu Y, Zhao X, Sun H, Zheng X, et al. (2020). Micheliolide suppresses the viability, migration and invasion of U251MG cells via the NF- κ B signaling pathway. *Oncol Lett*, 20:67.
- [49] Jia Y, Zhou L, Tian C, Shi Y, Wang C, Tong Z (2015). Dynamin-related protein 1 is involved in micheliolide-induced breast cancer cell death. *Onco Targets Ther*, 8:3371-3381.
- [50] Lei X, Wang J, Zhang F, Tang X, He F, Cheng S, et al. (2024). Micheliolide ameliorates lipopolysaccharide-induced acute kidney injury through suppression of NLRP3 activation by promoting mitophagy via Nrf2/PINK1/Parkin axis. *Int Immunopharmacol*, 138:112527.
- [51] Bai Y, Pan Y, Liu X (2025). Mechanistic insights into gasdermin-mediated pyroptosis. *Nat Rev Mol Cell Biol*, 26:501-521.
- [52] Yuan J, Ofengeim D (2024). A guide to cell death pathways. *Nat Rev Mol Cell Biol*, 25:379-395.
- [53] Wu DM, Li J, Shen R, Li J, Yu Y, Li L, et al. (2021). Autophagy Induced by Micheliolide Alleviates Acute Irradiation-Induced Intestinal Injury via Inhibition of the NLRP3 Inflammasome. *Front Pharmacol*, 12:773150.
- [54] Mishima E, Nakamura T, Doll S, Proneth B, Fedorova M, Pratt DA, et al. (2025). Recommendations for robust and reproducible research on ferroptosis. *Nat Rev Mol Cell Biol*, 26:615-630.
- [55] Zhao Y, Linkermann A, Takahashi M, Li Q, Zhou X (2025). Ferroptosis in cardiovascular disease: regulatory mechanisms and therapeutic implications. *Eur Heart J*, 46:3247-3260.
- [56] Liang D, Minikes AM, Jiang X (2022). Ferroptosis at the intersection of lipid metabolism and cellular signaling. *Mol Cell*, 82:2215-2227.
- [57] Oršolić N, Jembrek MJ (2025). Targeting Oxidative Stress for Disease. *Int J Mol Sci*, 26.
- [58] Wang J, Han D, Han L, Zhang Q, Liu S, Hu J, et al. (2025). Protective effect of micheliolide against inflammation and oxidative stress in asthma through the MAPK/NF- κ B signalling pathway. *World Allergy Organ J*, 18:101091.
- [59] Wu CY, Wang KQ, Qin YY, Wang HW, Wu MM, Zhu XD, et al. (2024). Micheliolide ameliorates severe acute pancreatitis in mice through potentiating Nrf2-mediated anti-inflammation and anti-oxidation effects. *Int Immunopharmacol*, 143:113490.
- [60] Guo J, Liu K, Wang J, Jiang H, Zhang M, Liu Y, et al. (2022). A rational foundation for micheliolide-based combination strategy by targeting redox and metabolic circuit in cancer cells. *Biochem Pharmacol*, 200:115037.
- [61] Li Q, Sun Y, Liu B, Li J, Hao X, Ge W, et al. (2020). ACT001 modulates the NF- κ B/MnSOD/ROS axis by targeting IKK β to inhibit glioblastoma cell growth. *J Mol Med (Berl)*, 98:263-277.
- [62] Adlimoghaddam A (2025). Mitochondrial Dysfunction in Aging and Age-related Disorders. *Aging Dis*, 16:2495-2497.
- [63] Li Y, Berliocchi L, Li Z, Rasmussen LJ (2024). Interactions between mitochondrial dysfunction and other hallmarks of aging: Paving a path toward interventions that promote healthy old age. *Aging Cell*, 23:e13942.
- [64] Liu Y, Yao L, Liu Y, Yang Y, Liang A, He H, et al. (2025). Micheliolide Alleviates Hepatic Fibrosis by Inhibiting Autophagy in Hepatic Stellate Cells via the TrxR1/2-Mediated ROS/MEK/ERK Pathway. *Pharmaceuticals (Basel)*, 18.
- [65] Zhang H, Gu W, Wu G, Yu Y (2024). Aging and Autophagy: Roles in Musculoskeletal System Injury. *Aging Dis*, 16:1438-1451.
- [66] Liu S, Yao S, Yang H, Liu S, Wang Y (2023). Autophagy: Regulator of cell death. *Cell Death Dis*, 14:648.
- [67] Minami S, Yamamoto T, Yamamoto-Imoto H, Isaka Y, Hamasaki M (2023). Autophagy and kidney aging. *Prog Biophys Mol Biol*, 179:10-15.
- [68] Cai L, Wu ZR, Cao L, Xu JD, Lu JL, Wang CD, et al. (2022). ACT001 inhibits pituitary tumor growth by inducing autophagic cell death via MEK4/MAPK pathway. *Acta Pharmacol Sin*, 43:2386-2396.
- [69] Wang Y, Zhang J, Yang Y, Liu Q, Xu G, Zhang R, et al. (2019). ROS generation and autophagosome accumulation contribute to the DMAMCL-induced inhibition of glioma cell proliferation by regulating the ROS/MAPK signaling pathway and suppressing the Akt/mTOR signaling pathway. *Onco Targets Ther*, 12:1867-1880.
- [70] Zhu J, Tang C, Cong Z, Yuan F, Cai X, Yang J, et al. (2021). ACT001 reverses resistance of prolactinomas via AMPK-mediated EGR1 and mTOR pathways. *Endocr Relat Cancer*, 29:33-46.
- [71] Tyrrell DJ, Goldstein DR (2021). Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6. *Nat Rev Cardiol*, 18:58-68.
- [72] Tasouli-Drakou V, Ogurek I, Shaikh T, Ringor M, DiCaro MV, Lei K (2025). Atherosclerosis: A

- Comprehensive Review of Molecular Factors and Mechanisms. *Int J Mol Sci*, 26.
- [73] Monaco C, McNamara CA, Slütter B, Foks AC, Bekiranov S, Mulder WJM, et al. (2025). Immunotherapy for atherosclerosis. *Physiol Rev*, 105:2141-2230.
- [74] De Meyer GRY, Zurek M, Puylaert P, Martinet W (2024). Programmed death of macrophages in atherosclerosis: mechanisms and therapeutic targets. *Nat Rev Cardiol*, 21:312-325.
- [75] Luo X, Wang Y, Zhu X, Chen Y, Xu B, Bai X, et al. (2024). Corrigendum to "MCL attenuates atherosclerosis by suppressing macrophage ferroptosis via targeting KEAP1/NRF2 interaction" [*Redox Biol*. 69 (2024) 102987]. *Redox Biol*, 69:103009.
- [76] Xiang Q, Yi X, Zhu XH, Wei X, Jiang DS (2024). Regulated cell death in myocardial ischemia-reperfusion injury. *Trends Endocrinol Metab*, 35:219-234.
- [77] Liu Z, Jin Q, Yin J (2026). Micheliolide alleviates myocardial ischemia/reperfusion injury in rats via KEAP1/NRF2-mediated suppression of oxidative stress and inflammation. *Immunopharmacol Immunotoxicol*, 48:104-110.
- [78] Santisteban MM, Iadecola C (2025). The pathobiology of neurovascular aging. *Neuron*, 113:49-70.
- [79] Joynt Maddox KE, Elkind MSV, Aparicio HJ, Commodore-Mensah Y, de Ferranti SD, Dowd WN, et al. (2024). Forecasting the Burden of Cardiovascular Disease and Stroke in the United States Through 2050-Prevalence of Risk Factors and Disease: A Presidential Advisory From the American Heart Association. *Circulation*, 150:e65-e88.
- [80] Chen Q, Wu M, Tang Q, Yan P, Zhu L (2024). Age-Related Alterations in Immune Function and Inflammation: Focus on Ischemic Stroke. *Aging Dis*, 15:1046-1074.
- [81] Tu WJ, Wang LD (2023). China stroke surveillance report 2021. *Mil Med Res*, 10:33.
- [82] Feigin VL, Brainin M, Norrving B, Martins SO, Pandian J, Lindsay P, et al. (2025). World Stroke Organization: Global Stroke Fact Sheet 2025. *Int J Stroke*, 20:132-144.
- [83] Wang J, Cai W, Yang G, Liang R, Wang Z, Li S, et al. (2025). Micheliolide interferes PKM2-P65 interaction and inhibits inflammasome activation in macrophage after ischemic stroke. *Int Immunopharmacol*, 163:115231.
- [84] Eisenmenger LB, Peret A, Famakin BM, Spahic A, Roberts GS, Bockholt JH, et al. (2023). Vascular contributions to Alzheimer's disease. *Transl Res*, 254:41-53.
- [85] Bhardwaj V, Kumari S, Dhapola R, Sharma P, Beura SK, Singh SK, et al. (2025). Shedding light on microglial dysregulation in Alzheimer's disease: exploring molecular mechanisms and therapeutic avenues. *Inflammopharmacology*, 33:679-702.
- [86] Li F, Eteleeb AM, Buchser W, Sohn C, Wang G, Xiong C, et al. (2022). Weakly activated core neuroinflammation pathways were identified as a central signaling mechanism contributing to the chronic neurodegeneration in Alzheimer's disease. *Front Aging Neurosci*, 14:935279.
- [87] Tolosa E, Garrido A, Scholz SW, Poewe W (2021). Challenges in the diagnosis of Parkinson's disease. *Lancet Neurol*, 20:385-397.
- [88] Liu Q, Zhang S, Zhu D, Tang X, Che Y, Feng X (2020). The parthenolide derivative ACT001 synergizes with low doses of L-DOPA to improve MPTP-induced Parkinson's disease in mice. *Behav Brain Res*, 379:112337.
- [89] Liu Q, Guo X, Huang Z, He Q, Zhu D, Zhang S, et al. (2020). Anti-neuroinflammatory effects of dimethylaminomylide (DMAMCL, i.e., ACT001) are associated with attenuating the NLRP3 inflammasome in MPTP-induced Parkinson disease in mice. *Behav Brain Res*, 383:112539.
- [90] Drake SS, Zaman A, Gianfelice C, Hua EM, Heale K, Afanasiev E, et al. (2024). Senolytic treatment diminishes microglia and decreases severity of experimental autoimmune encephalomyelitis. *J Neuroinflammation*, 21:283.
- [91] Xiao J, Meng Z, Lu Y, Nie Z, Liu Y, Yao Z, et al. (2025). Targeting microglia-Th17 feed-forward loop to suppress autoimmune neuroinflammation. *J Neuroinflammation*, 22:118.
- [92] Barbour MA, Whitehead B, Gumbo C, Karelina K, Weil ZM (2025). Traumatic brain injury persistently increases the incidence of both ischemic and hemorrhagic strokes: Potential mechanisms. *Prog Neurobiol*, 248:102749.
- [93] Cai L, Gong Q, Qi L, Xu T, Suo Q, Li X, et al. (2022). ACT001 attenuates microglia-mediated neuroinflammation after traumatic brain injury via inhibiting AKT/NF- κ B/NLRP3 pathway. *Cell Commun Signal*, 20:56.
- [94] Thouaye M, Yalcin I (2023). Neuropathic pain: From actual pharmacological treatments to new therapeutic horizons. *Pharmacol Ther*, 251:108546.
- [95] Zhang T, Lin C, Wu S, Jin S, Li X, Peng Y, et al. (2022). ACT001 Inhibits TLR4 Signaling by Targeting Co-Receptor MD2 and Attenuates Neuropathic Pain. *Front Immunol*, 13:873054.
- [96] Lei W, Jia L, Wang Z, Liang Z, Zhao A, Liu Y, et al. (2023). CC chemokines family in fibrosis and aging: From mechanisms to therapy. *Ageing Res Rev*, 87:101900.
- [97] Hu HH, Cao G, Wu XQ, Vaziri ND, Zhao YY (2020). Wnt signaling pathway in aging-related tissue fibrosis and therapies. *Ageing Res Rev*, 60:101063.
- [98] Ming X, Yang Z, Huang Y, Wang Z, Zhang Q, Lu C, et al. (2025). A chimeric peptide promotes immune surveillance of senescent cells in injury, fibrosis, tumorigenesis and aging. *Nat Aging*, 5:28-47.
- [99] Rieder F, Nagy LE, Maher TM, Distler JHW, Kramann R, Hinz B, et al. (2025). Fibrosis: cross-organ biology and pathways to development of innovative drugs. *Nat Rev Drug Discov*, 24:543-569.
- [100] Di X, Li Y, Wei J, Li T, Liao B (2025). Targeting Fibrosis: From Molecular Mechanisms to Advanced Therapies. *Adv Sci (Weinh)*, 12:e2410416.

- [101] Zhao X, Kwan JYY, Yip K, Liu PP, Liu FF (2020). Targeting metabolic dysregulation for fibrosis therapy. *Nat Rev Drug Discov*, 19:57-75.
- [102] Wu L, Lin H, Li S, Huang Y, Sun Y, Shu S, et al. (2024). Macrophage iron dyshomeostasis promotes aging-related renal fibrosis. *Aging Cell*, 23:e14275.
- [103] Hassanein EHM, Ibrahim IM, Abd-Alhameed EK, Sharawi ZW, Jaber FA, Althagafy HS (2023). Nrf2/HO-1 as a therapeutic target in renal fibrosis. *Life Sci*, 334:122209.
- [104] (2020). Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*, 395:709-733.
- [105] Wang S, Zhang B, Wang Y, Lan Q, Lv L, Xiao T, et al. (2025). Acetylation-regulated DUSP1 deficiency contributes to renal fibrosis progression. *Theranostics*, 15:3781-3796.
- [106] Schafer S, Viswanathan S, Widjaja AA, Lim WW, Moreno-Moral A, DeLaughter DM, et al. (2017). IL-11 is a crucial determinant of cardiovascular fibrosis. *Nature*, 552:110-115.
- [107] Ng B, Dong J, D'Agostino G, Viswanathan S, Widjaja AA, Lim WW, et al. (2019). Interleukin-11 is a therapeutic target in idiopathic pulmonary fibrosis. *Sci Transl Med*, 11.
- [108] Huang Q, Sun Y, Peng L, Sun J, Sha Z, Lin H, et al. (2023). Extracellular vesicle-packaged ILK from mesothelial cells promotes fibroblast activation in peritoneal fibrosis. *J Extracell Vesicles*, 12:e12334.
- [109] Meng Y, Zhao T, Zhang Z, Zhang D (2022). The role of hepatic microenvironment in hepatic fibrosis development. *Ann Med*, 54:2830-2844.
- [110] Jin C, Jiang A, Chang J, Hu Y, Wang Y, Zhao R, et al. (2025). SIRT1 Enhancement is Required for the Induction of Anti-Inflammatory Effect of Micheliolide in Carbon Tetrachloride Induced Liver Fibrosis in Mice. *J Inflamm Res*, 18:16231-16245.
- [111] Koudstaal T, Funke-Chambour M, Kreuter M, Molyneaux PL, Wijsenbeek MS (2023). Pulmonary fibrosis: from pathogenesis to clinical decision-making. *Trends Mol Med*, 29:1076-1087.
- [112] Koudstaal T, Wijsenbeek MS (2023). Idiopathic pulmonary fibrosis. *Presse Med*, 52:104166.
- [113] Chen G, Li J, Liu H, Zhou H, Liu M, Liang D, et al. (2023). Cepharanthine Ameliorates Pulmonary Fibrosis by Inhibiting the NF- κ B/NLRP3 Pathway, Fibroblast-to-Myofibroblast Transition and Inflammation. *Molecules*, 28.
- [114] Jaffar J, Glaspole I, Symons K, Westall G (2021). Inhibition of NF- κ B by ACT001 reduces fibroblast activity in idiopathic pulmonary fibrosis. *Biomed Pharmacother*, 138:111471.
- [115] Yi M, Yuan Y, Ma L, Li L, Qin W, Wu B, et al. (2024). Inhibition of TGF β 1 activation prevents radiation-induced lung fibrosis. *Clin Transl Med*, 14:e1546.
- [116] Yu M, Qian X, Wang Y, Li Q, Peng C, Chen B, et al. (2024). Emerging role of NEDD8-mediated neddylation in age-related metabolic diseases. *Ageing Res Rev*, 94:102191.
- [117] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*, 183:109119.
- [118] (2023). Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet*, 402:203-234.
- [119] Yu Q, Zuo X, Bai H, Zhang S, Luan J, Zhao Q, et al. (2024). Alleviative effects of the parthenolide derivative ACT001 on insulin resistance induced by sodium propionate combined with a high-fat diet and its potential mechanisms. *Eur J Pharmacol*, 971:176529.
- [120] Zhou Y, Chen Z, Zhou H, Niu B, Liu J, Li Y, et al. (2023). ACT001 Alleviates chronic kidney injury induced by a high-fat diet in mice through the GPR43/AMPK pathway. *Lipids Health Dis*, 22:198.
- [121] Eslam M, Sanyal AJ, George J (2020). MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology*, 158:1999-2014.e1991.
- [122] Wei S, Wang L, Evans PC, Xu S (2024). NAFLD and NASH: etiology, targets and emerging therapies. *Drug Discov Today*, 29:103910.
- [123] Jiang Y, Wu L, Zhu X, Bian H, Gao X, Xia M (2024). Advances in management of metabolic dysfunction-associated steatotic liver disease: from mechanisms to therapeutics. *Lipids Health Dis*, 23:95.
- [124] Liu J, Zhou Y, Li Y, Chen Z, Jiao X, Hu J, et al. (2025). Integrative transcriptomic and metabolomic analysis explores the mechanisms by which ACT001 treats MAFLD in mice. *Sci Rep*, 15:12494.
- [125] Niu B, Liu J, Zhou H, Chu W, Wu X, Zhou Y, et al. (2025). ACT001 alleviates MASLD through gut microbiota-bile acid-FXR axis in mice. *Ann Med*, 57:2580773.
- [126] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. (2018). The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*, 67:328-357.
- [127] Zhou H, Niu B, Wu X, Chu W, Zhou Y, Chen Z, et al. (2023). iTRAQ-based quantitative proteomics analysis of the effect of ACT001 on non-alcoholic steatohepatitis in mice. *Sci Rep*, 13:11336.
- [128] Gustafsson T, Ulfhake B (2024). Aging Skeletal Muscles: What Are the Mechanisms of Age-Related Loss of Strength and Muscle Mass, and Can We Impede Its Development and Progression? *Int J Mol Sci*, 25.
- [129] Johnston CB, Dagar M (2020). Osteoporosis in Older Adults. *Med Clin North Am*, 104:873-884.
- [130] Di Matteo A, Bathon JM, Emery P (2023). Rheumatoid arthritis. *Lancet*, 402:2019-2033.
- [131] Chiu YM, Lu YP, Lan JL, Chen DY, Wang JD (2021). Lifetime Risks, Life Expectancy, and Health Care Expenditures for Rheumatoid Arthritis: A Nationwide Cohort Followed Up From 2003 to 2016. *Arthritis Rheumatol*, 73:750-758.

- [132] Chen L, Wu B, Mo L, Chen H, Zhao Y, Tan T, et al. (2024). Associations between biological ageing and the risk of, genetic susceptibility to, and life expectancy associated with rheumatoid arthritis: a secondary analysis of two observational studies. *Lancet Healthy Longev*, 5:e45-e55.
- [133] Liu Y, Chen L, Chen Z, Liu M, Li X, Kou Y, et al. (2023). Multifunctional Janus Nanoplatfor for Efficiently Synergistic Theranostics of Rheumatoid Arthritis. *ACS Nano*, 17:8167-8182.
- [134] Yang L, Chang C, Tam W, Liang Y, Chen M, Zhang J, et al. (2025). Investigating Potential Biomarkers of Ankylosing Spondylitis: A Study on Mitochondrial and Senescence Pathways Using Machine Learning. *J Inflamm Res*, 18:15479-15500.
- [135] Tian ZG, Yao M, Chen J (2020). Micheliolide alleviates ankylosing spondylitis (AS) by suppressing the activation of the NLRP3 inflammasome and maintaining the balance of Th1/Th2 via regulating the NF-κB signaling pathway. *Ann Transl Med*, 8:991.
- [136] Montégut L, López-Otín C, Kroemer G (2024). Aging and cancer. *Mol Cancer*, 23:106.
- [137] (2024). Global age-sex-specific mortality, life expectancy, and population estimates in 204 countries and territories and 811 subnational locations, 1950-2021, and the impact of the COVID-19 pandemic: a comprehensive demographic analysis for the Global Burden of Disease Study 2021. *Lancet*, 403:1989-2056.
- [138] McHugh D, Durán I, Gil J (2025). Senescence as a therapeutic target in cancer and age-related diseases. *Nat Rev Drug Discov*, 24:57-71.
- [139] Kroemer G, McQuade JL, Merad M, André F, Zitvogel L (2023). Bodywide ecological interventions on cancer. *Nat Med*, 29:59-74.
- [140] Tong L, Li J, Li Q, Wang X, Medikonda R, Zhao T, et al. (2020). ACT001 reduces the expression of PD-L1 by inhibiting the phosphorylation of STAT3 in glioblastoma. *Theranostics*, 10:5943-5956.
- [141] Xi X, Liu N, Wang Q, Chu Y, Yin Z, Ding Y, et al. (2019). ACT001, a novel PAI-1 inhibitor, exerts synergistic effects in combination with cisplatin by inhibiting PI3K/AKT pathway in glioma. *Cell Death Dis*, 10:757.
- [142] Zhou X, Xia Q, Wang B, Li J, Liu B, Wang S, et al. (2025). USP14 modulates stem-like properties, tumorigenicity, and radiotherapy resistance in glioblastoma stem cells through stabilization of MST4-phosphorylated ALKBH5. *Theranostics*, 15:2293-2314.
- [143] Hou Y, Sun B, Liu W, Yu B, Shi Q, Luo F, et al. (2021). Targeting of glioma stem-like cells with a parthenolide derivative ACT001 through inhibition of AEBP1/PI3K/AKT signaling. *Theranostics*, 11:555-566.
- [144] Xi X, Ding X, Wang Q, Liu N, Wang B, Wang G, et al. (2025). Targeting the MDK/c-Myc complex to overcome temozolomide resistance in glioma. *Clin Transl Med*, 15:e70359.
- [145] Xu KQ, Gong Z, Yang JL, Xia CQ, Zhao JY, Chen X (2024). B-cell-specific signatures reveal novel immunophenotyping and therapeutic targets for hepatocellular carcinoma. *World J Gastroenterol*, 30:3894-3925.
- [146] Yu L, Chen W, Tang Q, Ji KY (2019). Micheliolide Inhibits Liver Cancer Cell Growth Via Inducing Apoptosis And Perturbing Actin Cytoskeleton. *Cancer Manag Res*, 11:9203-9212.
- [147] Li Z, Lai X, Fu S, Ren L, Cai H, Zhang H, et al. (2022). Immunogenic Cell Death Activates the Tumor Immune Microenvironment to Boost the Immunotherapy Efficiency. *Adv Sci (Weinh)*, 9:e2201734.
- [148] Xi Z, Dai R, Ze Y, Jiang X, Liu M, Xu H (2025). Traditional Chinese medicine in lung cancer treatment. *Mol Cancer*, 24:57.
- [149] Deng H, Liu H, Yang G, Wang D, Luo Y, Li C, et al. (2023). ACT001 inhibited CD133 transcription by targeting and inducing Olig2 ubiquitination degradation. *Oncogenesis*, 12:19.
- [150] Zhao M, Qin T, Huang D (2022). ACT001 inhibits the proliferation of non-small cell lung cancer cells by upregulating NKTR expression. *Thorac Cancer*, 13:1772-1782.
- [151] Shi Z, Li Y, Hou H, Wang Z, Guo H, Yu Y, et al. (2025). ACT001 Inhibits Tumor Progression and Modulates Immune Responses in Non-Small Cell Lung Cancer. *J Clin Lab Anal*, 39:e70066.
- [152] Kong P, Yu KN, Yang M, Almahi WA, Nie L, Chen G, et al. (2020). Micheliolide Enhances Radiosensitivities of p53-Deficient Non-Small-Cell Lung Cancer via Promoting HIF-1α Degradation. *Int J Mol Sci*, 21.
- [153] Kim SY, Park HS, Chiang AC (2025). Small Cell Lung Cancer: A Review. *Jama*, 333:1906-1917.
- [154] Ding XJ, Mei T, Xi XN, Wang JY, Wang WJ, Chen Y, et al. (2025). ACT001 Suppresses the Malignant Progression of Small-Cell Lung Cancer by Inhibiting Lactate Production and Promoting Anti-Tumor Immunity. *Thorac Cancer*, 16:e70028.
- [155] Tsoi H, You CP, Leung MH, Man EPS, Khoo US (2022). Targeting Ribosome Biogenesis to Combat Tamoxifen Resistance in ER+ve Breast Cancer. *Cancers (Basel)*, 14.
- [156] Jin XH, Jia YS, Shi YH, Li QY, Bao SQ, Lu WP, et al. (2019). ACT001 can prevent and reverse tamoxifen resistance in human breast cancer cell lines by inhibiting NF-κB activation. *J Cell Biochem*, 120:1386-1397.
- [157] Jia Y, Zhang C, Zhou L, Xu H, Shi Y, Tong Z (2015). Micheliolide overcomes KLF4-mediated cisplatin resistance in breast cancer cells by downregulating glutathione. *Onco Targets Ther*, 8:2319-2327.
- [158] Zhou L, Yu CW (2024). Epigenetic modulations in triple-negative breast cancer: Therapeutic implications for tumor microenvironment. *Pharmacol Res*, 204:107205.
- [159] Liu Y, Wang L, Liu J, Xie X, Hu H, Luo F (2020). Anticancer Effects of ACT001 via NF-κB Suppression in Murine Triple-Negative Breast Cancer Cell Line 4T1. *Cancer Manag Res*, 12:5131-5139.
- [160] Venugopal S, Sekeres MA (2024). Contemporary Management of Acute Myeloid Leukemia: A Review. *JAMA Oncol*, 10:1417-1425.
- [161] Zhang Q, Lu Y, Ding Y, Zhai J, Ji Q, Ma W, et al. (2012). Guaianolide sesquiterpene lactones, a source to discover

- agents that selectively inhibit acute myelogenous leukemia stem and progenitor cells. *J Med Chem*, 55:8757-8769.
- [162] Deng X, Xu X, Lin D, Zhang X, Yu L, Sheng H, et al. (2020). Real-World Impact of Surgical Excision on Overall Survival in Primary Central Nervous System Lymphoma. *Front Oncol*, 10:131.
- [163] Liu Z, Wang G, Liu H, Ding K, Song J, Fu R (2024). ACT001 inhibits primary central nervous system lymphoma tumor growth by enhancing the anti-tumor effect of T cells. *Biomed Pharmacother*, 178:117133.
- [164] Yang J, Li Y, Han X, Pei X, Lin Z, Li C (2023). The antitumor effect of the novel agent MCL/ACT001 in pancreatic ductal adenocarcinoma. *J Cancer Res Clin Oncol*, 149:5717-5728.
- [165] Tang X, Ding Q, Chen C, Chen F, Zhou X, Hong CJ, et al. (2019). Micheliolide inhibits gastric cancer growth in vitro and in vivo via blockade of the IL-6/STAT3 pathway. *Pharmazie*, 74:175-178.
- [166] Xi XN, Liu N, Wang QQ, Wu HT, He HB, Wang LL, et al. (2019). Pharmacokinetics, tissue distribution and excretion of ACT001 in Sprague-Dawley rats and metabolism of ACT001. *J Chromatogr B Analyt Technol Biomed Life Sci*, 1104:29-39.
- [167] Jackson PA, Widen JC, Harki DA, Brummond KM (2017). Covalent Modifiers: A Chemical Perspective on the Reactivity of α,β -Unsaturated Carbonyls with Thiols via Hetero-Michael Addition Reactions. *J Med Chem*, 60:839-885.
- [168] Ziegler DS, Hassall T, Valvi S, Cai D (2024). DIPG-84. Phase 1 dose-escalation study of act001 in pediatric patients with advanced brain and solid tumors shows activity in diffuse midline glioma (DMG). *Neuro-Oncology*, 26:0-0.
- [169] Wang Y, Liu Y, Zhang J, Wang G, Tao R, Cai L, et al. (2024). CTNI-17. A phase 2 study of safety and efficacy of concurrent act001 and radiation treatment followed by act001 maintenance therapy in newly diagnosed pediatric patients with diffuse intrinsic pontine glioma (DIPG). *Neuro-Oncology*, 26:viii99-viii99.
- [170] Lickliter JD, Jennens R, Lemech CR, Kichenadasse G, Cai D, Su SY-C (2021). Phase 1 dose-escalation study of ACT001 in patients with recurrent glioblastoma and other advanced solid tumors. *Journal of Clinical Oncology*, 39:2037-2037.
- [171] Lickliter JD, Jennens R, Lemech CR, Su SY-C, Chen Y (2018). Phase 1 dose-escalation study of ACT001 in patients with recurrent glioblastoma and other advanced solid tumors. *Journal of Clinical Oncology*, 36:e14048-e14048.
- [172] Li W, Li Q, Wang L, Cai D, Yang X (2023). CTNI-29. A phase 2 study to evaluate efficacy and safety of act001 and temozolomide combination treatment in patients with recurrent glioblastoma multiforme. *Neuro-Oncology*, 25:v80-v80.
- [173] Zhang T, Zhang W, Zhang K, Chen X, Cai D, Wang P, et al. (2022). Safety and efficacy evaluation of ACT001 and WBRT treatment in patients with solid tumor and brain metastasis. *Journal of Clinical Oncology*, 40:e14006-e14006.
- [174] Glaspole I, Cai D, Chambers D. 2023. Safety and efficacy study of ACT001 in IPF and fILD patients. *European Respiratory Society*.
- [175] Wick W, Gorlia T, Bendszus M, Taphoorn M, Sahm F, Harting I, et al. (2017). Lomustine and Bevacizumab in Progressive Glioblastoma. *N Engl J Med*, 377:1954-1963.
- [176] Lickliter JD, Jennens R, Lemech CR, Kichenadasse G, Cai D, Su SY-C. 2021. Phase 1 dose-escalation study of ACT001 in patients with recurrent glioblastoma and other advanced solid tumors. *Wolters Kluwer Health*.