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Review
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Enhancing Anti-Cancer Therapy with Selective Autophagy Inhibitors by Targeting Protective Autophagy

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Abstract

Autophagy is a process of eliminating damaged or unnecessary proteins and organelles, thereby maintaining intracellular homeostasis. Deregulation of autophagy is associated with several diseases including cancer. Contradictory dual roles of autophagy have been well established in cancer. Cytoprotective mechanism of autophagy has been extensively investigated for overcoming resistance to cancer therapies including radiotherapy, targeted therapy, immunotherapy, and chemotherapy. Selective autophagy inhibitors that directly target autophagic process have been developed for cancer treatment. Efficacies of autophagy inhibitors have been tested in various pre-clinical cancer animal models. Combination therapies of autophagy inhibitors with chemotherapeutics are being evaluated in clinal trials. In this review, we will focus on genetical and pharmacological perturbations of autophagy-related proteins in different steps of autophagic process and their therapeutic benefits. We will also summarize combination therapies of autophagy inhibitors with chemotherapies and their outcomes in pre-clinical and clinical studies. Understanding of current knowledge of development, progress, and application of cytoprotective autophagy inhibitors in combination therapies will open new possibilities for overcoming drug resistance and improving clinical outcomes.

Key Words: Autophagy, Autophagy inhibitor, Anticancer agent, Resistance, Combination therapy

INTRODUCTION

Macroautophagy (hereafter referred to as autophagy) is a highly conserved catabolic process by which damaged or unnecessary proteins or organelles are delivered to lysosomes for degradation, leading to maintenance of intracellular homeostasis (Levy *et al*., 2017). Autophagic process involves formation of double-membraned vesicles known as autophagosomes that can engulf proteins and organelles prior to delivery to lysosome (Mizushima, 2007; Mizushima *et al*., 2011). Autophagy occurs at a basal level in all cells. It is induced by various signals and cellular stresses such as hypoxia, starvation, and different cancer therapies as a cytoprotective mechanism. Autophagy has a context-dependent role in cancer. It is closely related to the occurrence and drug resistance of cancer (Eskelinen, 2011; Towers and Thorburn, 2016; Chang and Zou, 2020). Autophagy can limit oxidative stress, chronic tissue damage, and oncogenic signaling by preventing toxic accumulation of damaged proteins and organelles, particularly mitochondria, thereby inhibiting tumorigenesis in the early stage of tumor formation (White *et al*., 2015). In contrast, some cancers are dependent on autophagy for survival by using autophagy-mediating recycling to maintain mitochondria function and energy homeostasis because of elevated metabolic demand of cancer growth. In established tumors, autophagy can be induced as a response to nutrient deprivation, energy deficits, hypoxia, and chemotherapeutics drugs, finally resulting in acquired resistance in tumors. Some tumor cell types with high basal autophagic flux might show intrinsic drug resistance. Conversely, persistent or excessive autophagy can induce autophagic cell death in cancer therapy (Puissant *et al*., 2010; Aryal *et al*., 2014). Clinical interventions to manipulate autophagy in cancer treatment are underway by mainly focusing on inhibiting autophagy, although such interventions are in contradiction with dual roles of autophagy.

In this review, we will focus on application of autophagy in-

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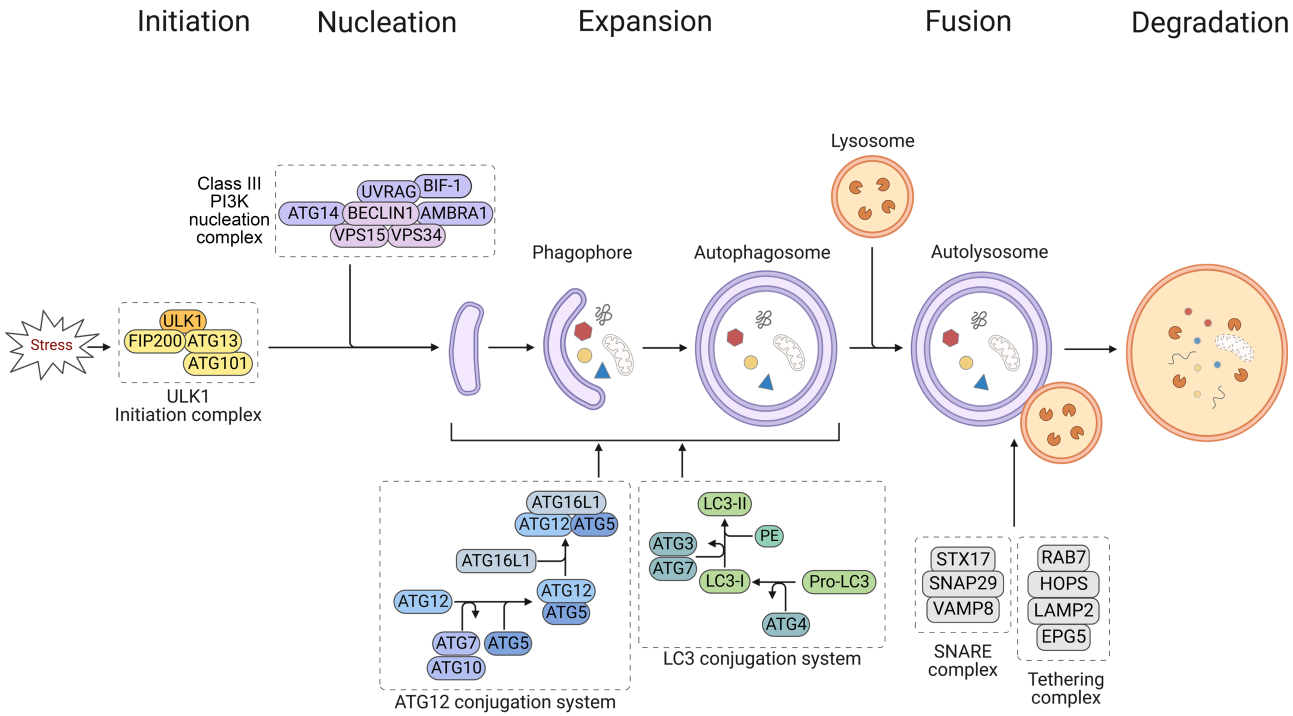

Fig. 1. Schematic overview of core autophagic process.

hibitors in cancer treatment. First, we will provide important targets of autophagic process and impact of autophagy-related gene deficiency in genetically engineered mice as basic information. Pharmacological inhibitors developed for targeting autophagic process and their efficacies in preclinical and clinical studies are then reviewed. Specially, we will focus on combination therapies of autophagy inhibitors with chemotherapeutic agents to improve therapeutic benefits of current cancer therapies.

CORE PROCESS OF AUTOPHAGY

Autophagic process can be divided into distinct stages: initiation, nucleation of the autophagosome, expansion of the autophagosome membrane and maturation, docking and fusion with the lysosome for cargo degradation, and degradation (Fig. 1). Formation and turnover of autophagosome are executed by highly conserved autophagy-related (ATG) proteins (Mizushima *et al*., 2011). Initiating signals of autophagy to form the autophagosome originate from activated Unc-51-like kinase 1 (ULK1, human homolog of yeast ATG1). ULK1 forms a pre-initiation complex with ATG13, ATG101, and focal adhesion kinase family-interacting protein 200 (FIP200) under stress conditions (Carlsson and Simonsen, 2015). ULK1 initiation complex then recruits class III PI3K nucleation complex composed of BECLIN 1 (human homolog of yeast ATG6), ATG14, type III phosphatidylinositol 3-kinase/vacuolar protein sorting 34 (class III PI3K/VPS34), autophagy and BECLIN 1 regulator-1 (AMBRA1), and UV radiation resistance-associated gene protein (UVRAG) by phosphorylating BECLIN 1 to activate autophagy-specific VPS34 (Russell *et al*., 2013). BIF-1 is also involved in the formation of nucleation complex by binding to UVRAG (Takahashi *et al*., 2007). The formed phosphatidylinositol 3-phosphate (PI3P)-binding complex can then direct distribution of the machinery that enable autophagosome formation (Hansen *et al*., 2018). In the ATG12 conjugation system, ATG12 is attached to ATG5, which is then attached to ATG16L1, followed by dimerization and interaction with the PI3P-binding complex through WD repeat domain phosphoinositide-interacting proteins (WIPIs; ATG18 in yeast) and zinc-finger FYVE domain-containing protein 1 (DFCP1). Under catalysis of E1-like enzyme ATG7 and E2-like enzyme ATG10, the formed ATG5/ATG12/ATG16L1 (E3) complex can facilitate recruitment and conversion of precursor pro-microtubule-associated protein 1 light chain 3 (LC3; ATG8 in yeast) to membrane bound LC3-II form (LC3 conjugation). In the LC3 conjugation system, pro-LC3 is cleaved by protease ATG4 to form cytosolic LC3-I, which is then recognized by E1-like enzyme ATG7 and E2-like enzyme ATG3, leading to conjugation with phosphatidylethanolamine (PE) to form LC3-II (Ichimura *et al*., 2000; Kabeya *et al*., 2000; Hanada *et al*., 2007; Aman *et al*., 2021). This conjugation is incorporated into pre-autophagosomal and autophagosomal membranes, where LC3 can interact with cargo receptors such as sequestosome 1 (SQSTM1/p62) and neighbor of BRCA1 gene 1 (Nbr1) carrying LIRs (LC3-interacting regions) to target them for autophagic degradation (Birgisdottir *et al*., 2013; Slobodkin and Elazar, 2013). LC3-II is widely used as a marker for assessing autophagy due to its abundance in autophagosomal membranes (Schaaf *et al*., 2016). Following expansion and maturation, LC3-I is released from autophagosomes by deconjugation through the action of ATG4 (Tanida *et al*., 2004). Sealed autophagosome then merges with lysosome through

Table 1. *In vivo* genetic studies of autophagy-related genes

Autophagic stage	Target	Genetic modification	Phenotypes	Reference
Initiation	ULK1	KO	Viable without overt development defects; delayed mitochondrial clearance in reticulocytes	Kundu *et al*., 2008
	ULK2	KO	Normal development and fertile	Lee and Tournier, 2011
	ATG13	KO	Embryonic lethality with growth retardation and myocardial growth defects (E17.5)	Kaizuka and Mizushima, 2016
	ATG101	N/A		
	FIP200	KO	Embryonic lethality with defective heart and liver development (E14.5)	Gan *et al*., 2006
Nucleation	BECLIN 1	KO	Embryonic lethality (E8.5) High incidence of spontaneous tumors as in heterozygote	Yue *et al*., 2003
	ATG14	KO	Neonatal lethality	https://www.mousephenotype.org/
	VPS15	KO	Embryonic lethality (E7.5)	Nemazanyy *et al*., 2013
	VPS34	KO	Embryonic lethality with abnormal embryogenesis (E7.5-E8.5)	Zhou *et al*., 2011
	UVRAG	KO	Embryonic lethality (E7.5)	Afzal *et al*., 2015
	BIF-1	KO	Normal development and high incidence of spontaneous tumorigenesis	Takahashi *et al*., 2007
	AMBRA1	KO	Embryonic lethality with neural tube defect (E18.5*)	Fimia *et al*., 2007
Expansion	LC3B	KO	Normal development and fertile	Cann *et al*., 2008
	ATG3	KO	Neonatal lethality (1 d)	Sou *et al*., 2008
	ATG4A	KO	Close-to-normal development	https://www.mousephenotype.org/
	ATG4B	KO	Close-to-normal development	Marino *et al*., 2010
	ATG4C	KO	Close-to-normal development	https://www.mousephenotype.org/
	ATG5	KO	Neonatal lethality (1 d)	Kuma *et al*., 2004
	ATG7	KO	Neonatal lethality (1 d)	Komatsu *et al*., 2005
	ATG10	N/A		
	ATG12	KO	Neonatal lethality (1 d)	Malhotra *et al*., 2015
	ATG16L1	KO	Neonatal lethality (1 d)	Saitoh *et al*., 2008
Fusion	STX17	N/A		
	SNAP29	KO	Neonatal lethality with ichthyotic phenotype (1 d)	Schiller *et al*., 2016
	VAMP8	KO	Partial lethality and growth retardation with defect in secretion of the pancreas	Wang *et al*., 2004
	RAB7	KO	Embryonic lethality (E7-8)	Kawamura *et al*., 2012
	LAMP2	KO	Increased mortality between (20-40 d) with cardiomyopathy	Tanaka *et al*., 2000
	EPG5	KO	Growth retardation and reduced survival with selective neuronal vulnerability to degeneration	Zhao *et al*., 2013

N/A, not available; *, available at https://www.mousephenotype.org/.

assistance of SNARE complex (STX17, SNAP29, VAMP8) and tethering complex (HOPS complex, RAB7, EPG5, and LAMP2) to form autolysosome (Yu *et al*., 2018; Xiao *et al*., 2021). Sequestered autophagic bodies and the inner membrane are then released into the lumen, where they are exposed to acidic hydrolases and lipases for degradation (Mizushima, 2007). Finally, autophagy is completed by allowing the resulting macromolecules to be recycled for reuse in the biosynthesis of essential components required for survival under stress conditions (Yorimitsu and Klionsky, 2005).

PHENOTYPES OF AUTOPHAGY-DEFICIENT MICE

Atg gene knockout mice are useful for understanding physiological roles of autophagy *in vivo*. Among core *Atg* genes involved in autophagosome formation in mammals, 25 of them have been knocked out in mice (Table 1). Three mortality patterns of *Atg* knockout mice have been observed. Some die at embryonic period. Some die within 1 d after birth. Some show vitality without obvious abnormalities. *Atg* gene (such as *Atg4a*, *Atg4b*, *Atg4c*, *Lc3b*, *Ulk1*, and *Ulk2*) knockout mice show no obvious defective phenotypes with close-to-normal development partly due to functional redundancy (Cann *et al*., 2008; Kundu *et al*., 2008; Marino *et al*., 2010; Lee and Tournier, 2011; Groza *et al*., 2022) (International Mouse Phenotyping Consortium [IMPC], https://www.mousephenotype.org/). However, *Ulk1*/*Ulk2* double knockout mice show neonatal lethality (Cheong *et al*., 2014). Mice with deletion of nonredundant genes (*Atg3*, *Atg5*, *Atg7*, *Atg12*, *Atg14*, and *Atg16l1*) involved

in ATG12 and LC3 conjugation systems after nucleation stage show neonatal lethality (Kuma *et al*., 2004; Komatsu *et al*., 2005; Saitoh *et al*., 2008; Sou *et al*., 2008; Malhotra *et al*., 2015) (https://www.mousephenotype.org/), whereas mice with knockout of nonredundant genes (*Fip200*, *Atg13*, *Beclin 1*, *Vps15*, *Vps34*, *Uvrag*, and *Ambra1*) involved in earlier stages before expansion stage are embryonic lethal (Yue *et al*., 2003; Gan *et al*., 2006; Fimia *et al*., 2007; Zhou *et al*., 2011; Nemazanyy *et al*., 2013; Afzal *et al*., 2015; Kaizuka and Mizushima, 2016). Among genes involved in fusion stage, knockout of *Snap29* gene encoding a component of SNARE complex shows neonatal lethality and deletion of *Vamp8* gene encoding another component of SNARE complex shows partial lethality and growth retardation with defect in secretion of the pancreas (Wang *et al*., 2004; Schiller *et al*., 2016). Deletion of genes (*Lamp2* and *Epg5*) of the tethering complex shows partial lethality, retardation, or reduced survival (Tanaka *et al*., 2000; Zhao *et al*., 2013). Loss of *Rab7* gene encoding another factor of the tethering complex leads to embryonic lethality (Kawamura *et al*., 2012). Thus, most autophagy-related genes are very important for embryonic and neonatal development. Reasons for phenotypic differences between whole-body knockout mice of *Atg* genes are still questionable. No genetic study has been reported for *Atg101*, *Atg10*, or *Stx17* gene.

While biallelic deletion of *Beclin 1* gene in mice shows embryonic lethality, monoallelic deletion of *Beclin 1* shows normal development with high incidence of spontaneous tumorigenesis and reduced autophagy, indicating that *Beclin 1* gene is essential for early embryonic development and a haploinsufficient tumor suppressor (Qu *et al*., 2003; Yue *et al*., 2003). In case of *Bif-1* gene, biallelic deletion in whole body leads to normal development with high incidence of spontaneous tumorigenesis (Takahashi *et al*., 2007). Mice with systemic mosaic deletion of *Atg5* and mice with liver-specific *Atg7* homologous knockout also develop benign liver adenomas, which originate from autophagy-deficient hepatocytes (Takamura *et al*., 2011). Thus, some *Atg* genes are necessary for suppression of spontaneous tumorigenesis through a cell-intrinsic protective mechanism. Conversely, autophagy can promote tumor growth by suppressing p53 response, maintaining mitochondrial function, sustaining metabolic homeostasis and survival during stress, and preventing progression of tumor to benign oncocytomas (Kimmelman, 2011; Guo *et al*., 2013b). Deletion of *Atg5* or *Atg7* gene in KRAS-transformed cells with proficient autophagy can impair their tumorigenicity by failing to maintain levels of tricarboxylic acid cycle metabolite and mitochondrial respiration under nutrient starvation, which creates an energy crisis that threatens survival (Guo *et al*., 2011; Yang *et al*., 2011). Deletion of *Atg7* also alters progression of lung cancer cells with KRAS (G12D) and Trp53 mutations by developing into oncocytomas instead of adenomas and carcinomas with suppressed proliferation and reduced tumor burden (Guo *et al*., 2013a). These observations support that autophagy plays a double-edged sword role in suppressing tumor initiation and in promoting survival and growth of tumors.

PHARMACOLOGICAL INHIBITORS DIRECTLY TARGETING AUTOPHAGY FORMATION

The process of autophagy is divided into four distinct stages (Fig. 1). Each stage has potential targets for inhibiting autophagy. Pharmacological inhibitors that target tumor growth and autophagy formation are summarized in Table 2. At the initiation stage, ULK1 has been mainly studied to develop inhibitors to interfere with growth of various cancer types including lung cancer and leukemia both *in vitro* and *in vivo* (Tang *et al*., 2017; Qiu *et al*., 2020). SBI-0206965, MRT68921, and ULK101 have been found as ULK1 kinase inhibitors showing cytotoxicity against various cancer cells *in vitro* (Tang *et al*., 2017; Martin *et al*., 2018; Chen *et al*., 2020; Qiu *et al*., 2020). In case of MRT68921, its effects on tumor growth inhibition and prolonged survival have been shown in H460 lung cancer and MNK45 gastric cancer xenografted animal models. It has dual targets, NUAK1 and ULK1 (Martin *et al*., 2018).

At the nucleation stage, VPS34/class III PI3K has been extensively studied as a main target for autophagy formation. Several kinase inhibitors including 3-MA, SAR405, SB02024, and VPS34-IN1 have been developed for inhibiting autophagy and tumor growth as shown in Table 2. These compounds show a good relevance to inhibition of autophagy formation and suppression of *in vitro* tumor cell growth in several cancer types including breast cancer and leukemia. 3-MA, SAR405, and SB02024 show inhibitory effects on tumor growth *in vivo* with extended survival in xenograft animal models (Dyczynski *et al*., 2018; Noman *et al*., 2020; Chen and Yao, 2021).

At the expansion stage, only ATG4B protease has been targeted for the development of autophagy inhibitors. NSC185058, S130, and tioconazole have shown autophagy formation-inhibiting and tumor-suppressive effects on various cancers including glioblastoma and colorectal cancer both *in vitro* and *in vivo* (Akin *et al*., 2014; Huang *et al*., 2017; Liu *et al*., 2018; Fu *et al*., 2019; El-Gowily *et al*., 2021). NSC185058 has been found to prolong survival of JK83 primary cancer-xenografted mice (Huang *et al*., 2017). Tioconazole has a survival benefit in MCF-7 breast cancer-xenografted mice (El-Gowily *et al*., 2021). UAMC-2526 can dose-dependently inhibit HT-29 cancer cells with potent inhibition of autophagy (Kurdi *et al*., 2017). FMK-9a has a weak cytotoxicity to HeLa cells although it can potently inhibit autophagy formation (Chu *et al*., 2018b). Its *in vivo* efficacy has not been reported yet.

At the fusion stage, EACC can inhibit STX17, resulting in inhibition of autolysosome formation (Vats and Manjithaya, 2019). At present, its inhibitory effect on tumor growth has not been reported yet. Several other inhibitors can also inhibit autolysosome formation. Among them, chloroquine (CQ) and hydroxychloroquine (HCQ) originally developed as anti-malaria drugs have been found to be able to inhibit autolysosome formation by increasing lysosomal pH and lysosomal membrane permeability (Homewood *et al*., 1972). They can also inhibit autophagic flux by decreasing autophagosome-lysosome fusion presumably by interfering with SNAP29 recruitment (Mauthe *et al*., 2018). Both compounds have been intensively studied for inhibiting tumor growth of many cancer types. They also have beneficial effects by prolonging survival in preclinical animal models (Hu *et al*., 2016; Liu *et al*., 2019; Chen *et al*., 2021; El-Gowily *et al*., 2021). Besides these inhibitors, bafilomycin A1, ROC-325, LS-1-10, BRD1240, cytochalasin E, Lys05, and DC661 can also inhibit autolysosome formation during autophagic process. Bafilomycin A1, ROC-325, LS-1-10, BRD1240, cytochalasin E, Lys05, and DC661 compounds can inhibit autolysosome formation by elevating lysosomal pH (Yamamoto *et al*., 1998; McAfee *et al*., 2012; Aldrich *et al*., 2015; Carew *et al*., 2017; Fu *et al*., 2017; Takanezawa *et al*.,

Table 2. Autophagy inhibitors targeting core autophagic process

Autophagic stage	Target	Inhibitor	Cancer type (cell lines)	Working concentrations/ *in vivo* dose	Mechanism of autophagy inhibition	Inhibitory effects on cancer			Reference
						In vitro	*In vivo*		
						Cyto-toxicity	Tumor growth	Survival	
Initiation	ULK1/2	SBI-0206965	Non-small cell lung cancer (A549, H460, HCC827)	0.01-100 μM	Inhibits ULK1 kinase activity (Egan *et al*., 2015)	+	ND	ND	Tang *et al*., 2017
			Acute myeloid leukemia (HL60, U937)	2-15 μM		+	ND	ND	Qiu *et al*., 2020
		MRT68921	Various cancer (A549, **H460**, H1299, SW480, SW620, U251, U266, HT-29, HCT-116, Colo320, PC-3, **MNK45, 4T1**)	1.76-8.91 μM (IC_{50})/ 10-40 mg/kg/d, 7 times	Inhibits ULK1 kinase activity (Petherick *et al*., 2015)	+	+	+	Chen *et al*., 2020
		ULK101	Various cancer (A549, U2OS, H838, H727, H2030)	11.3-56.8 μM (IC_{50})	Inhibits ULK1 kinase activity (Martin *et al*., 2018)	+	ND	ND	Martin *et al*., 2018
Nucleation	VPS34	3-MA	Uterine sarcoma (FU-MMT-1, **MES-SA**)	200-300 μM/ 15 mg/kg/d, 7 times	Inhibits VPS34 kinase activity (Petiot *et al*., 2000)	+	+	+	Chen and Yao, 2021
		SAR405	Various cancer (**B16-F10, CT26, Renca, YUMM**)	10 mg/kg/d, 10 times	Inhibits VPS34 kinase activity (Ronan *et al*., 2014)	ND	+	+	Noman *et al*., 2020
		SB02024	Various cancer (**B16-F10, CT26, Renca, YUMM**)	20 mg/kg/d, 10 times	Inhibits VPS34 kinase activity (Dyczynski *et al*., 2018)	ND	+	+	Noman *et al*., 2020
			Breast cancer (**MCF-7, MDA-MB-231**)	0.1-100 μM/ 20-50 mg/kg/d, 21-30 times		+	+	ND	Dyczynski *et al*., 2018
		VPS34-IN1	Acute myeloid leukemia (HL60, U937, K562, MOLM-14, MV4-11, THP1, KASUMI, OCI-AML2, OCI-AML3)	1.4-9.6 μM (IC_{50})	Inhibits VPS34 kinase activity (Bago *et al*., 2014)	+	ND	ND	Meunier *et al*., 2020
		VPS34-IN2 (PIK-III)	Not studied on tumor cell growth and death		Inhibits VPS34 kinase activity (Dowdle *et al*., 2014)				
Expansion	ATG4B	NSC185058	Glioblastoma (Patient-derived glioma stem-like cell **JK83, 23**)	10-100 μM/ 150 mg/kg/d, 9 times	Inhibits ATG4B protease activity (Akin *et al*., 2014)	+	+	+	Huang *et al*., 2017
			Osteosarcoma (**Saos-2**)	3-100 μM/ 100 mg/kg, ~15 times		+	+	ND	Akin *et al*., 2014
		UAMC-2526	Colorectal cancer (**HT-29**)	1-100 μM	Inhibits ATG4B protease activity (Kurdi *et al*., 2017)	+	+	ND	Kurdi *et al*., 2017
		FMK-9a	Ovarian cancer (HeLa)	100 μM	Inhibits ATG4B protease activity (Qiu *et al*., 2016)	+	ND	ND	Chu *et al*., 2018b
		S130	Various cancer (HeLa, **HCT116**, HL60)	4.7-16.1 μM (IC_{50})/ 20 mg/kg/d, 21 times	Inhibits ATG4B activity (Fu *et al*., 2019)	+	+	ND	Fu *et al*., 2019
		Tioconazole	Various cancer (H4, **HCT116**, MDA-MB-231)	40 μM/60 mg/kg/d, ~9 times	Inhibits ATG4B protease activity (Liu *et al*., 2018)	+	+	ND	Liu *et al*., 2018
			Breast cancer (**MCF-7**)	14.5 μM (IC_{50})/ 60 mg/kg/d, ~12 times		+	+	+	El-Gowily *et al*., 2021
Fusion	STX17	EACC	Not studied on tumor cell growth and death		Inhibits translocation of Stx17 onto autophagosome (Vats and Manjithaya, 2019)				

Table 2. Continued

Autophagic stage	Target	Inhibitor	Cancer type (cell lines)	Working concentrations/ *in vivo* dose	Mechanism of autophagy inhibition	Inhibitory effects on cancer			Reference
						In vitro	*In vivo*		
						Cyto-toxicity	Tumor growth	Survival	
	Lysosome	Chloroquine	Hepatocellular carcinoma (**HepG2**, Huh7)	5-80 μM/ 160 mg/kg/d, ~15 times	Deacidifies lysosome and increases lysosomal membrane permeability (Homewood *et al.*, 1972)	+	+	ND	Hu *et al.*, 2016
			Breast cancer (**MCF-7**)	32.5 μM (IC_{50})/ 50 mg/kg/d, ~12 times		+	+	+	El-Gowily *et al.*, 2021
		Hydroxy-chloro-quine	Glioblastoma (LN18, LN229)	~15 μM		+	ND	ND	Liu *et al.*, 2019
			Hepatocellular carcinoma (HepG2, **Huh7**)	12.69-13.60 μM (IC_{50})/ 30 mg/kg/d, 20 times		+	+	ND	Chen *et al.*, 2021
		Bafilomycin A1	Gastric cancer (SGC-7901)	0.1 μM	Deacidifies lysosome by inhibiting the lysosomal V-ATPase (Yamamoto *et al.*, 1998)	+	ND	ND	Li *et al.*, 2016
		ROC-325	Acute myeloid leukemia (**MV4-11**, HL-60, KG-1, MOLM-13, NOMO-1, PL-21)	1-10 μM/ 50 mg/kg, ~12 times	Deacidifies lysosome and increases lysosomal membrane permeability (Carew *et al.*, 2017)	+	+	+	Nawrocki *et al.*, 2019
			Various cancer (A498, A549, CFPAC-1, COLO-205, DLD-1, IGROV-1, MCF-7, MiaPaCa-2, NC1-H69, PC-3, RL, UACC-62, **786-O**, Caki-2, Achn)	4.6-11 μM (IC_{50})/ 25-50 mg/kg/d, 30 times		+	+	ND	Carew *et al.*, 2017
		LS-1-10	Colon cancer (LoVo, **DLD1**, HT29, HCT116, SW480)	0.82-1.31 μM (IC_{50})/ 40-80 mg/kg/d, 15 times	Increases lysosomal membrane permeability (Fu *et al.*, 2017)	+	+	ND	Fu *et al.*, 2017
		BRD1240	Not studied on tumor cell growth and death		Inhibits lysosomal acidification (Aldrich *et al.*, 2015)	ND			
		Cytochalasin E	Lung cancer (A549)	0.25-1 μM	Deacidifies lysosome and increases lysosomal membrane permeability (Takanezawa *et al.*, 2018)	+	ND	ND	Takanezawa *et al.*, 2018
		Lys05	Various cancer (LN229, **C8161, 1205Lu, HT-29**)	3.6-7.9 μM (IC_{50})/ 10-80 mg/kg, ~7 times	Deacidifies lysosome and increases lysosomal membrane permeability (McAfee *et al.*, 2012; Zhou *et al.*, 2020)	+	+	ND	McAfee *et al.*, 2012
			Glioblastoma (U251, LN229)	6.0-9.1 μM (IC_{50})		+	ND	ND	Zhou *et al.*, 2020
		DC661	Hepatocellular carcinoma (Hep3B, **Hep1-6**)	0.5-0.6 μM (IC_{50})/ 3 mg/kg/d, 21 times	Increases lysosomal membrane permeability by inhibiting PPT1 (Xu *et al.*, 2022)	+	+	+	Xu *et al.*, 2022
			Various cancer (A375P, WM3918, WM983B, PANC1, **HT-29**)	0.1-1 μM/ 3 mg/kg/d, 10 times		+	+	ND	Rebecca *et al.*, 2019

ND, not determined; Bold letters indicate cell lines used in *in vivo* experiments.

2018; Zhou *et al*., 2020; Xu *et al*., 2022). DC661 is a dimeric CQ derivative (Xu *et al*., 2022). ROC-325, LS-1-10, Lys05, and DC661 have been tested as possible cancer therapeutics in pre-clinical animal models. It was found that they could suppress tumor growth in several cancer models (McAfee *et al*., 2012; Carew *et al*., 2017; Fu *et al*., 2017; Nawrocki *et al*., 2019; Rebecca *et al*., 2019; Xu *et al*., 2022). ROC-325 and DC661 have a survival benefit in MV4-11 acute myeloid leukemia or Hep1-6 hepatocellular carcinoma (HCC) xenografted mice (Nawrocki *et al*., 2019; Xu *et al*., 2022).

So far, ULK1/2, VPS34, ATG4B, and fusion with lysosome have been mainly targeted to develop inhibitors to block autophagic process. Their inhibitors can suppress tumor growth and prolong survival in various cancers in preclinical setting. Notably, most autophagy inhibitors have been developed to block activities of enzymes such as kinase and protease rather than protein-protein interactions except for inhibitors blocking autophagosome fusion with lysosome.

SYNERGISTIC EFFECTS OF AUTOPHAGY INHIBITORS WITH ANTI-CANCER DRUGS IN PRE-CLINICAL STUDIES

Combination of autophagy inhibitors with various anti-cancer therapeutics have been tested in various cancer cell lines and pre-clinical cancer animal models to increase their efficacies (Table 3). In the initiation stage, SBI-0206965, a ULK1 inhibitor, showed an anti-tumor effect on non-small cell lung cancer (NSCLC) cells (Tang *et al*., 2017). It has been reported that SBI-0206965 can sensitize these cells to cisplatin (a platinum-based chemotherapeutic agent causing DNA damage) by modulating both autophagy and apoptosis pathways. The sensitivity of acute myeloid leukemia (AML) cell lines to daunorubicin (a DNA alkylating agent) can also be enhanced by SBI-0206965 (Qiu *et al*., 2020).

In the nucleation stage, 3-MA has been tested for combination with several kinase inhibitors such as apatinib, gefitinib, and sorafenib, a HDAC inhibitor (vorinostat), and a platinum-based chemotherapeutic agent (cisplatin). Apatinib, a highly selective inhibitor of vascular endothelial growth factor receptor 2 (VEGFR2) tyrosine kinase, can induce cell cycle arrest, apoptosis, and autophagy in osteosarcoma cells lines. By inhibiting autophagy with 3-MA, apoptosis can be increased in apatinib-treated cells (Liu *et al*., 2017a). Apatinib also shows combinatorial effect with 3-MA by significantly inhibiting the growth and migration of uterine sarcoma cells (Chen and Yao, 2021). For triple negative breast cancers (TNBCs), effective targeted therapy is lacking. Since epidermal growth factor receptor (EGFR) is over-expressed in about 50% of TNBCs, EGFR inhibitors such as gefitinib treatment have been attempted. However, their effects were disappointing (Nakai *et al*., 2016). Autophagy was thought to be related to drug resistance. By autophagy inhibition with 3-MA or bafilomycin A1, the sensitivity of gefitinib could be improved (Liu *et al*., 2017b). Autophagy inhibition by 3-MA can enhance the synergistic effect of a combination of vorinostat with sorafenib in HCC cells (Yuan *et al*., 2014). Treatment with 3-MA can also enhance cisplatin sensitivity in ovarian cancer cells (Zhang *et al*., 2012). SB02024, another inhibitor of VPS34, can significantly potentiate cytotoxicities of sunitinib (broad kinase inhibitor) and erlotinib (EGFR kinase inhibitor) to breast cancer cells (Dyczynski *et al*., 2018). For immunologically cold tumors, antibodies targeting programmed cell death 1 (PD-1) or programmed death-ligand 1 (PD-L1) have limited efficacies (Jiang *et al*., 2019; Wu *et al*., 2022). Combination of SB02024 or SAR405 can improve the therapeutic benefit of anti-PD-L1/PD-1 in melanoma and colorectal cancer cells by inhibiting VPS34 (Noman *et al*., 2020). PIK-III, a recently developed inhibitor of lipid kinase VPS34, can also inhibit tyrosine kinase inhibitor (TKI)-induced autophagy when used in combination with nilotinib (Baquero *et al*., 2019), showing an enhanced anti-cancer effect when it is combined with sunitinib or erlotinib (Dyczynski *et al*., 2018).

For the expansion stage, ATG4B inhibiting compound UAMC-2526 is a benzotropolone derivative with fair plasma stability (Kurdi *et al*., 2017). It has been demonstrated that UAMC-2526 can improve inhibition of tumor growth with oxaliplatin (a platinum-based chemotherapeutic agent) in colorectal cancer cells. It has been shown that tioconazole, a clinical anti-fungal drug, can inhibit activities of ATG4A and ATG4B in a drug repurposing study (Liu *et al*., 2018). In HCT116 colorectal cancer cells, tioconazole combined with doxorubicin (a DNA alkylating agent) resulted in significantly enhanced chemotherapeutic efficacy in spheroid cell culture and xenografted tumors. In MCF breast cancer cells, combination of tioconazole with doxorubicin significantly inhibited PI3K/AKT/mTOR and ATG4B pathways, resulting in tumor growth inhibition with various antioxidant effects (El-Gowily *et al*., 2021).

In the late autophagy stage, multiple inhibitors can affect the fusion process. CQ and HCQ are clinically approved antimalarial agents. They have been tested for combination with various anti-cancer drugs. Anti-microtubule drug [paclitaxel, mebendazole (MBZ)], DNA alkylating agents [doxorubicin, daunorubicin, temozolomide (TMZ)], platinum-based chemotherapeutic agent (cisplatin), a Raf kinase inhibitor (sorafenib), and an anti-VEGF antibody (bevacizumab) have been successfully used for combination with CQ or HCQ to sensitize cancer cells to anti-cancer drugs. In endometrial carcinoma cell lines, paclitaxel-mediated cell death is further potentiated by pretreatment with CQ (Liu and Li, 2015). Combination of CQ with doxorubicin can also significantly sensitize various cancer cells to doxorubicin treatment *in vitro* (Liu *et al*., 2018; El-Gowily *et al*., 2021) and *in vivo* (El-Gowily *et al*., 2021). Cisplatin-based chemotherapy is the first line treatment for bladder cancer. Cisplatin-induced autophagy is considered to be responsible for cisplatin resistance. Autophagy inhibitors bafilomycin A1 and CQ can significantly enhance cytotoxicity of cisplatin toward bladder cancer cells (Lin *et al*., 2017). CQ treatment can also sensitize ovarian cancer cells to cisplatin *in vitro* (Zhang *et al*., 2012). TMZ is the first line chemotherapeutic drug of choice in glioblastoma. It can induce autophagy (Singh *et al*., 2021). However, glioblastoma with a grim prognosis (median overall survival (OS) of 14.6 months) demands further therapeutic modalities. MBZ, a widely used anthelmintic drug, has shown cytotoxic effects on several cancer cells including melanoma, gastric cancer, lung cancer, and glioblastomas (Guerini *et al*., 2019). Addition of CQ can also enhance anti-proliferative effect of TMZ or MBZ (Kanzawa *et al*., 2004; Lee *et al*., 2015). Such effect is further potentiated by triple combination with TMZ (Jo *et al*., 2022). Combination of CQ with apatinib (VEGFR2 inhibitor) can also effectively inhibit *in vivo* growth of thyroid cancer cells (KHM-5M) xenografted in mice (Feng *et al*., 2018).

Table 3. Synergistic effects of autophagy inhibitors with anti-cancer drugs in pre-clinical models

Autophagic stage	Autophagy inhibitor	Anticancer drug	Cancer type (cell lines)	Synergistic effect		Reference
				In vitro	*In vivo*	
Initiation	SBI-0206965	Cisplatin	Non-small cell lung cancer (A549, H460)	+	ND	Tang *et al*., 2017
		Daunorubicin	Acute myeloid leukemia (HL60, U937)	+	ND	Qiu *et al*., 2020
Nucleation	3-MA	Apatinib	Osteosarcoma (**KHOS**)	+	+	Liu *et al*., 2017a
			Uterine sarcoma cancer (**MES-SA**, FU-MMT-1)	+	+*	Chen and Yao, 2021
		Gefitinib	Triple negative breast cancer (**MDA-MB-468**, MDA-MB-231)	+	+	Liu *et al*., 2017b
		Sorafenib, Vorinostat	Hepatocellular carcinoma (Hep3B, HepG2, PLC/PRF/5)	+	ND	Yuan *et al*., 2014
		Cisplatin	Ovarian cancer (A2780, OVCAR3)	+	ND	Zhang *et al*., 2012
	SB02024	Sunitinib, Erlotinib	Breast cancer (MDA-MB-231, MCF-7)	+	ND	Dyczynski *et al*., 2018
		Anti-PD-1, Anti-PD-L1	Various tumor (**B16-F10, CT26**)	ND	+*	Noman *et al*., 2020
	SAR405	Anti-PD-1, Anti-PD-L1	Various tumor (**B16-F10, CT26**)	ND	+*	Noman *et al*., 2020
	PIK-III	Nilotinib	Chronic myeloid leukemia (Patient-derived $CD34^+$ CML cell)	+	ND	Baquero *et al*., 2019
		Sunitinib, Erlotinib	Breast cancer (MDA-MB-231, MCF-7)	+	ND	Dyczynski *et al*., 2018
Expansion	UAMC-2526	Oxaliplatin	Colorectal cancer (**HT-29**)	+	+	Kurdi *et al*., 2017
	Tioconazole	Doxorubicin	Colorectal cancer (**HCT116**, H4, MDA-MB-231)	+	+	Liu *et al*., 2018
			Breast cancer (**MCF-7**)	+	+	El-Gowily *et al*., 2021
Fusion	Chloroquine	Paclitaxel	Endometrial carcinoma (HEC-1A, JEC)	+	ND	Liu and Li, 2015
		Doxorubicin	Various cancer (HCT116, H4, MDA-MB-231)	+	ND	Liu *et al*., 2018
			Breast cancer (**MCF-7**)	+	+	El-Gowily *et al*., 2021
		Cisplatin	Bladder cancer (5637, T24)	+	ND	Lin *et al*., 2017
			Ovarian cancer (A2780, OVCAR3)	+	ND	Zhang *et al*., 2012
		Temozolomide, Mebendazole	Glioblastoma (U87, U373)	+	ND	Jo *et al*., 2022
		Apatinib	Anaplastic thyroid cancer (C643, **KHM-5M**)	+	+	Feng *et al*., 2018
	Hydroxy-chloro-quine	Sorafenib	Hepatocellular carcinoma (**Huh7**, HepG2)	+	+	Chen *et al*., 2021
		Bevacizumab	Glioblastoma (LN18, LN229)	+	ND	Liu *et al*., 2019
	Bafilomycin A1	Cisplatin	Tongue squamous cell carcinoma (Tca8113, Tscca)	+	ND	Chu *et al*., 2018a
			Bladder cancer (5637, T24)	+	ND	Lin *et al*., 2017
		Gefitinib	Triple negative breast cancer (**MDA-MB-468**, MDA-MB-231)	+	+	Liu *et al*., 2017b
		5-Fluorouracil	Gastric cancer (SGC-7901)	+	ND	Li *et al*., 2016
	ROC-325	Azacitidine	Acute myeloid leukemia (**MV4-11**, HL-60, MOLM-13, KG-1)	+	+*	Nawrocki *et al*., 2019
	Cytochalasin E	Bortezomib	Lung cancer (A549)	+	ND	Takanezawa *et al*., 2018
	Lys05	Nilotinib	Chronic myeloid leukemia (**Patient-derived $CD34^+$ CML cell**)	+	+	Baquero *et al*., 2019
	DC661	Sorafenib	Hepatocellular carcinoma (Hep 3B, **Hep 1-6**)	+	+*	Xu *et al*., 2022

ND, not determined; Bold letters indicate cell lines used in *in vivo* experiments; *, increased survival rate in *in vivo* mouse models.

HCQ has also been studied for combination with sorafenib or bevacizumab to inhibit cancer cell growth. While sorafenib is an effective chemotherapeutic agent in advanced HCC, sorafenib resistance can lead to treatment failure. A combination therapy of sorafenib with HCQ provides better therapeutic outcomes even for sorafenib-resistant HCC cells partly by modulating autophagy (Chen *et al*., 2021). For recurrent glioblastomas, bevacizumab (BEV) is widely used for disease control. However, BEV treatment only shows extended progression free survival (PFS). OS benefit could not be gained for patients (Wick *et al*., 2017). Recent evidence has demonstrated that BEV-induced cytoprotective autophagy is a cause of treatment failure (Huang *et al*., 2018). By combining HCQ with BEV for glioblastoma cell lines, the anti-cancer effect of BEV can be enhanced by blocking the autophagic process (Liu *et al*., 2019). Bafilomycin A1 can also increase cisplatin cytotoxicity in tongue squamous cell carcinoma (TSCC) and bladder cancer cells by inhibiting lysosomal uptake of platinum and enhancing intracellular platinum ion binding to DNA (Lin *et al*., 2017; Chu *et al*., 2018a). Combination of bafilomycin A1 with gefitinib (EGFR kinase inhibitor) can also enhance anti-tumor effects *in vitro* and *in vivo* (Liu *et al*., 2017b). When gastric cancer cell line was treated with 5-fluorouracil, chemotherapy-induced autophagy was recognizable. Bafilomycin A1 decreased the viability and clone formation, inhibited the invasive and migratory ability, and increased apoptosis (Li *et al*., 2016).

ROC-325, a novel autophagy inhibitor, can effectively in-

hibit autophagy in AML cells. Azacitidine (AZA), a hypomethylating agent, is frequently used in the management of myelodysplastic syndromes and AML. AZA treatment can trigger autophagy in AML cells. AZA in combination with ROC-325 can significantly increase the benefit in both *in vitro* and *in vivo* studies (Nawrocki *et al*., 2019). Cytochalasin E in combination with bortezomib, an inhibitor of the 26S proteasome, has also been used to treat human lung cancer cells (Takanezawa *et al*., 2018). In chronic myeloid leukemia (CML) patients, TKI treatment could induce autophagy that leads to treatment failure. To overcome such resistance, the effect of Lys05, a highly potent lysosomotropic agent, has been studied (Baquero *et al*., 2019). Lys05-mediated autophagy inhibition can reduce numbers of leukemic stem cells both *in vivo* and *in vitro*. Furthermore, Lys05 can sensitize patient-derived CMLs to TKI treatment. Palmitoyl-protein thioesterase 1 (PPT1) plays a critical role in various cancers (Rebecca *et al*., 2019; Sharma *et al*., 2020; Luo *et al*., 2021). It is significantly upregulated in HCC tissues compared with that in normal tissues (Xu *et al*., 2022). Increased PPT1 levels are also associated with poor prognosis. DC661, a selective and potent small-molecule PPT1-inhibitor, can inhibit autophagy and enhance sensitivity of HCC cells to sorafenib by inducing lysosomal membrane permeabilization, leading to lysosomal deacidification.

Synergy in anti-tumor effects has been observed by combining chemotherapeutics with all autophagy inhibitors to block each stage of autophagic process. In addition, all cytotoxic drugs ranging from platinum-based chemotherapeutic agents and DNA alkylating drugs to anti-angiogenic agent (bevacizumab) and immune modulating drug (anti-PD-1/anti-PD-L1) have been effectively combined with various autophagy inhibitors. In the future, it is necessary to evaluate the best combinations by examining which chemotherapeutics can be more effectively combined with which type of autophagy inhibitors.

CLINICAL TRIALS OF AUTOPHAGY INHIBITORS WITH OR WITHOUT CHEMOTHERAPEUTICS FOR CANCER TREATMENT

Various phases of clinical trials have been performed regarding autophagy inhibitors in combination with or without several chemotherapeutic drugs (Table 4). Although autophagy inhibitors showed potential benefits from pre-clinical studies, only those affecting the late autophagy stage are studied as potential candidates for clinical trials. Since most trials were designed as phase 1 or 2 studies, majority of trials were single arm trials without masking. At present, several solid tumors have been subjected to ongoing or completed clinical trials of CQ or HCQ by single treatment or combination treatment with various anti-cancer agents (https://clinicaltrials.gov/). Only published results or recognizable results from completed clinical trials will be reviewed.

In a single-center, randomized, double blinded, placebo-controlled trial of single treatment of CQ for glioblastoma patient, median OS after surgery was extended to 24 months for CQ-treated patients compared to 11 months for controls, although that trial failed to show statistical significance probably due to a small sample size (n=30) (Sotelo *et al*., 2006). Although this result warrants further a larger scale study, it provides implications that CQ, in conjunction with other treatments, might prolong survival of patients with glioblastoma.

Combination therapy of CQ with taxane or taxane-like chemotherapeutic agents (Docetaxel, paclitaxel, nab-paclitaxel, ixabepilone) against advanced or metastatic breast cancer which is refractory to anthracycline-based therapy has demonstrated a higher objective response rate (ORR) of 45 % than the expected ORR of 30% (Anand *et al*., 2021). In addition, the combination was well-tolerated without showing significant toxicity. A phase 1B/2 clinical trial of metformin and CQ has been registered for a dose-finding study in patients with IDH1-mutated or IDH2-mutated solid tumors (Molenaar *et al*., 2017).

In a phase 1 trial for surgically removable early-stage solid tumors, oral HCQ of 200 or 400 mg twice daily for 14 days as a neoadjuvant regimen showed no serious adverse events (Wang *et al*., 2018). It elevated plasma prostate apoptosis response- 4 (Par-4) levels over basal levels. Four patients had prostate adenocarcinomas. Two patients had NSCLC. Others had papillary thyroid carcinoma, squamous cell carcinoma of larynx, or carcinoid tumor of the lung. All nine HCQ-treated patients showed p62 induction indicative of autophagy inhibition by HCQ. Resected tumors from eight patients with elevated plasma Par-4 levels all exhibited TUNEL-positivity indicative of apoptosis. A single administration of HCQ was also performed for previously treated metastatic pancreatic cancer patients as a phase 2 trial (Wolpin *et al*., 2014). The primary endpoint was 2-month PFS. Among 20 patients enrolled, only 2 (10%) had no disease progression. Median PFS and OS were 46.5 days and 69.0 days, respectively. The HCQ monotherapy failed to show therapeutic efficacy. Thus, further combinatorial treatment strategies are needed.

In a phase 1 dose-escalation study of HCQ in combination with carboplatin and gemcitabine, HCQ 100 mg daily was found to be the maximum tolerated dose (MTD) (Karim *et al*., 2022). Dose-limiting toxicity was thrombocytopenia and/or neutropenia. This MTD was lower than that from previously reported outcomes with concomitant use of chemotherapeutics probably due to the myelosuppressive nature of these agents and previous treatment history of patients. When response rate was assessed in that study, one patient showed partial response (PR), 15 patients showed stable disease (SD), and six patients had progressive disease (PD). The disease control rate (DCR) was 48% for more than 6 months duration, 21% for more than 12 months, and 14% for more than 18 months. Combinatorial effects of HCQ on pancreatic cancer, gastrointestinal cancer, HCC, breast cancer, prostate cancer, and lung cancer are also under investigation in various phase 1-2 clinical trials (Table 4). A randomized phase 2 trial of pancreatic cancer to examine the ability of HCQ combined with a pre-operative regimen of gemcitabine and nab-paclitaxel (GA) was completed. The exact clinical impact of this study is yet to be determined. Further reports with proper analysis are warranted.

In a phase 2 trial, untreated metastatic NSCLC patients underwent a single arm designed study of HCQ in combination with carboplatin, paclitaxel, or bevacizumab (Malhotra *et al*., 2019). The ORR was 33% in 30 patients evaluable for response. It was found that 20% of patients demonstrated SD. The medium PFS was 3.3 months. In nine patients with KRAS positive tumors, the ORR was 44 % with median PFS higher than 6.4 months. Addition of HCQ provided a beneficial effect on clinical response. The benefit seemed to be higher for a certain subgroup of molecularly targeted patients.

Table 4. Clinical trials of autophagy inhibitors with or without anti-cancer drugs for cancer treatment

Autophagic stage	Autophagy inhibitor	Anti-cancer drugs	Cancer type	Phase	Study design	Masking	Enrollment	Trial no.	Reference
Fusion	Chloroquine	NA	Glioblastoma	3	Randomized	Double	30	NCT00224978*	Sotelo *et al*., 2006
		NA	Breast cancer	1/2	Single arm	None	12	NCT01023477	https://clinicalTrials.gov/
		Docetaxel, paclitaxel, nab-paclitaxel, ixabepilone	Breast cancer	2	Single arm	None	38	NCT01446016*	Anand *et al*., 2021
		Metformin	IDH1/2-mutated solid tumors	1/2	Single arm	None	15	NCT02496741	Molenaar *et al*., 2017
		Gemcitabine	Pancreatic cancer	1	Single arm	None	9	NCT01777477	https://clinicalTrials.gov/
		DT01	Melanoma	1	Single arm	None	27	NCT01469455	https://clinicalTrials.gov/
		Temozolomide	Glioblastoma	1	Single arm	None	13	NCT02378532	https://clinicalTrials.gov/
		Temozolomide	Glioblastoma	2	Single arm	None	10	NCT04397679	https://clinicalTrials.gov/
		Bortezomib, cyclophosphamide	Multiple myeloma	2	Single arm	None	11	NCT01438177	https://clinicalTrials.gov/
	Hydroxychloroquine	NA	Solid tumor	1	Single arm	None	10	NCT02232243*	Wang *et al*., 2018
		NA	Prostate cancer	2	Single arm	None	64	NCT00726596	https://clinicalTrials.gov/
		NA	Colorectal cancer	2	Single arm	None	38	NCT01006369	https://clinicalTrials.gov/
		NA	Pancreatic cancer	2	Single arm	None	20	NCT01273805*	Wolpin *et al*., 2014
		Carboplatin, gemcitabine	Advanced solid tumors	1	Single arm	None	22	NCT02071537*	Karim *et al*., 2022
		Gemcitabine, nab-paclitaxel	Pancreatic cancer	2	Randomized	None	104	NCT01978184	https://clinicalTrials.gov/
		Paclitaxel, carboplatin, bevacizumab	Lung cancer	2	Single arm	None	32	NCT01649947*	Malhotra *et al*., 2019
		Gemcitabine, nab-paclitaxel	Pancreatic cancer	1/2	Randomized	None	119	NCT01506973*	Karasic *et al*., 2019
		Temozolomide	Brain and CNS tumors	1/2	Single arm	None	92	NCT00486603*	Rosenfeld *et al*., 2014
		Gemcitabine	Pancreatic cancer	1/2	Single arm	None	35	NCT01128296	https://clinicalTrials.gov/
		IL-2	Renal cell cancer	1/2	Single arm	None	30	NCT01550367*	https://clinicalTrials.gov/
		NA	Prostate cancer	2	Single arm	None	20	NCT04011410	https://clinicalTrials.gov/
		Ulixertinib	Gastrointestinal cancer	2	Single arm	None	215	NCT05221320	https://clinicalTrials.gov/
		Sorafenib	Hepatocellular carcinoma	2	Single arm	None	68	NCT03037437	https://clinicalTrials.gov/
		Abemaciclib	Breast cancer	2	Randomized	None	66	NCT04523857	https://clinicalTrials.gov/
		Binimetinib	Lung cancer	2	Single arm	None	29	NCT04735068	https://clinicalTrials.gov/
		Chlorphensin carbamate, mFOLFIRINOX	Pancreatic cancer	1	Single arm	None	40	NCT05083780	https://clinicalTrials.gov/
		Trametinib	Pancreatic cancer	2	Single arm	None	22	NCT05518110	https://clinicalTrials.gov/

NA, not applicable; *, Clinical trials with published results.

Another phase 2 trial of GA regimen with or without HCQ on patients with advanced pancreatic cancer has been performed (Karasic *et al*., 2019). Primary end point of OS at 12 months was not improved by HCQ treatment. However, ORR was 38.2% (n=21) in the HCQ group and 21.1% (n=12) in the non-HCQ group. Treatment-related grade 3 or 4 adverse events that were higher in the HCQ group were neutropenia, fatigue, nausea, peripheral neuropathy, visual changes, and neuropsychiatric symptoms. Although HCQ combination failed to provide enhanced OS, higher response rate indicated that HCQ combination could be beneficial under certain clinical circumstances such as locally advanced tumor that might be potentially resectable upon treatment response.

A phase 1/2 trial of HCQ in combination with the standard of care for newly diagnosed glioblastoma has also been performed (Rosenfeld *et al*., 2014). Regarding phase 1 trial results, 3/3 subjects experienced Grade 3 or 4 neutropenia and thrombocytopenia. The MTD for HCQ was found to be 600 mg/d in this combination. Phase 2 trial results revealed that the median survival was 15.6 months with survival rates of 70%, 36%, and 25% at 12, 18, and 24 months, respectively. Pharmacokinetics analysis demonstrated a dose-proportional exposure for HCQ. However, since the MTD for HCQ was 600 mg/d, autophagy inhibition was not constantly achieved. Further development of compounds with lower toxicities and/or more inhibitory potential for autophagy is mandatory.

Another phase 1/2 trial of HCQ in combination with IL-2, a standard treatment for metastatic renal cell cancer, has been performed (https://clinicaltrials.gov/). Among 30 enrolled participants, initial 13 patients were administered with 1200 mg/d of HCQ. However, due to severe unexpected adverse events, the dose of HCQ was reduced to 600 mg/d. Overall, 29 patients were analyzed. Control rate (CR), PR, and SD were achieved in 3 (10.3%), 3 (10.3%), and 14 (48.3%) patients, respectively. Interestingly, 3/3 CR patients and 2/3 PR patients belonged to the 600 mg/d HCQ cohort. Proper interpretation of these results by relevant authorities has not been reported yet. The combinatorial effect of HCQ should be discussed in further details.

Up to date, CQ and its derivative HCQ are the only autophagy inhibitors that have been investigated in clinical trials for cancer treatment. However, most studies are still in phase 1 or 2. Clinical benefits of single and combinatorial treatments are not clearly demonstrated yet. Several dozens of clinical trials are still on-going or planned. We hope that more positive results would follow to provide cancer patients better treatment options.

FUTURE PERSPECTIVES

Despite various autophagy inhibitors targeting each autophagy stage have been tested in pre-clinical *in vitro* and *in vivo* studies, only CQ and HCQ have been translated into clinical trials. While CQ and HCQ are clinically approved antimalarial agents, other autophagy inhibitors do not have clinical implications yet. Since anti-cancer effect of CQ and HCQ might also come from other activities besides inhibition of autophagy, more specific autophagy inhibitors with safety that allow use in clinics should be developed. At present, clinically available inhibitors that can act in early stages of autophagic process are very limited. Such inhibitors also should be developed as effective adjuvant therapeutics for blocking cytoprotective autophagy to cure cancers. Thus, the development of more clinically effective and more selective autophagy inhibitors with various modes of action and acceptable toxicities is mandatory.

In the future, the positive impact of autophagy inhibition on cancer treatment needs further clarification. In order to determine whether the combinatorial strategy is beneficial or not, measures to identify autophagy inhibition needs to be standardized. Different MTDs from various clinical trials with different measures for autophagy inhibition can lead to confused interpretation for the presence of autophagy inhibition and their effects on clinical outcomes.

Utilizing an autophagy inhibitor in combination with chemotherapeutics may show potential benefits in cancer treatment because treatment resistance is in part correlated with increased autophagy reaction to cancer treatment. However, limited pool of autophagy inhibitors applicable to real world practice cripples our capability of validating autophagy inhibition in oncology practice. Further development of clinically available autophagy inhibitors with sufficient efficacy and safety is mandatory. By properly assessing autophagy inhibition in cancer treatment, more sophisticated clinical trials can be designed, leading to more informative results regarding combinatorial effects of autophagy inhibitors.

CONFLICT OF INTEREST

The authors have no competing financial interests relevant to this study to disclose.

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REFERENCES

Afzal, S., Hao, Z., Itsumi, M., Abouelkheer, Y., Brenner, D., Gao, Y., Wakeham, A., Hong, C., Li, W. Y., Sylvester, J., Gilani, S. O., Brustle, A., Haight, J., You-Ten, A. J., Lin, G. H., Inoue, S. and Mak, T. W. (2015) Autophagy-independent functions of UVRAG are essential for peripheral naive T-cell homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 1119-1124.

Akin, D., Wang, S. K., Habibzadegah-Tari, P., Law, B., Ostrov, D., Li, M., Yin, X. M., Kim, J. S., Horenstein, N. and Dunn, W. A., Jr. (2014) A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Autophagy* **10**, 2021-2035.

Aldrich, L. N., Kuo, S. Y., Castoreno, A. B., Goel, G., Kuballa, P., Rees, M. G., Seashore-Ludlow, B. A., Cheah, J. H., Latorre, I. J., Schreiber, S. L., Shamji, A. F. and Xavier, R. J. (2015) Discovery of a small-molecule probe for V-ATPase function. *J. Am. Chem. Soc.* **137**, 5563-5568.

Aman, Y., Schmauck-Medina, T., Hansen, M., Morimoto, R. I., Simon, A. K., Bjedov, I., Palikaras, K., Simonsen, A., Johansen, T., Tavernarakis, N., Rubinsztein, D. C., Partridge, L., Kroemer, G., Labbadia, J. and Fang, E. F. (2021) Autophagy in healthy aging and disease. *Nat. Aging* **1**, 634-650.

Anand, K., Niravath, P., Patel, T., Ensor, J., Rodriguez, A., Boone, T., Wong, S. T. and Chang, J. C. (2021) A phase II study of the efficacy and safety of chloroquine in combination with taxanes in the

treatment of patients with advanced or metastatic anthracycline-refractory breast cancer. *Clin. Breast Cancer* **21**, 199-204.
Aryal, P., Kim, K., Park, P. H., Ham, S., Cho, J. and Song, K. (2014) Baicalein induces autophagic cell death through AMPK/ULK1 activation and downregulation of mTORC1 complex components in human cancer cells. *FEBS J.* **281**, 4644-4658.
Bago, R., Malik, N., Munson, M. J., Prescott, A. R., Davies, P., Sommer, E., Shpiro, N., Ward, R., Cross, D., Ganley, I. G. and Alessi, D. R. (2014) Characterization of VPS34-IN1, a selective inhibitor of Vps34, reveals that the phosphatidylinositol 3-phosphate-binding SGK3 protein kinase is a downstream target of class III phosphoinositide 3-kinase. *Biochem. J.* **463**, 413-427.
Baquero, P., Dawson, A., Mukhopadhyay, A., Kuntz, E. M., Mitchell, R., Olivares, O., Ianniciello, A., Scott, M. T., Dunn, K., Nicastri, M. C., Winkler, J. D., Michie, A. M., Ryan, K. M., Halsey, C., Gottlieb, E., Keaney, E. P., Murphy, L. O., Amaravadi, R. K., Holyoake, T. L. and Helgason, G. V. (2019) Targeting quiescent leukemic stem cells using second generation autophagy inhibitors. *Leukemia* **33**, 981-994.
Birgisdottir, A. B., Lamark, T. and Johansen, T. (2013) The LIR motif - crucial for selective autophagy. *J. Cell Sci.* **126**, 3237-3247.
Cann, G. M., Guignabert, C., Ying, L., Deshpande, N., Bekker, J. M., Wang, L., Zhou, B. and Rabinovitch, M. (2008) Developmental expression of LC3alpha and beta: absence of fibronectin or autophagy phenotype in LC3beta knockout mice. *Dev. Dyn.* **237**, 187-195.
Carew, J. S., Espitia, C. M., Zhao, W., Han, Y., Visconte, V., Phillips, J. and Nawrocki, S. T. (2017) Disruption of autophagic degradation with ROC-325 antagonizes renal cell carcinoma pathogenesis. *Clin. Cancer Res.* **23**, 2869-2879.
Carlsson, S. R. and Simonsen, A. (2015) Membrane dynamics in autophagosome biogenesis. *J. Cell Sci.* **128**, 193-205.
Chang, H. and Zou, Z. (2020) Targeting autophagy to overcome drug resistance: further developments. *J. Hematol. Oncol.* **13**, 159.
Chen, M. Y., Yadav, V. K., Chu, Y. C., Ong, J. R., Huang, T. Y., Lee, K. F., Lee, K. H., Yeh, C. T. and Lee, W. H. (2021) Hydroxychloroquine (HCQ) modulates autophagy and oxidative DNA damage stress in hepatocellular carcinoma to overcome sorafenib resistance via TLR9/SOD1/hsa-miR-30a-5p/Beclin-1 axis. *Cancers (Basel)* **13**, 3227.
Chen, S. and Yao, L. (2021) Autophagy inhibitor potentiates the antitumor efficacy of apatinib in uterine sarcoma by stimulating PI3K/Akt/mTOR pathway. *Cancer Chemother. Pharmacol.* **88**, 323-334.
Chen, Y., Xie, X., Wang, C., Hu, Y., Zhang, H., Zhang, L., Tu, S., He, Y. and Li, Y. (2020) Dual targeting of NUAK1 and ULK1 using the multitargeted inhibitor MRT68921 exerts potent antitumor activities. *Cell Death Dis.* **11**, 712.
Cheong, H., Wu, J., Gonzales, L. K., Guttentag, S. H., Thompson, C. B. and Lindsten, T. (2014) Analysis of a lung defect in autophagy-deficient mouse strains. *Autophagy* **10**, 45-56.
Chu, H. Y., Wang, W., Chen, X., Jiang, Y. E., Cheng, R., Qi, X., Zhong, Z. M., Zeng, M. S., Zhu, X. F. and Sun, C. Z. (2018a) Bafilomycin A1 increases the sensitivity of tongue squamous cell carcinoma cells to cisplatin by inhibiting the lysosomal uptake of platinum ions but not autophagy. *Cancer Lett.* **423**, 105-112.
Chu, J., Fu, Y., Xu, J., Zheng, X., Gu, Q., Luo, X., Dai, Q., Zhang, S., Liu, P., Hong, L. and Li, M. (2018b) ATG4B inhibitor FMK-9a induces autophagy independent on its enzyme inhibition. *Arch. Biochem. Biophys.* **644**, 29-36.
Dowdle, W. E., Nyfeler, B., Nagel, J., Elling, R. A., Liu, S., Triantafellow, E., Menon, S., Wang, Z., Honda, A., Pardee, G., Cantwell, J., Luu, C., Cornella-Taracido, I., Harrington, E., Fekkes, P., Lei, H., Fang, Q., Digan, M. E., Burdick, D., Powers, A. F., Helliwell, S. B., D'Aquin, S., Bastien, J., Wang, H., Wiederschain, D., Kuerth, J., Bergman, P., Schwalb, D., Thomas, J., Ugwonali, S., Harbinski, F., Tallarico, J., Wilson, C. J., Myer, V. E., Porter, J. A., Bussiere, D. E., Finan, P. M., Labow, M. A., Mao, X., Hamann, L. G., Manning, B. D., Valdez, R. A., Nicholson, T., Schirle, M., Knapp, M. S., Keaney, E. P. and Murphy, L. O. (2014) Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis *in vivo*. *Nat. Cell Biol.* **16**, 1069-1079.
Dyczynski, M., Yu, Y., Otrocka, M., Parpal, S., Braga, T., Henley, A. B., Zazzi, H., Lerner, M., Wennerberg, K., Viklund, J., Martinsson, J., Grander, D., De Milito, A. and Pokrovskaja Tamm, K. (2018) Targeting autophagy by small molecule inhibitors of vacuolar protein sorting 34 (Vps34) improves the sensitivity of breast cancer cells to Sunitinib. *Cancer Lett.* **435**, 32-43.
Egan, D. F., Chun, M. G., Vamos, M., Zou, H., Rong, J., Miller, C. J., Lou, H. J., Raveendra-Panickar, D., Yang, C. C., Sheffler, D. J., Teriete, P., Asara, J. M., Turk, B. E., Cosford, N. D. and Shaw, R. J. (2015) Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. *Mol. Cell* **59**, 285-297.
El-Gowily, A. H., Loutfy, S. A., Ali, E. M. M., Mohamed, T. M. and Mansour, M. A. (2021) Tioconazole and chloroquine act synergistically to combat doxorubicin-induced toxicity via inactivation of PI3K/AKT/mTOR signaling mediated ROS-dependent apoptosis and autophagic flux inhibition in MCF-7 breast cancer cells. *Pharmaceuticals (Basel)* **14**, 254.
Eskelinen, E. L. (2011) The dual role of autophagy in cancer. *Curr. Opin. Pharmacol.* **11**, 294-300.
Feng, H., Cheng, X., Kuang, J., Chen, L., Yuen, S., Shi, M., Liang, J., Shen, B., Jin, Z., Yan, J. and Qiu, W. (2018) Apatinib-induced protective autophagy and apoptosis through the AKT-mTOR pathway in anaplastic thyroid cancer. *Cell Death Dis.* **9**, 1030.
Fimia, G. M., Stoykova, A., Romagnoli, A., Giunta, L., Di Bartolomeo, S., Nardacci, R., Corazzari, M., Fuoco, C., Ucar, A., Schwartz, P., Gruss, P., Piacentini, M., Chowdhury, K. and Cecconi, F. (2007) Ambra1 regulates autophagy and development of the nervous system. *Nature* **447**, 1121-1125.
Fu, W., Li, X., Lu, X., Zhang, L., Li, R., Zhang, N., Liu, S., Yang, X., Wang, Y., Zhao, Y., Meng, X. and Zhu, W. G. (2017) A novel acridine derivative, LS-1-10 inhibits autophagic degradation and triggers apoptosis in colon cancer cells. *Cell Death Dis.* **8**, e3086.
Fu, Y., Hong, L., Xu, J., Zhong, G., Gu, Q., Gu, Q., Guan, Y., Zheng, X., Dai, Q., Luo, X., Liu, C., Huang, Z., Yin, X. M., Liu, P. and Li, M. (2019) Discovery of a small molecule targeting autophagy via ATG4B inhibition and cell death of colorectal cancer cells *in vitro* and *in vivo*. *Autophagy* **15**, 295-311.
Gan, B., Peng, X., Nagy, T., Alcaraz, A., Gu, H. and Guan, J. L. (2006) Role of FIP200 in cardiac and liver development and its regulation of TNFalpha and TSC-mTOR signaling pathways. *J. Cell Biol.* **175**, 121-133.
Groza, T., Gomez, F. L., Mashhadi H. H., Munoz-Fuentes V., Gunes O., Wilson R., Cacheiro P., Frost A., Keskivali-Bond P., Vardal B., McCoy A., Cheng T. K., Santos L., Wells S., Smedley D., Mallon A. M. and Parkinson H. (2022) The International Mouse Phenotyping Consortium: comprehensive knockout phenotyping underpinning the study of human disease. *Nucleic Acids Res.* gkac972.
Guerini, A. E., Triggiani, L., Maddalo, M., Bonu, M. L., Frassine, F., Baiguini, A., Alghisi, A., Tomasini, D., Borghetti, P., Pasinetti, N., Bresciani, R., Magrini, S. M. and Buglione, M. (2019) Mebendazole as a candidate for drug repurposing in oncology: an extensive review of current literature. *Cancers (Basel)* **11**, 1284.
Guo, J. Y., Chen, H. Y., Mathew, R., Fan, J., Strohecker, A. M., Karsli-Uzunbas, G., Kamphorst, J. J., Chen, G., Lemons, J. M., Karantza, V., Coller, H. A., Dipaola, R. S., Gelinas, C., Rabinowitz, J. D. and White, E. (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev.* **25**, 460-470.
Guo, J. Y., Karsli-Uzunbas, G., Mathew, R., Aisner, S. C., Kamphorst, J. J., Strohecker, A. M., Chen, G., Price, S., Lu, W., Teng, X., Snyder, E., Santanam, U., Dipaola, R. S., Jacks, T., Rabinowitz, J. D. and White, E. (2013a) Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev.* **27**, 1447-1461.
Guo, J. Y., Xia, B. and White, E. (2013b) Autophagy-mediated tumor promotion. *Cell* **155**, 1216-1219.
Hanada, T., Noda, N. N., Satomi, Y., Ichimura, Y., Fujioka, Y., Takao, T., Inagaki, F. and Ohsumi, Y. (2007) The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. *J. Biol. Chem.* **282**, 37298-37302.
Hansen, M., Rubinsztein, D. C. and Walker, D. W. (2018) Autophagy as a promoter of longevity: insights from model organisms. *Nat. Rev. Mol. Cell Biol.* **19**, 579-593.
Homewood, C. A., Warhurst, D. C., Peters, W. and Baggaley, V. C. (1972) Lysosomes, pH and the anti-malarial action of chloro-

quine. *Nature* **235**, 50-52.
Hu, T., Li, P., Luo, Z., Chen, X., Zhang, J., Wang, C., Chen, P. and Dong, Z. (2016) Chloroquine inhibits hepatocellular carcinoma cell growth *in vitro* and *in vivo*. *Oncol. Rep.* **35**, 43-49.
Huang, H., Song, J., Liu, Z., Pan, L. and Xu, G. (2018) Autophagy activation promotes bevacizumab resistance in glioblastoma by suppressing Akt/mTOR signaling pathway. *Oncol. Lett.* **15**, 1487-1494.
Huang, T., Kim, C. K., Alvarez, A. A., Pangeni, R. P., Wan, X., Song, X., Shi, T., Yang, Y., Sastry, N., Horbinski, C. M., Lu, S., Stupp, R., Kessler, J. A., Nishikawa, R., Nakano, I., Sulman, E. P., Lu, X., James, C. D., Yin, X. M., Hu, B. and Cheng, S. Y. (2017) MST4 phosphorylation of ATG4B regulates autophagic activity, tumorigenicity, and radioresistance in glioblastoma. *Cancer Cell* **32**, 840-855.e8.
Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Ohsumi, M., Noda, T. and Ohsumi, Y. (2000) A ubiquitin-like system mediates protein lipidation. *Nature* **408**, 488-492.
Jiang, Y., Chen, M., Nie, H. and Yuan, Y. (2019) PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations. *Hum. Vaccin. Immunother.* **15**, 1111-1122.
Jo, S. B., Sung, S. J., Choi, H. S., Park, J. S., Hong, Y. K. and Joe, Y. A. (2022) Modulation of autophagy is a potential strategy for enhancing the anti-tumor effect of mebendazole in glioblastoma cells. *Biomol. Ther. (Seoul)* **30**, 616-624.
Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y. and Yoshimori, T. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.* **19**, 5720-5728.
Kaizuka, T. and Mizushima, N. (2016) Atg13 is essential for autophagy and cardiac development in mice. *Mol. Cell. Biol.* **36**, 585-595.
Kanzawa, T., Germano, I. M., Komata, T., Ito, H., Kondo, Y. and Kondo, S. (2004) Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. *Cell Death Differ.* **11**, 448-457.
Karasic, T. B., O'Hara, M. H., Loaiza-Bonilla, A., Reiss, K. A., Teitelbaum, U. R., Borazanci, E., De Jesus-Acosta, A., Redlinger, C., Burrell, J. A., Laheru, D. A., Von Hoff, D. D., Amaravadi, R. K., Drebin, J. A. and O'Dwyer, P. J. (2019) Effect of gemcitabine and nab-paclitaxel with or without hydroxychloroquine on patients with advanced pancreatic cancer: a phase 2 randomized clinical trial. *JAMA Oncol.* **5**, 993-998.
Karim, N. A., Ullah, A., Ahmad, I., Bahassi, E., Olowokure, O., Khaled, A., Davis, H. and Morris, J. C. (2022) A phase I trial to determine the safety and tolerability of autophagy inhibition using chloroquine or hydroxychloroquine in combination with carboplatin and gemcitabine in patients with advanced solid tumors. *Front. Oncol.* **12**, 811411.
Kawamura, N., Sun-Wada, G. H., Aoyama, M., Harada, A., Takasuga, S., Sasaki, T. and Wada, Y. (2012) Delivery of endosomes to lysosomes via microautophagy in the visceral endoderm of mouse embryos. *Nat. Commun.* **3**, 1071.
Kimmelman, A. C. (2011) The dynamic nature of autophagy in cancer. *Genes Dev.* **25**, 1999-2010.
Komatsu, M., Waguri, S., Ueno, T., Iwata, J., Murata, S., Tanida, I., Ezaki, J., Mizushima, N., Ohsumi, Y., Uchiyama, Y., Kominami, E., Tanaka, K. and Chiba, T. (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J. Cell Biol.* **169**, 425-434.
Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T. and Mizushima, N. (2004) The role of autophagy during the early neonatal starvation period. *Nature* **432**, 1032-1036.
Kundu, M., Lindsten, T., Yang, C. Y., Wu, J., Zhao, F., Zhang, J., Selak, M. A., Ney, P. A. and Thompson, C. B. (2008) Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. *Blood* **112**, 1493-1502.
Kurdi, A., Cleenewerck, M., Vangestel, C., Lyssens, S., Declercq, W., Timmermans, J. P., Stroobants, S., Augustyns, K., De Meyer, G. R. Y., Van Der Veken, P. and Martinet, W. (2017) ATG4B inhibitors with a benzotropolone core structure block autophagy and augment efficiency of chemotherapy in mice. *Biochem. Pharmacol.* **138**, 150-162.
Lee, E. J. and Tournier, C. (2011) The requirement of uncoordinated 51-like kinase 1 (ULK1) and ULK2 in the regulation of autophagy. *Autophagy* **7**, 689-695.
Lee, S. W., Kim, H. K., Lee, N. H., Yi, H. Y., Kim, H. S., Hong, S. H., Hong, Y. K. and Joe, Y. A. (2015) The synergistic effect of combination temozolomide and chloroquine treatment is dependent on autophagy formation and p53 status in glioma cells. *Cancer Lett.* **360**, 195-204.
Levy, J. M. M., Towers, C. G. and Thorburn, A. (2017) Targeting autophagy in cancer. *Nat. Rev. Cancer* **17**, 528-542.
Li, L. Q., Xie, W. J., Pan, D., Chen, H. and Zhang, L. (2016) Inhibition of autophagy by bafilomycin A1 promotes chemosensitivity of gastric cancer cells. *Tumour Biol.* **37**, 653-659.
Lin, J. F., Lin, Y. C., Tsai, T. F., Chen, H. E., Chou, K. Y. and Hwang, T. I. (2017) Cisplatin induces protective autophagy through activation of BECN1 in human bladder cancer cells. *Drug Des. Devel. Ther.* **11**, 1517-1533.
Liu, K., Ren, T., Huang, Y., Sun, K., Bao, X., Wang, S., Zheng, B. and Guo, W. (2017a) Apatinib promotes autophagy and apoptosis through VEGFR2/STAT3/BCL-2 signaling in osteosarcoma. *Cell Death Dis.* **8**, e3015.
Liu, L. Q., Wang, S. B., Shao, Y. F., Shi, J. N., Wang, W., Chen, W. Y., Ye, Z. Q., Jiang, J. Y., Fang, Q. X., Zhang, G. B. and Xuan, Z. X. (2019) Hydroxychloroquine potentiates the anti-cancer effect of bevacizumab on glioblastoma via the inhibition of autophagy. *Biomed. Pharmacother.* **118**, 109339.
Liu, P. F., Tsai, K. L., Hsu, C. J., Tsai, W. L., Cheng, J. S., Chang, H. W., Shiau, C. W., Goan, Y. G., Tseng, H. H., Wu, C. H., Reed, J. C., Yang, L. W. and Shu, C. W. (2018) Drug repurposing screening identifies tioconazole as an ATG4 inhibitor that suppresses autophagy and sensitizes cancer cells to chemotherapy. *Theranostics* **8**, 830-845.
Liu, S. and Li, X. (2015) Autophagy inhibition enhances sensitivity of endometrial carcinoma cells to paclitaxel. *Int. J. Oncol.* **46**, 2399-2408.
Liu, Z., He, K., Ma, Q., Yu, Q., Liu, C., Ndege, I., Wang, X. and Yu, Z. (2017b) Autophagy inhibitor facilitates gefitinib sensitivity *in vitro* and *in vivo* by activating mitochondrial apoptosis in triple negative breast cancer. *PLoS One* **12**, e0177694.
Luo, Q., Li, X., Gan, G., Yang, M., Chen, X. and Chen, F. (2021) PPT1 reduction contributes to erianin-induced growth inhibition in oral squamous carcinoma cells. *Front. Cell Dev. Biol.* **9**, 764263.
Malhotra, J., Jabbour, S., Orlick, M., Riedlinger, G., Guo, Y., White, E. and Aisner, J. (2019) Phase Ib/II study of hydroxychloroquine in combination with chemotherapy in patients with metastatic non-small cell lung cancer (NSCLC). *Cancer Treat. Res. Commun.* **21**, 100158.
Malhotra, R., Warne, J. P., Salas, E., Xu, A. W. and Debnath, J. (2015) Loss of Atg12, but not Atg5, in pro-opiomelanocortin neurons exacerbates diet-induced obesity. *Autophagy* **11**, 145-154.
Marino, G., Fernandez, A. F., Cabrera, S., Lundberg, Y. W., Cabanillas, R., Rodriguez, F., Salvador-Montoliu, N., Vega, J. A., Germana, A., Fueyo, A., Freije, J. M. and Lopez-Otin, C. (2010) Autophagy is essential for mouse sense of balance. *J. Clin. Invest.* **120**, 2331-2344.
Martin, K. R., Celano, S. L., Solitro, A. R., Gunaydin, H., Scott, M., O'Hagan, R. C., Shumway, S. D., Fuller, P. and MacKeigan, J. P. (2018) A potent and selective ULK1 inhibitor suppresses autophagy and sensitizes cancer cells to nutrient stress. *iScience* **8**, 74-84.
Mauthe, M., Orhon, I., Rocchi, C., Zhou, X., Luhr, M., Hijlkema, K. J., Coppes, R. P., Engedal, N., Mari, M. and Reggiori, F. (2018) Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. *Autophagy* **14**, 1435-1455.
McAfee, Q., Zhang, Z., Samanta, A., Levi, S. M., Ma, X. H., Piao, S., Lynch, J. P., Uehara, T., Sepulveda, A. R., Davis, L. E., Winkler, J. D. and Amaravadi, R. K. (2012) Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 8253-8258.
Meunier, G., Birsen, R., Cazelles, C., Belhadj, M., Cantero-Aguilar, L., Kosmider, O., Fontenay, M., Azar, N., Mayeux, P., Chapuis, N., Tamburini, J. and Bouscary, D. (2020) Antileukemic activity of the VPS34-IN1 inhibitor in acute myeloid leukemia. *Oncogenesis* **9**,

94.
Mizushima, N. (2007) Autophagy: process and function. *Genes Dev.* **21**, 2861-2873.
Mizushima, N., Yoshimori, T. and Ohsumi, Y. (2011) The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell Dev. Biol.* **27**, 107-132.
Molenaar, R. J., Coelen, R. J. S., Khurshed, M., Roos, E., Caan, M. W. A., van Linde, M. E., Kouwenhoven, M., Bramer, J. A. M., Bovée, J., Mathôt, R. A., Klümpen, H. J., van Laarhoven, H. W. M., van Noorden, C. J. F., Vandertop, W. P., Gelderblom, H., van Gulik, T. M. and Wilmink, J. W. (2017) Study protocol of a phase IB/II clinical trial of metformin and chloroquine in patients with IDH1-mutated or IDH2-mutated solid tumours. *BMJ Open* **7**, e014961.
Nakai, K., Hung, M. C. and Yamaguchi, H. (2016) A perspective on anti-EGFR therapies targeting triple-negative breast cancer. *Am. J. Cancer Res.* **6**, 1609-1623.
Nawrocki, S. T., Han, Y., Visconte, V., Przychodzen, B., Espitia, C. M., Phillips, J., Anwer, F., Advani, A., Carraway, H. E., Kelly, K. R., Sekeres, M. A., Maciejewski, J. P. and Carew, J. S. (2019) The novel autophagy inhibitor ROC-325 augments the antileukemic activity of azacitidine. *Leukemia* **33**, 2971-2974.
Nemazanyy, I., Blaauw, B., Paolini, C., Caillaud, C., Protasi, F., Mueller, A., Proikas-Cezanne, T., Russell, R. C., Guan, K. L., Nishino, I., Sandri, M., Pende, M. and Panasyuk, G. (2013) Defects of Vps15 in skeletal muscles lead to autophagic vacuolar myopathy and lysosomal disease. *EMBO Mol. Med.* **5**, 870-890.
Noman, M. Z., Parpal, S., Van Moer, K., Xiao, M., Yu, Y., Viklund, J., De Milito, A., Hasmim, M., Andersson, M., Amaravadi, R. K., Martinsson, J., Berchem, G. and Janji, B. (2020) Inhibition of Vps34 reprograms cold into hot inflamed tumors and improves anti-PD-1/PD-L1 immunotherapy. *Sci. Adv.* **6**, eaax7881.
Petherick, K. J., Conway, O. J., Mpamhanga, C., Osborne, S. A., Kamal, A., Saxty, B. and Ganley, I. G. (2015) Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. *J. Biol. Chem.* **290**, 11376-11383.
Petiot, A., Ogier-Denis, E., Blommaart, E. F., Meijer, A. J. and Codogno, P. (2000) Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J. Biol. Chem.* **275**, 992-998.
Puissant, A., Robert, G., Fenouille, N., Luciano, F., Cassuto, J. P., Raynaud, S. and Auberger, P. (2010) Resveratrol promotes autophagic cell death in chronic myelogenous leukemia cells via JNK-mediated p62/SQSTM1 expression and AMPK activation. *Cancer Res.* **70**, 1042-1052.
Qiu, L., Zhou, G. and Cao, S. (2020) Targeted inhibition of ULK1 enhances daunorubicin sensitivity in acute myeloid leukemia. *Life Sci.* **243**, 117234.
Qiu, Z., Kuhn, B., Aebi, J., Lin, X., Ding, H., Zhou, Z., Xu, Z., Xu, D., Han, L., Liu, C., Qiu, H., Zhang, Y., Haap, W., Riemer, C., Stahl, M., Qin, N., Shen, H. C. and Tang, G. (2016) Discovery of fluoromethylketone-based peptidomimetics as covalent ATG4B (autophagin-1) inhibitors. *ACS Med. Chem. Lett.* **7**, 802-806.
Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., Rosen, J., Eskelinen, E. L., Mizushima, N., Ohsumi, Y., Cattoretti, G. and Levine, B. (2003) Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J. Clin. Invest.* **112**, 1809-1820.
Rebecca, V. W., Nicastri, M. C., Fennelly, C., Chude, C. I., Barber-Rotenberg, J. S., Ronghe, A., McAfee, Q., McLaughlin, N. P., Zhang, G., Goldman, A. R., Ojha, R., Piao, S., Noguera-Ortega, E., Martorella, A., Alicea, G. M., Lee, J. J., Schuchter, L. M., Xu, X., Herlyn, M., Marmorstein, R., Gimotty, P. A., Speicher, D. W., Winkler, J. D. and Amaravadi, R. K. (2019) PPT1 promotes tumor growth and is the molecular target of chloroquine derivatives in cancer. *Cancer Discov.* **9**, 220-229.
Ronan, B., Flamand, O., Vescovi, L., Dureuil, C., Durand, L., Fassy, F., Bachelot, M. F., Lamberton, A., Mathieu, M., Bertrand, T., Marquette, J. P., El-Ahmad, Y., Filoche-Romme, B., Schio, L., Garcia-Echeverria, C., Goulaouic, H. and Pasquier, B. (2014) A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nat. Chem. Biol.* **10**, 1013-1019.
Rosenfeld, M. R., Ye, X., Supko, J. G., Desideri, S., Grossman, S. A., Brem, S., Mikkelson, T., Wang, D., Chang, Y. C., Hu, J., McAfee, Q., Fisher, J., Troxel, A. B., Piao, S., Heitjan, D. F., Tan, K. S., Pontiggia, L., O'Dwyer, P. J., Davis, L. E. and Amaravadi, R. K. (2014) A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy* **10**, 1359-1368.
Russell, R. C., Tian, Y., Yuan, H., Park, H. W., Chang, Y. Y., Kim, J., Kim, H., Neufeld, T. P., Dillin, A. and Guan, K. L. (2013) ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.* **15**, 741-750.
Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B. G., Satoh, T., Omori, H., Noda, T., Yamamoto, N., Komatsu, M., Tanaka, K., Kawai, T., Tsujimura, T., Takeuchi, O., Yoshimori, T. and Akira, S. (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* **456**, 264-268.
Schaaf, M. B., Keulers, T. G., Vooijs, M. A. and Rouschop, K. M. (2016) LC3/GABARAP family proteins: autophagy-(un)related functions. *FASEB J.* **30**, 3961-3978.
Schiller, S. A., Seebode, C., Wieser, G. L., Goebbels, S., Mobius, W., Horowitz, M., Sarig, O., Sprecher, E. and Emmert, S. (2016) Establishment of two mouse models for CEDNIK syndrome reveals the pivotal role of SNAP29 in epidermal differentiation. *J. Invest. Dermatol.* **136**, 672-679.
Sharma, G., Ojha, R., Noguera-Ortega, E., Rebecca, V. W., Attanasio, J., Liu, S., Piao, S., Lee, J. J., Nicastri, M. C., Harper, S. L., Ronghe, A., Jain, V., Winkler, J. D., Speicher, D. W., Mastio, J., Gimotty, P. A., Xu, X., Wherry, E. J., Gabrilovich, D. I. and Amaravadi, R. K. (2020) PPT1 inhibition enhances the antitumor activity of anti-PD-1 antibody in melanoma. *JCI Insight* **5**, e133225.
Singh, N., Miner, A., Hennis, L. and Mittal, S. (2021) Mechanisms of temozolomide resistance in glioblastoma - a comprehensive review. *Cancer Drug Resist.* **4**, 17-43.
Slobodkin, M. R. and Elazar, Z. (2013) The Atg8 family: multifunctional ubiquitin-like key regulators of autophagy. *Essays Biochem.* **55**, 51-64.
Sotelo, J., Briceño, E. and López-González, M. A. (2006) Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* **144**, 337-343.
Sou, Y. S., Waguri, S., Iwata, J., Ueno, T., Fujimura, T., Hara, T., Sawada, N., Yamada, A., Mizushima, N., Uchiyama, Y., Kominami, E., Tanaka, K. and Komatsu, M. (2008) The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. *Mol. Biol. Cell* **19**, 4762-4775.
Takahashi, Y., Coppola, D., Matsushita, N., Cualing, H. D., Sun, M., Sato, Y., Liang, C., Jung, J. U., Cheng, J. Q., Mule, J. J., Pledger, W. J. and Wang, H. G. (2007) Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat. Cell Biol.* **9**, 1142-1151.
Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., Eishi, Y., Hino, O., Tanaka, K. and Mizushima, N. (2011) Autophagy-deficient mice develop multiple liver tumors. *Genes Dev.* **25**, 795-800.
Takanezawa, Y., Nakamura, R., Kojima, Y., Sone, Y., Uraguchi, S. and Kiyono, M. (2018) Cytochalasin E increased the sensitivity of human lung cancer A549cells to bortezomib via inhibition of autophagy. *Biochem. Biophys. Res. Commun.* **498**, 603-608.
Tanaka, Y., Guhde, G., Suter, A., Eskelinen, E. L., Hartmann, D., Lullmann-Rauch, R., Janssen, P. M., Blanz, J., von Figura, K. and Saftig, P. (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* **406**, 902-906.
Tang, F., Hu, P., Yang, Z., Xue, C., Gong, J., Sun, S., Shi, L., Zhang, S., Li, Z., Yang, C., Zhang, J. and Xie, C. (2017) SBI0206965, a novel inhibitor of Ulk1, suppresses non-small cell lung cancer cell growth by modulating both autophagy and apoptosis pathways. *Oncol. Rep.* **37**, 3449-3458.
Tanida, I., Sou, Y. S., Ezaki, J., Minematsu-Ikeguchi, N., Ueno, T. and Kominami, E. (2004) HsAtg4B/HsApg4B/autophagin-1 cleaves the carboxyl termini of three human Atg8 homologues and delipidates microtubule-associated protein light chain 3- and GABAA receptor-associated protein-phospholipid conjugates. *J. Biol. Chem.* **279**,

36268-36276.
Towers, C. G. and Thorburn, A. (2016) Therapeutic targeting of autophagy. *EBioMedicine* **14**, 15-23.
Vats, S. and Manjithaya, R. (2019) A reversible autophagy inhibitor blocks autophagosome-lysosome fusion by preventing Stx17 loading onto autophagosomes. *Mol. Biol. Cell* **30**, 2283-2295.
Wang, C. C., Ng, C. P., Lu, L., Atlashkin, V., Zhang, W., Seet, L. F. and Hong, W. (2004) A role of VAMP8/endobrevin in regulated exocytosis of pancreatic acinar cells. *Dev. Cell* **7**, 359-371.
Wang, P., Burikhanov, R., Jayswal, R., Weiss, H. L., Arnold, S. M., Villano, J. L. and Rangnekar, V. M. (2018) Neoadjuvant administration of hydroxychloroquine in a phase 1 clinical trial induced plasma Par-4 levels and apoptosis in diverse tumors. *Genes Cancer* **9**, 190-197.
White, E., Mehnert, J. M. and Chan, C. S. (2015) Autophagy, metabolism, and cancer. *Clin. Cancer Res.* **21**, 5037-5046.
Wick, W., Gorlia, T., Bendszus, M., Taphoorn, M., Sahm, F., Harting, I., Brandes, A. A., Taal, W., Domont, J., Idbaih, A., Campone, M., Clement, P. M., Stupp, R., Fabbro, M., Le Rhun, E., Dubois, F., Weller, M., von Deimling, A., Golfinopoulos, V., Bromberg, J. C., Platten, M., Klein, M. and van den Bent, M. J. (2017) Lomustine and bevacizumab in progressive glioblastoma. *N. Engl. J. Med.* **377**, 1954-1963.
Wolpin, B. M., Rubinson, D. A., Wang, X., Chan, J. A., Cleary, J. M., Enzinger, P. C., Fuchs, C. S., McCleary, N. J., Meyerhardt, J. A., Ng, K., Schrag, D., Sikora, A. L., Spicer, B. A., Killion, L., Mamon, H. and Kimmelman, A. C. (2014) Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. *Oncologist* **19**, 637-638.
Wu, M., Huang, Q., Xie, Y., Wu, X., Ma, H., Zhang, Y. and Xia, Y. (2022) Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation. *J. Hematol. Oncol.* **15**, 24.
Xiao, M., Benoit, A., Hasmim, M., Duhem, C., Vogin, G., Berchem, G., Noman, M. Z. and Janji, B. (2021) Targeting cytoprotective autophagy to enhance anticancer therapies. *Front. Oncol.* **11**, 626309.
Xu, J., Su, Z., Cheng, X., Hu, S., Wang, W., Zou, T., Zhou, X., Song, Z., Xia, Y., Gao, Y. and Zheng, Q. (2022) High PPT1 expression predicts poor clinical outcome and PPT1 inhibitor DC661 enhances sorafenib sensitivity in hepatocellular carcinoma. *Cancer Cell Int.* **22**, 115.
Yamamoto, A., Tagawa, Y., Yoshimori, T., Moriyama, Y., Masaki, R. and Tashiro, Y. (1998) Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct. Funct.* **23**, 33-42.
Yang, S., Wang, X., Contino, G., Liesa, M., Sahin, E., Ying, H., Bause, A., Li, Y., Stommel, J. M., Dell'antonio, G., Mautner, J., Tonon, G., Haigis, M., Shirihai, O. S., Doglioni, C., Bardeesy, N. and Kimmelman, A. C. (2011) Pancreatic cancers require autophagy for tumor growth. *Genes Dev.* **25**, 717-729.
Yorimitsu, T. and Klionsky, D. J. (2005) Autophagy: molecular machinery for self-eating. *Cell Death Differ.* **12 Suppl 2**, 1542-1552.
Yu, L., Chen, Y. and Tooze, S. A. (2018) Autophagy pathway: cellular and molecular mechanisms. *Autophagy* **14**, 207-215.
Yuan, H., Li, A. J., Ma, S. L., Cui, L. J., Wu, B., Yin, L. and Wu, M. C. (2014) Inhibition of autophagy signi fi cantly enhances combination therapy with sorafenib and HDAC inhibitors for human hepatoma cells. *World J. Gastroenterol.* **20**, 4953-4962.
Yue, Z., Jin, S., Yang, C., Levine, A. J. and Heintz, N. (2003) Beclin **1**, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 15077-15082.
Zhang, Y., Cheng, Y., Ren, X., Zhang, L., Yap, K. L., Wu, H., Patel, R., Liu, D., Qin, Z. H., Shih, I. M. and Yang, J. M. (2012) NAC1 modulates sensitivity of ovarian cancer cells to cisplatin by altering the HMGB1-mediated autophagic response. *Oncogene* **31**, 1055-1064.
Zhao, H., Zhao, Y. G., Wang, X., Xu, L., Miao, L., Feng, D., Chen, Q., Kovacs, A. L., Fan, D. and Zhang, H. (2013) Mice deficient in Epg5 exhibit selective neuronal vulnerability to degeneration. *J. Cell Biol.* **200**, 731-741.
Zhou, W., Guo, Y., Zhang, X. and Jiang, Z. (2020) Lys05 induces lysosomal membrane permeabilization and increases radiosensitivity in glioblastoma. *J. Cell. Biochem.* **121**, 2027-2037.
Zhou, X., Takatoh, J. and Wang, F. (2011) The mammalian class 3 PI3K (PIK3C3) is required for early embryogenesis and cell proliferation. *PLoS One* **6**, e16358.