Chapter 17

Autophagy Modulation for Organelle-Targeting Therapy

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Additional information is available at the end of the chapter

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Abstract

Autophagy is a crucial metabolic pathway that sustains cellular homeostasis in health and that can also play either a protective or a destructive role in disease. During the last decade, progress made in understanding of the molecular basis of autophagy has uncovered an exciting opportunity to target it for the treatment of several human illnesses. In fact, there is emerging interest in autophagy-modulating and autophagy-targeted therapy with a variety of pharmacologic agents. However, to develop effective autophagy-targeted therapy, it is essential to identify the pharmacologic key targets in the autophagy pathway. In this chapter, we reviewed the cases of success and pitfalls of activating or inhibiting autophagy attempting therapeutic intervention of diseases, including cancer, neurologic disorders, and infectious diseases. In all these histopathologic states, autophagy is considered as the principal cellular mechanisms of defense and immunochemical homeostasis. In the last section of this chapter, we discuss main directions that may be of particular use in the future investigations, including a promissory avenue for autophagy modulation for organelle-targeting therapy through a promotion of parallel damage in lysosomal and mitochondrial membranes.

Keywords: autophagy-targeted therapy, activation/inhibition of autophagy, triterpenoids, lysosomal and mitochondrial membranes

1. Introduction

The impact of autophagy in human pathogenesis comprises its critical function for the degradation and recycling of long-lived proteins, lipid droplets, protein aggregates, mature ribosomes, glycogen, and even entire organelles such as the endoplasmic reticulum, mitochondria, and Golgi apparatus [1, 2]. For example, when the efficiency of mitophagy (a type of autophagy that specifically targets dysfunctional mitochondria [2–7]) is reduced, the maintenance of cellular homeostasis decreases, leading to cell aging, genomic instability, and senescence [4, 8, 9]. However, the molecular mechanism by which deficient mitophagy jeopardizes genomic stability are unclear [10].

To develop effective autophagy-based therapy, it has been essential to identify the pharmacologic key targets in the autophagy pathway for the development of new therapeutic agents. As discussed earlier in this book, autophagy can play either a protective or a destructive role in disease states and thus for therapeutic purposes is valuable to identify and develop pharmacologic agents that might activate or inhibit this cellular process [11].

We will evolve this part of the chapter to review the cases of success and pitfalls of activating or inhibiting autophagy attempting therapeutic intervention of diseases, including cancer, neurologic disorders, and infectious diseases. In the following sections, we discuss a few directions that may be of particular use in future investigations. As recently proposed by our group, the promotion of parallel damage in lysosomes and mitochondria represents a promissory avenue for therapeutic autophagy targeting and aiding controlled cell death and senescence [12, 13].

2. Autophagy-modulating drugs

During the last decade, progress made in understanding of the molecular basis of autophagy has uncovered an exciting opportunity to target it for the treatment of human illnesses [14]. In principle, understanding the role of autophagy in diseases has helped identify new avenues of pharmacologic modulation of autophagy as novel therapeutic intervention. Thus, knowing the process of autophagy targeting might facilitate the search of new drugs or concepts for the treatment of several types of diseases whose etiology or progression is associated with autophagy including cancer [8, 15–17], degenerative diseases 25 [18–23], and lysosomal storage disorders [24–26].

2.1. Autophagy inhibitors

The pharmacologic inhibition targeting the early or later autophagy process has been demonstrated to play pivotal roles in cellular outcome and may affect disease processes. Because inhibition of autophagy by pharmacologic agents also may have some off-target effects on cellular functions, the question of whether, for example, cell death can truly occur due to autophagy alone remains to be clarified [11, 27]. **Table 1** lists the compounds identified as inhibitors of autophagy.

The inhibitors that target the early stage of autophagy include 3-MA, Wortmannin, and LYS294002, all of which inhibit the class III PI3K (VSP34) and disable the formation of autophagosome. The inhibitors act on the later stage of autophagy, including compounds (see **Table 1**) that are capable of preventing lysosomal degradation or blocking the fusion of autophagosomes with lysosomes. For example, the neutralization of intralysosomal pH by lysosomotropic agents such as Bafilomycin A_1, Chloroquine (CQ), Hydroxychloroquine (HCQ), or NH_4Cl prevents the digestive activity of hydrolases, leading to inhibition of degradative activity of autolysosomes [33, 34, 37, 44]. In their unprotonated form, CQ and HCQ can diffuse across cell membranes to become protonated and accumulated only in acidic organelles. Once trapped within lysosomes, they interfere with prosurvival autophagy, resulting in controlled cell death [57–60]. This unique property has established CQ as the most widely used drug to inhibit autophagy in vitro and in vivo. Bafilomycin A_1 is a selective vacuolar-type H^+-ATPase [V-ATPase] inhibitor responsible for acidifying lysosomes and endosomes [33, 34]. Of note Bafilomycin A_1 blocks the fusion of autophagosomes with lysosomes, which results from inhibition of ATP2A/SERCA activity independently of its effect on intralysosomal pH [35, 61].

Compounds	Autophagy signaling pathway
3-methyladenine	An inhibitor of autophagic/lysosomal protein degradation [28], but not a specific autophagy inhibitor [29] may also inhibit the activity of Phosphatidylinositol 3-kinase [30], effectively blocking the early stage of autophagy. 3-MA does not inhibit BECN1-independent autophagy [29].
ARN5187	4-[[[1-(2-fluorophenyl)cyclopentyl]amino]methyl]-2-[(4-methylpiperazin-1 -yl)methyl]phenol, 1 is a lysosomotropic compound with a dual inhibitory activity against the circadian regulator NR1D2/REV-ERBβ and autophagy [31, 32].
Bafilomycin A_1	A V-ATPase inhibitor that causes an increase in lysosomal/vacuolar pH and, ultimately, blocks fusion of autophagosomes with the vacuole; the latter may result from inhibition of ATP2A/SERCA [33–35].
Betulinic acid	A pentacyclic triterpenoid that promotes parallel damage in mitochondrial and lysosomal compartments and, ultimately, triggers autophagy associated cell death in human keratinocytes [12].
CA074	*N*-(L-3-trans-propylcarbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline is a potent and specific inhibitor of cathepsin B in vitro [36].
Chloroquine	Chloroquine and its analog Hydroxychloroquine are lysosomotropic compounds that elevate/neutralize the lysosomal/vacuolar pH [37].
Colchicine	A microtubule depolarizing agent that may block autophagosome maturation to autolysosomes and increased LC3II protein levels [38].
Desmethyl clomipramine	3-(2-chloro-5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N-methylpropan-1 -Amine, an active metabolite of clomipramine inhibits late autophagy through a significant blockage of the degradation of autophagic cargo [39]; may induce an increase in the steady-state levels of p62/SQSTM1 by inhibiting the autophagic flux as opposed to an activation of the autophagic pathway [39].
E64d	Inhibits papain-like cathepsin cysteine proteases and calpain-activated neutral proteases [40]; should be used in combination with pepstatin A to inhibit lysosomal protein degradation [29].
Eflornithine	2,5-diamino-2-(difluoromethyl)pentanoic acid, an irreversible inhibitor of ODC1 (ornithine decarboxylase 1) that blocks spermidine synthesis and *ATG5* gene expression acting as a novel autophagy inhibitor [41].

Compounds	Autophagy signaling pathway
Leupeptin	An inhibitor of cysteine, serine, and threonine proteases that causes significant inhibition of the intracellular maturation of cathepsin B, L, and H [37, 42, 43]; decreases the degradation of short- and long-lived proteins [44]; should be used in combination with pepstatin A and/or E-64d to block lysosomal protein degradation [29].
Lucanthone	Interferes with lysosomal function and leads to the accumulation of undegraded proteins and induces a cathepsin D-mediated apoptosis [45].
LYS294002	2-(4-morpholi-nyl)-8-phenylchromone may prevent autophagic sequestration by inhibiting phosphatidylinositol 3-kinase activity [30].
Monensin	An inhibitor of protein transport, acts as proton exchange for potassium or sodium and inhibits autophagy by preventing the fusion of the autophagosome with the lysosome [46].
NH4Cl	Lysosomotropic compound that elevate/neutralize the lysosomal/vacuolar pH, inhibiting the lysosomal pathway of protein degradation [37].
Nocodazole	A depolymerizer of nonacetylated microtubules and impairs tubulin acetylation but does not affect polymerized acetylated microtubules; may impair the conversion of LC3I to LC3II but does not block the degradation of LC3II-associated autophagosomes [47].
Pepstatin A	An aspartyl protease inhibitor that can be used to partially block lysosomal degradation [44]; should be used in combination with other inhibitors such as E-64d [29].
PES	2-Phenyl-ethynesulfonamide , a small molecule inhibitor of heat shock protein 70 (HSP70), impairs autophagy through its inhibitory effects on lysosomal functions showing an accumulation of the precursor procathepsin L and a markedly reduced abundance of the smaller, mature form of the enzyme [48].
Propofol	May exert protective effects on neuronal cells and cardiomyocytes, in part through the inhibition of early autophagy [49–51].
Spautin	A specific and potent autophagy inhibitor 1 that promotes degradation of the Vps34 (a phosphoinositide 3-kinase class III isoform) via inhibiting ubiquitin-specific processing protease 10 (USP10) and USP13, two ubiquitin-specific peptidases that target the deubiquitination of Beclin1 [52].
Thapsigargin	A sarco/endoplasmic reticulum Ca (2+)-ATPase inhibitor that inhibits autophagic sequestration of cytosolic material through the depletion of intracellular Ca2+ stores [53, 54]; also may lead to the accumulation of mature autophagosomes by blocking autophagosome fusion with the endocytic system by interfering with the recruitment of RAB7 [55].
Vacuolin-1	2-N-[(3-iodophenyl)methylideneamino]-6-morpholin-4-yl-4-N,4-N-diphenyl-1,3,5-triazine-2,4-diamine, an activator of RAB5A GTPase activity that potently and reversibly inhibits autophagosome-lysosome fusion; also may alkalinize lysosomal pH and decrease lysosomal Ca^{2+} content [56].
Vinblastine	A depolymerizer of both nonacetylated and acetylated microtubules that interferes with both LC3I-LC3II conversion and LC3II-associated autophagosome fusion with lysosomes [47].
Wortmannin	An inhibitor of PI3K and PtdIns3K that blocks autophagy, but not a specific inhibitor that prevents autophagic sequestration such as 3-methyladenine [30].

Table 1. Compounds known to inhibit autophagy.

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Other inhibitors of autophagy that impair the autolysosome formation include the antidepressant drug Desmethylclomipramine, the anti-schistome agent Lucanthone, Eflornithine, Monensin, PES, Spautin, Thapsigargin, Vacuolin-1, and Vinblastine (see **Table 1**).

The digestive phase of autophagy may also be blocked by lessening lysosome-mediated proteolysis such as the cysteine protease inhibitor E-64d; the aspartic protease inhibitor Pepstatin A; the active inhibitor of cathepsin B CA074; and the cysteine, serine, and threonine protease inhibitor Leupeptin [37, 44, 62]. Autophagosomes and lysosomes move along the microtubules to fuse, so microtubule-disrupting agents, including taxanes, Nocodazole, Colchicine, and Vinca alkaloids, may inhibit the fusion of autophagosomes with lysosomes [11, 63, 64].

2.2. Autophagy activators

It is now generally believed that modulating the activity of autophagy through targeting specific regulatory molecules in the autophagy machinery may improve clinical outcome for diverse diseases [11, 14]. In this context, mTOR inhibitors has been considered as the most potent activators of autophagy by playing pivotal key negative regulatory role [29]. **Table 2** lists the compounds that have been identified as activators of autophagy.

Compounds	Autophagy signaling pathway
10-NCP	10-(4′-N-diethylamino)butyl)-2-chlorophenoxazine that may promote potential and safe upregulation of autophagy in neurons in an AKT- and mTOR-independent fashion [65].
17-AAG	17-Allylamino-17-Demethoxygeldanamycin that may inhibit the HSP90 CDC37 chaperone complex activating autophagy in certain systems (e.g., neurons), but impairs starvation-induced autophagy and mitophagy in others by promoting the turnover of ULK1 [66].
Akti-1/2	Akt inhibitor VIII isozyme-selective Akti-1/2 can promote allosteric inhibition of AKT1 and AKT2 and activates autophagy in B-cell lymphoma [67].
AUTEN-67	An inhibitor of MTMR14, a myotubularin-related phosphatase that may antagonize the formation of autophagic membrane [68].
AZD8055	5-[2,4-bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3- d]pyrimidin-7-yl]-2-methoxyphenyl methanol that may inhibit both mTORC1 and mTORC2 [69].
Everolimus	An inhibitor of mTORC1 that induces both autophagy and apoptosis in B-cell lymphoma primary cultures [67].
KU-0063794	A specific mTOR inhibitor that may bind the catalytic site and activates autophagy [70, 71].
MLN4924	A small molecule inhibitor of NEDD8 activating enzyme (NAE) [72] that triggers autophagy through the blockage of mTOR signals via DEPTOR as well as the HIF1A-DDIT4/REDD1-TSC1/2 axis [73] as a result of inactivation of cullin-RING ligases [74].

Compounds	Autophagy signaling pathway
Oleanolic acid	A pentacyclic triterpenoid that promotes damage in mitochondrial compartments, and ultimately, activates prosurvival autophagy in human keratinocytes [12].
NAADP-AM	Nicotinic acid adenine dinucleotide phosphate (NAADP) can mobilize Ca^{2+} from acidic Ca^{2+} stores through lysosomal two-pore channels (TPCs) in primary cultured rat astrocytes and present evidence that NAADP-evoked Ca^{2+} signals regulate autophagy [75].
NVP-BEZ235	NVP-BEZ235 is an imidazo[4,5-c]quinoline derivative that can inhibit the activity of target proteins in the PI3K/AKT/mTOR cascade and activates autophagy in human gliomas [76, 77].
PMI	Is a pharmacological P62-mediated mitophagy inducer (PMI) that activates mitophagy without recruiting Parkin or collapsing the mitochondrial membrane potential [78].
PP242	2-(4-amino-1-isopropyl-1H- pyrazolo[3,4-d]pyrimidin-3-yl)-1H-indol-5-ol is a ATP-competitive inhibitor of mTORC1 and mTORC2 [79, 80]; should be more effective mTORC1 inhibitor than rapamycin [81].
PP30	3-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(4,5-dihydrothiazol-2-yl) benzamide is a ATP-competitive inhibitor of mTORC1 and mTORC2 [81].
Rapamycin	Binds to FKBP1A/FKBP12 and inhibits mTORC1; the complex binds to the FRB domain of mTOR and limits its interaction with RPTOR, thus inducing autophagy, but only providing partial mTORC1 inhibition [82].
Resveratrol	A natural polyphenol that affects many proteins [83] via both AMPK activation and JNK-mediated p62/SQSTM1 expression activates autophagy [84, 85].
Ridaforolimus	Binds to and inhibits the mammalian target of Rapamycin (mTOR), which may result in cell cycle arrest and, consequently, the inhibition of tumor cell growth and proliferation.
RSVAs	Synthetic small-molecule analogs of resveratrol that potently activate AMPK and induce autophagy [86].
Saikosaponin-d	A natural small-molecule inhibitor of ATP2A/SERCA that induces autophagy via direct inhibition of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), leading to the increase of intracellular calcium ion levels and activating the Ca^{2+}/calmodulin-dependent kinase kinase-b (CaMKKβ)/AMPK signaling cascade [87].
Tat-Beclin 1	A cell penetrating peptide that potently induces autophagy [88, 89].
Temsirolimus	Is Rapamycin ester analog CCI-779 with better stability and pharmacological properties compared to rapamycin that activates autophagy in neurons in Alzheimer's disease [90–92].
TMS	Trans-3,5,4-trimethoxystilbene upregulates the expression of the transient receptor potential canonical channel 4 (TRPC4), resulting in mTOR inhibition and autophagy activation [93].

Compounds	Autophagy signaling pathway
Torin1	1-[4-(4-propanoylpiperazin-1-yl)- 3-(trifluoromethyl)phenyl]-9-quinolin-3-ylbenzo [h][1, 6]naphthyridin-2-one, a catalytic mTORC1 and mTORC2 inhibitor that induces autophagy [94].
Trehalose	mTOR-independent, autophagic enhancer that may be relevant for the treatment of different neurodegenerative diseases [20, 95, 96].
Tunicamycin	A glycosylation inhibitor that induces autophagy due to endoplasmic reticulum stress [97].
WYE-125132	1-[4-[1-(1,4-dioxaspiro[4.5]decan- 8-yl)-4-(8-oxa-3- azabicyclo[3.2.1]octan-3-yl)pyrazolo [3,4- d]pyrimidin-6-yl]phenyl]-3-methylurea is an ATP-competitive and specific inhibitor of mTORC1 and mTORC2 [98].

Table 2. Compounds known to activate autophagy.

There are several other agents that negatively regulate autophagy, such as inositol 1,4,5-trisphosphate (IP3), epidermal growth factor receptor (EGFR), Bcl-2, and Bcl-xl. The mTORC1 inhibitor Rapamycin and its analogs Temsirolimus (CCI-79, Torisel), Everolimus (RAD001, Afinitor), and Ridaforolimus (AP-23573, Deferolimus, MK-8669) are strong inducers of autophagy [14], as are the ATP-competitive inhibitors of mTOR such as Torin 1 [94], PP242 [79, 80], PP30 [81] and AZD8055 [69], and WYE-125132 [98], but their autophagy-inducing efficacy has not been well documented [11]. Other autophagic enhancers that induces autophagy via a mTOR-independent pathway, include AUTEN-67 [68], 10-NCP [65], PMI [78], Resveratrol [84, 85], Trehalose [20, 95, 96], and Tunicamycin [97].

3. Modulation of autophagy as a cancer therapy

The human cancer represents a significant worldwide public health problem considered as the main cause of death [99]. Its worldwide incidence is expected to show more than 21 thousand million new cases in 2030 [100]. To deal with such increased incidence, 47,608 clinical 15 trials have been currently carried out according to *Clinical Trials.Gov* [101]. Therapeutic targeting of the autophagy pathway as a new anticancer strategy has been under extensive investigation [11, 64, 102, 103]. Several data indicate that prosurvival autophagy confers a tumor growth advantage through the supplementation of required nutrition of growth, and thus it represents a novel therapeutic target [46, 104]. Actually, autophagy may represent a major impediment to successful cancer therapy by radiation, drugs (e.g., Doxorubicin, Temozolomide, and Etoposide), histone deaceltylase inhibitors, Arsenic trioxide, TNF-alpha, IFN-gamma, Imatinib, and Rapamycin and the anti-estrogen hormonal therapy Tamoxifen as reviewed [46, 104, 105]. Dalby and colleagues propose that inhibitors of autophagy may either enhance the efficacy of anti-tumor therapy or promote cell death not only in primary cancer types but also in advanced-stage cancers and metastatic tumors that are considered drug resistant or apoptosis resistant, such as chemotherapy-resistant cancer [105]. However, depending on the context, such as tumor type or stage, the autophagy-enhancing agents believed to induce a type II programmed cell death mechanism through an extensive autophagic degradation of intracellular content may also elicit beneficial effects in the treatment of cancer [105]. Both the approaches, inhibition of either the prosurvival or induction of prodeath mechanism of autophagy, will be discussed further.

3.1. Use of autophagy activators in cancer treatment

Several evidence have suggested that increased autophagy may kill cells. However, the weakness of many studies has been that the demonstration of autophagy after a cytotoxic treatment does not prove that autophagy contributed to cell death, only that it was associated with it [11]. It is equally plausible that increased autophagy in these settings was more a failed effort to maintain cell survival than triggering per se cell death. If autophagy would act more definitively as a prodeath cell role than a prosurvival one, its inhibition would have to increase survival. In fact, most studies have showed that a cell death had been counterbalanced by an autophagic salvage response rather than demonstrate a causative role for autophagy in the promotion of cell death [106, 107]. Nonetheless, induction of autophagy-associated cell death has been suggested as a potential strategy to eradicate human cancers [108]. In fact, several anticancer drugs have been reported to kill tumor cells through autophagy-mediated mechanisms, include Photodynamic Therapy, Cisplatin, 5-Fluoroacil, Etoposide, Imatinib, and Paclitaxel, as reviewed [109].

Rapamycin and its more soluble analog Temsirolimus trigger autophagy, as does KU-63794, whose selective mTOR inhibition has been attributed to its antitumor mechanism regardless apoptosis induction [110–112] in which the disruption of the PI3K/Akt signaling pathway might greatly enhance the effectiveness of mTOR inhibitors [110]. Likewise, the inhibitory effect of the mTOR inhibitor Everolimus on acute lymphoblastic leukemia was associated with autophagy activation [113]. The combination of Temsirolimus, an mTOR inhibitor, and HCQ, an autophagy inhibitor, augments cell death in preclinical models [114].

The ATP-competitive inhibitors of mTORC1/mTORC2, WYE-125132 [98], and AZD8055 [69] have demonstrable anticancer activity by growth inhibition, and potentially autophagy both in vitro and in vivo. In case of rapamycin-resistant T37/46 phosphorylation sites on 4E-BP1, AZD8055 may fully inhibit mTOR [69]. AZD8055 is currently in Phase I clinical trials as an antitumor agent (NCT00973076, NCT01316809, NCT00999882 and NCT00731263). The tyrosine kinase inhibitor Dasatinib (BMS-354825) has been reported to enhance the antiglioma effect of Temozolomide through triggering significant decrease in cell proliferation while simultaneously increasing autophagy, and this action can be antagonized by the autophagy inhibitor 3-MA [115].

Other types of drugs possessing an autophagy-inducing effect have also found their potential application in cancer treatment (see **Table 3**). For instance, autophagy-associated cell death may contribute to the anticancer actions of the histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (Vorinostat) [116–118]. Vorinostat may induce autophagy through downregulation of Akt/mTOR signaling and induction of ER stress response, whose biological effects might be antagonized by the autophagy inhibitor 3-MA [117]. Coadministration of Vorinostat and a poly (ADP-ribose) polymerase (PARP) inhibitor Olaparib synergistically inhibits the growth of triple-negative breast cancer cells through increased apoptotic and autophagy-associated cell death [119]. In Tamoxifen-resistant MCF-7 breast cancer cells the HDAC inhibitor MHY218 induces apoptosis or autophagy-related cell death [120]. The estrogen receptor antagonist Raloxifene induces autophagy via the activation of AMPK by sensing decreases in ATP, leading to a nonapoptotic autophagy-associated cell death in breast cancer [121]. However, it has been proposed that autophagy is sterol-dependent and is associated with cell survival rather than cytotoxicity [122]. The natural products Resveratrol [84], triterpenoids Ursolic acid [123–125], and Saponin [126] promote cancer cell death associated with activation of autophagy. It is conceivable that some autophagy-inducing agents may also be useful in cancer therapies because of their ability to trigger autophagy-associated cell death [11, 113, 115]. The same attention given to inhibitors of autophagy should be given to autophagy-inducing or autophagy-enhancing agents [114, 127–131].

Cancer Type	Identifier	Study	Phase	Status	
Renal cell cancer	NCT00830895	Everolimus for nonclear cell renal cell carcinoma (RCC)	II	1	[132]
	NCT01090466	Gemcitabine Hydrochloride, Cisplatin, and Temsirolimus as first-line therapy to treat patients with locally advanced and/or metastatic transitional cell cancer of the urothelium	I/II	1	
Prostate cancer	NCT01313559	Pasireotide (SOM230) with or without Everolimus to treat patients with hormone-resistant chemotherapy naive prostate cancer	II	1	
	NCT00574769	Docetaxel with Everolimus and Bevacizumab in men with advanced prostate cancer	I/II	1	
	NCT02339168	Enzalutamide and Metformin Hydrochloride to treat patients with hormone-resistant prostate cancer	I	2	
	NCT01748500	Pantoprazole and Docetaxel for men with metastatic castration-resistant prostate cancer	II	2	
	NCT01497925	ADIPEG 20 and Docetaxel in solid tumors with emphasis on prostate cancer and nonsmall cell lung cancer	I	2	
Breast cancer	NCT00411788	Rapamycin and Trastuzumab for patients with HER-2 receptor positive metastatic breast cancer	II	3	
	NCT01111825	Temsirolimus and Neratinib for the treatment of patients with metastatic HER2-amplified or triple negative breast cancer	I/II	2	
	NCT00736970	Ridaforolimus in combination with Trastuzuma in patients with metastatic, HER2-positive breast cancer who have developed resistance to Trastuzumab.	III	1	[133]
	NCT01605396	Ridaforolimus and Exemestane, compared with Ridaforolimus, Dalotuzumab and Exemestane to	II	2	

Cancer Type	Identifier	Study	Phase	Status	
		treat breast cancer			
	NCT01234857	Ridaforolimus in combination with Dalotuzumab compared to the standard of care treatment in estrogen receptor positive breast cancer patients	II	1	
Nonsmall cell lung cancer	NCT00079235	Temsirolimus to treat patients with stage III-B (with pleural effusion) or stage IV nonsmall cell lung cancer	II	1	
	NCT00923273	Sirolimus and Pemetrexed to treat nonsmall cell lung cancer	I/II	1	
Small cell lung cancer	NCT00374140	Everolimus in previously treated small cell lung cancer	II	1	
	NCT01079481	Combination anticancer therapy of Everolimus and Paclitaxel for relapsed or refractory small cell lung cancer	I/II	1	
Pancreatic cancer	NCT01648465	Everolimus to treat newly diagnosed patients with advanced gastrointestinal neuroendocrine tumors	II	4	
	NCT01537107	Sirolimus and Vismodegib to treat patients with solid tumors or pancreatic cancer that is metastatic or cannot be removed by surgery	I	5	
Glioblastoma	NCT00329719	Temsirolimus and Sorafenib to treat patients with recurrent glioblastoma	I/II	1	
	NCT01062399	Everolimus, Temozolomide, and Radiation therapy to treat patients with newly diagnosed glioblastoma multiforme	I/II	2	
	NCT01956734	Virus DNX2401 and Temozolomide to treat recurrent glioblastoma	I	2	
Colorectal cancer	NCT00522665	Second-line therapy with Irinotecan, Cetuximab, and Everolimus to treat colorectal cancer	I/II	1	
	NCT01154335	Everolimus and Linsitinib to treat patients with refractory metastatic colorectal cancer	I	1	[134]
Chronic myeloid leukemia	NCT01188889	Everolimus to treat chronic phase chronic myeloid leukemia with persistent molecular disease.	I/II	6	
Chronic lymphocytic leukemia	NCT00935792	Everolimus and Alemtuzumab to treat patients with recurrent chronic lymphocytic leukemia or small lymphocytic lymphoma	I/II	1	
Advanced solid tumor	NCT00849550	Everolimus in combination with current standard treatment of XELOX-A (Bevacizumab, Oxaliplatin, Capecitabine) to treat advanced solid tumors	I	1	
	NCT01020305	Temsirolimus to reverse androgen insensitivity for	I/II	1	

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Cancer Type	Identifier	Study	Phase	Status	
		castration-resistant prostate cancer			
	NCT00657982	Everolimus in a neoadjuvant setting in men with intermediate or high risk prostate cancer	II	4	
	NCT01155258	Temsirolimus and Vinorelbine Ditartrate to treat patients with unresectable or metastatic solid tumors	I	2	
	NCT01295632	Ridaforolimus with MK-2206 or MK-0752 for participants with advanced cancer	I	1	[135]
	NCT01169532	Ridaforolimus and the HDAC inhibitor Vorinostat to treat patients with advanced cancer	I	1	[136]
	NCT00781846	Ridaforolimus in combination with Bevacizumab for patients with advanced cancers	I	1	[137]
Endometrial carcinoma	NCT00739830	Ridaforolimus in advanced endometrial carcinoma	II	1	[138]
Multiple myeloma	NCT00693433	Temsirolimus and Dexamethasone to treat patients with recurrent or refractory multiple myeloma	I	1	
	NCT00398515	Temsirolimus and Lenalidomide to treat patients with previously treated multiple myeloma	I	1	
	NCT00918333	Everolimus and Panobinostat to treat patients with recurrent multiple myeloma, non-Hodgkin lymphoma, or Hodgkin lymphoma	I/II	2	
	NCT00474929	Everolimus and Sorafenib to treat patients with relapsed or refractory lymphoma or multiple myeloma	I/II	2	
Ovarian cancer	NCT01460979	Temsirolimus to treat ovarian cancer of women who progressed during previous platinum chemotherapy or within 6 months after therapy or advanced endometrial carcinoma	II	1	
	NCT00982631	Temsirolimus and Pegylated Liposomal Doxorubicin to treat advanced or recurrent breast, endometrial and ovarian cancer	I	3	
	NCT01196429	Temsirolimus, Carboplatin, and Paclitaxel as first-line therapy to treat patients with newly diagnosed stage III–IV clear cell ovarian cancer	II	1	
	NCT01010126	Temsirolimus and Bevacizumab to treat advanced endometrial, ovarian, liver, carcinoid, or islet cell cancer	II	2	
	NCT01031381	Everolimus and Bevacizumab to treat recurrent ovarian, peritoneal, and fallopian tube cancer	II	1	
	NCT01281514	Everolimus and Carboplatin, Pegylated Liposomal Doxorubicin Hydrochloride to treat patients with relapsed ovarian	I	4	

Cancer Type	Identifier	Study	Phase	Status	
		epithelial, fallopian tube, or peritoneal cavity cancer			
Melanoma	NCT01166126	Temsirolimus and AZD 6244 to treat naive with BRAF mutant unresectable stage IV	II	1	
	NCT01014351	Everolimus with Paclitaxel and Carboplatin to treat metastatic melanoma	II	1	
	NCT01092728	Dasatinib to treat acral lentiginous, mucosal, or chronic sun-damaged melanoma	II	1	
Sarcoma	NCT00112372	Ridaforolimus to treat patients with refractory or advanced malignancies and sarcomas	I/II	1	[139]
	NCT00093080	Ridaforolimus to treat patients with advanced sarcoma	II	1	

1 Completed or terminated; 2 Active, not recruiting; 3 Unknown; 4 Recruiting; 5 Suspended; 6 Withdrawn.

Table 3. Clinical trials of the effects of autophagy activators on human cancers.

3.2. Use of autophagy inhibitors in cancer treatment

Autophagy confers stress tolerance that enables tumor cells to maintain metabolic homeostasis and the adaptation to hypoxic, nutrient-limiting, and metabolically stressed environments as well as resistance to therapy-induced stress, such as chemotherapy or radiotherapy [11, 64, 105]. Since autophagy activation confers an advantage to tumor growth, it would be one of the hallmarks of tumor progression [140]. For example, K-Ras^{V12} transforming malignant cells are capable of evading metabolic stress and cell death through activation of autophagy cascades. In an attempt to overcome this advantage of tumor behavior, the treatment with autophagy inhibitors Bafilomycin A_1 or 3-MA successfully decreases the growth of human breast epithelial cells in vitro [141]. Also, targeting autophagy inhibition using CQ suppressed growth and tumorigenicity of K-Ras mutation tumor cells leading to prolonged survival in pancreatic cancer xenografts and genetic mouse models [142]. These preclinical results suggest that autophagy might be exploited as a new therapeutic target in the setting of tumors driven by oncogenic RAS, which may improve clinical outcome of the patients with RAS-driven tumors, such as pancreatic cancer and malignant melanoma; however, recently reported KRAS-driven tumor lines may not require autophagy for growth [143]. By profiling 47 cell lines with pharmacological and genetic loss-of-function tools, Eng and colleagues suggested that KRAS mutation status would not predict the sensitivity of cancer cells to autophagy inhibition with CQ [143]. Accordingly, oncogenic B-RAF signaling in melanoma impairs the therapeutic advantage of autophagy inhibition [144].

Despite this controversial relation regarding the activation of MAPK pathway and the prediction of the efficacy of autophagy inhibition, the pharmacologic inhibition targeting the early or late autophagy process may increase controlled cell death of several other human tumors during chemotherapy aiding improved clinical outcomes [59, 102, 109]. The therapeutic modulation of autophagy for cancer treatment has been supported by preclinical models in

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which inhibition of autophagy restored chemosensitivity and enhanced tumor cell death [64]. For example, CQ and its analog HCQ given in combination with chemotherapy suppressed tumor growth and triggered cell death to a greater extent than did chemotherapy alone, both in vitro and in vivo as reviewed [64].

Moreover, suppression of autophagy via use of chemical inhibitors of autophagy such as 3-MA can sensitize tumor cells to the effects of chemotherapeutic drugs [11, 14], 5-Fluorouracil [128, 145], TNF-a [146], proteasome inhibitors [147], and Src family kinase (SFK) inhibitor Saracatinib [148].

The sensitizing effects of inhibiting autophagy on the antitumor efficacy of chemotherapeutic agents have been recapitulated in preclinical models of Myc-induced lymphoma [67, 149], colon cancer [45, 59, 127, 128, 131, 145, 150–153], ovarian cancer [154], breast cancer [31, 32, 155, 156] hepatocellular cancer [157], prostate cancer [148, 156], bladder cancer [156], melanoma [114], and glioma [152, 158]. Preclinical evidence reveals the efficacy of CQ to inhibit the genesis and self-renewal of cancer stem cells (CSC) and underlines the impact of this “old drug” as repurposing strategy to open a new CSC-targeted chemoprevention era [153].

Several clinical trials that have been conducted or are in progress have shown favorable effects of CQ as a novel antitumor drug as reviewed [159, 160]. Autophagy inhibition may contribute to the anticancer actions of the histone deacetylase (HDAC) inhibitor Vorinostat [127, 130, 136]. **Table 4** compiles recent clinical trials therapeutic targeting autophagy inhibition.

Cancer Type	Identifier	Study	Phase	Status
Renal cell cancer	NCT01144169	Hydroxychloroquine before surgery in patients with primary renal cell carcinoma	I	1
	NCT01480154	Akt Inhibitor MK2206 and Hydroxychloroquine to treat advanced solid tumors, melanoma, prostate, or kidney cancer	I	2
	NCT01510119	Everolimus and Hydroxychloroquine to treat renal cell carcinoma	I/II	4
Prostate cancer	NCT00726596	Hydroxychloroquine to treat patients with rising PSA levels after local therapy for prostate cancer	II	2
	NCT00786682	Docetaxel and Hydroxychloroquine to treat metastatic prostate cancer	II	1
	NCT01480154	Akt Inhibitor MK2206 and Hydroxychloroquine to treat advanced solid tumors, melanoma, prostate, or kidney cancer	I	2
	NCT01828476	Navitoclax and Abiraterone with or without Hydroxychloroquine to treat progressive metastatic castrate refractory prostate cancer	II	1
Breast cancer	NCT01292408	Hydrochloroquine to treat breast cancer patients	II	3
	NCT00765765	Hydroxychloroquine and Ixabepilone to treat metastatic breast cancer	I/II	1

Cancer Type	Identifier	Study	Phase	Status	
	NCT01023477	Chloroquine to treat ductal carcinoma *in situ*	I/II	4	
	NCT02333890	Chloroquine to treat breast cancer	II	4	
Non–small cell lung cancer	NCT00977470	Erlotinib with or without Hydroxychloroquine in chemo-naive advanced NSCLC and (EGFR) mutations	II	2	
	NCT00809237	Hydroxychloroquine and Gefitinib to treat lung cancer	I/II	3	
	NCT00933803	Hydroxychloroquine, Carboplatin, Paclitaxel, and Bevacizumab to treat recurrent advanced non-small cell lung cancer	I/II	1	
	NCT01649947	Hydroxychloroquine, Carboplatin, Paclitaxel, and Bevacizumab to treat advanced/recurrent nonsmall cell lung cancer	II	4	
	NCT00728845	Hydroxychloroquine, Carboplatin, Paclitaxel, and Bevacizumab to treat recurrent advanced non–small cell lung cancer	I/II	1	
Small cell lung cancer	NCT00969306	Chloroquine to treat stage IV small cell lung cancer	I	4	
Pancreatic cancer	NCT01273805	Hydroxychloroquine to treat patients with metastatic pancreatic cancer	II	2	
	NCT01128296	Study of presurgery Gemcitabine and Hydroxychloroquine to treat stage IIB or III adenocarcinoma of the pancreas	I/II	2	
	NCT01506973	Hydroxychloroquine in combination with Gemcitabine/ Abraxane to inhibit autophagy in pancreatic cancer	I/II	4	
	NCT01978184	Gemcitabine and Abraxane with or without Hydroxychloroquine	II	4	
Glioblastoma	NCT00486603	Hydroxychloroquine, radiation therapy, and Temozolomide to treat patients with newly diagnosed glioblastoma multiforme	I/II	1	[161]
	NCT00224978	Chloroquine to treat glioblastoma multiforme	III	1	[162]
	NCT02432417	Chloroquine and chemoradiation to treat glioblastoma	II	5	
	NCT02378532	Chloroquine and Chemoradiation to treat glioblastoma	I	5	
Colorectal cancer	NCT01206530	FOLFOX, Bevacizumab and Hydroxychloroquine to treat colorectal cancer	I/II	4	
	NCT01006369	Hydroxychloroquine, Capecitabine, Oxaliplatin, and Bevacizumab to treat metastatic colorectal cancer	II	6	
	NCT02316340	Vorinostat and Hydroxychloroquine versus Regorafenib to treat colorectal cancer	II	4	
Chronic myeloid leukemia	NCT01227135	Imatinib Mesylate with or without Hydroxychloroquine to treat patients with chronic myeloid leukemia	II	3	
	NCT00771056	Hydroxychloroquine in untreated B-CLL Patients	II	6	
Advanced solid tumor	NCT00813423	Sunitinib Malate and Hydroxychloroquine to treat patients with advanced solid tumors	I	2	

Cancer Type	Identifier	Study	Phase	Status
		that have not responded to chemotherapy		
	NCT00714181	Hydroxychloroquine and Temozolomide to treat patients with metastatic or unresectable solid tumors	I	1
	NCT00909831	Hydroxychloroquine and Temsirolimus to treat patients with metastatic solid tumors that have not responded to treatment	I	2
	NCT01023737	Hydroxychloroquine and with histone deacetylase (HDAC) inhibitor Vorinostat in patients with advanced solid tumors	I	4
	NCT01266057	Sirolimus or Vorinostat and Hydroxychloroquine in advanced solid tumors	I	4
Multiple myeloma	NCT00568880	Hydroxychloroquine and Bortezomib to treat patients with relapsed or refractory multiple myeloma	I/II	3
	NCT01689987	Hydroxychloroquine, Cyclophosphamide, Dexamethasone, and Sirolimus to treat patients with relapsed or refractory multiple myeloma	I	2
	NCT01438177	Chloroquine and VELCADE and Cyclophosphamide to treat relapsed and refractory multiple myeloma	II	1
Melanoma	NCT00962845	Hydroxychloroquine to treat patients with stage III or stage IV melanoma that can be removed by surgery	I	2
	NCT01480154	Akt Inhibitor MK2206 and Hydroxychloroquine to treat patients with advanced solid tumors, melanoma, prostate, or kidney cancer	I	2
	NCT02257424	Dabrafenib, Trametinib and Hydroxychloroquine in patients with advanced BRAF mutant melanoma	I/II	4

1 Completed or terminated; 2 Active, not recruiting; 3 Unknown; 4 Recruiting; 5 Not yet recruiting; 6 suspended.

Table 4. Clinical trials of the effects of autophagy inhibitors on human cancers.

4. Therapeutic effects of autophagy modulators on cardiovascular diseases

Autophagy plays a dichotomous role on many cardiac pathologic states in which it may exert both protective and detrimental effects through context-dependent mechanisms. As a protective mechanism, autophagy closely protects the heart from myocardial ischemia-reperfusion (I/R) and attenuates cardiac remodeling after myocardial infarction [49, 163]. In fact, as demonstrated in ischemia-reperfusion-induced heart injury, Parkin-mediated mitophagy showed a protective role against the cell death of cardiomyocytes [164]. Moreover, recent evidence indicates that basal levels of autophagy are required for the maintenance of normal cardiovascular function and morphology [165]. However, by contrast, excessive levels of autophagy—or perhaps distinct forms of autophagic flux—contribute to several types of cardiomyopathy by functioning as a controlled cell death pathway [165, 166]. In line with these findings, the selection of activators or inhibitors of autophagy for prevention or treatment of cardiovascular diseases will be complicated. Nevertheless, in practice successful therapeutic approaches that regulate autophagy have been reported recently, suggesting that the autophagic machinery may be properly manipulated to treat heart failure or to prevent rupture of atherosclerotic plaques and sudden death [165, 166]. Whereas there have been no clinical data reporting the efficacy of pharmacologic modulation of autophagy in cardiac diseases, as reviewed in 2011, nine patents have disclosed the pharmacologic modulation of autophagy as a new therapeutic strategy against cardiovascular diseases [166]. In this section, we will review the fundamental use of autophagy modulators on heart diseases, whose biological effects have been identified through both in vitro and in vivo models.

5. Use of autophagy inhibitors in treatment of heart disease

Pharmacologic suppression of autophagy pathway comprises potential new targets for treating cardiac disorders [11]. In vitro and in vivo studies demonstrated that the inhibitor of histone deacetylases Trichostatin A may attenuate both load- and agonist-induced hypertrophic growth and abolish the associated activation of autophagy, reducing pathologic cardiac remodeling during severe pressure overload [167]. Through negative modulation of early stage of autophagy process by inhibiting the expression of *Beclin-1* induced by myocardial I/R injury parallel to phosphorylation of mTOR, Propofol reduces autophagy-associated cell death induced by the myocardial I/R injury [49]. Although these strategies for suppressing the excessive activation of autophagy for treating cardiac disorders are in theory promising, a comprehensive view of myocardial autophagy will be obligatory to avoid disrupting homeostatic mechanisms [166]. Thus, although the major challenges remain, patients with heart disease are likely to benefit from these efforts.

In other situations, such as in response to stress, activation rather than suppressing autophagy might be beneficial, since it increases the clearance of misfolded and other harmful proteins. In fact, recent reports had established a requirement for autophagy for cardioprotection in rodent models mediated by a variety of agents including the adenosine A1 receptor agonist Chlorocyclopentyladenosine, Sulfaphenazole, and ischemic preconditioning [168]. On the fate of ischemic-reperfused cardiomyocytes, autophagy plays a protective role [169, 170].

The development of a pharmacological agent to salvage myocardium after an ischemic insult has been explored. For example, attempting to enhance the heart's tolerance to ischemia-reperfusion through inducing autophagy, the antimicrobial agent Sulfaphenazole might be used [163]. Likewise, chloramphenicol succinate has been shown to activate autophagy and reduce myocardial damage during I/R [169, 170]. In the case of regression of established increase in myocyte cell size (i.e., cardiac hypertrophy) induced by ascending aortic constriction (i.e., pressure overload), the administration of Rapamycin, a mTOR inhibitor, may improve cardiac function [171, 172]. In line with this finding, animal studies suggest that mTOR inhibition attenuates cardiac allograft remodeling secondary to downregulation of mTOR downstream targets and increased autophagy. To increase the paucity of data regarding effect of Sirolimus, a mTOR inhibitor, on human heart remodeling, a current clinical trial Phase 1 has been conducted (NCT01889992).

Macrophages play a central role in atherosclerotic plaque destabilization, leading to acute coronary syndromes and sudden death, and therefore their clearance from atherosclerotic plaques through autophagy has been suggested as an attractive therapeutic strategy for atherosclerosis [173]. In line with these findings, the stent-based delivery of Everolimus was shown to selectively clear macrophages from atherosclerotic plaques in rabbits by activating autophagy without altering smooth muscle cells [174]. As suggested recently, mTOR inhibition represents a promising strategy for stabilization of atherosclerotic plaques [175], as this also prevents adverse left ventricular remodeling and limits infarct size following myocardial infarction [176]. Though the benefits afforded by autophagic activation depend on cardiac pathologic states, vigilance for extra-cardiac effects may be critical [166].

6. Use of autophagy modulators for neurologic disorders

In contrast to other cell types, neurons for being nondividing cells are particularly sensitive to changes in autophagic degradation [177]. As most neurons must survive for the lifetime of the organism, maintenance of organelle function and clearance of aberrant, misfolded, and aggregate proteins are critical processes regulated by autophagy [19]. In fact, many aggregate-prone forms of such proteins, including tau [178], α-synuclein [179, 180], mutant huntingtin [181], and mutant ataxin 3 [178] have a higher dependency on autophagy for their clearance. While autophagy clears these aggregate-prone proteins, upregulation of autophagy may also contribute to amyloid-β pathology [182], as autophagic vacuoles may represent one site of amyloid-β generation. The intracellular accumulation of these aggregate proteins are features of many late-onset neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), tauopathies, and polyglutamine expansion diseases—such as Huntington's disease (HD) and various spinocerebellar ataxias (SCAs) [183]. Currently, there are no effective therapeutic strategies capable of attenuating or preventing the neurodegeneration resulting from these diseases in humans.

Autophagy has been considered as a potentially novel approach for treating neurodegenerative disorders [160, 183], although its role in neurodegenerative disorders remains unclear [11]. Even though autophagy may be initially induced as a neuroprotective response, due to excessive, imbalanced induction or defects in completing degradation, it may also contribute to neuronal atrophy, neurite degeneration, and cell death [19, 177]. It is noteworthy that the failed attempt of autophagy at neuron survival has been closely associated with age-related autophagy insufficiency and lysosomal aging [19]. In fact, several evidences recently suggest a possible role for autophagic dysfunction in the pathogenesis of neurodegenerative diseases [184]. Conversely, autophagy also has the ability to decrease the accumulation of toxic, aggregate-prone proteins that cause neurodegeneration [11, 183]. As summarized in the following paragraph, multiple studies provide proof of principle for the activation of autophagy as a therapy for neurodegenerative disease. To date, there are still very few reported clinical results demonstrating that modulation of autophagy indeed represents an effective therapeutic intervention for these devastating diseases [11].

HD disease is caused by a polyglutamine expansion mutation in the huntingtin protein (polyQ-expanded Htt) that confers a toxic gain-of-function and causes the protein to become aggregate-prone proteins, which are cleared by autophagy. It is noteworthy that upregulating this process by Rapamycin attenuates their toxicity in various HD models [181, 185]. The autophagy inducer Rapamycin or its analog CCI-779 has been reported to promote autophagic clearance of polyQ-expanded Htt protein [178, 181, 186]. Likewise, Rapamycin increases the clearance of α-synuclein and lessens the formation of aggregates (Lewy bodies) in neurons [179]. Rapamycin in combination with lithium showed a greater protection against neurodegeneration in an HD fly model [185]. Interestingly, the disaccharide bilayer membrane-protector Trehalose [187] accelerates the autophagic clearance of mutant Huntingtin and α-synuclein through an mTOR-independent pathway [95]. However, in a combination of mTOR inhibitor, Rapamycin Trehalose's effect on autophagic activity increases, resulting in an additive effect on the clearance of the above proteins [95]. Together these studies demonstrate that autophagy upregulation and promotion of aggregation-prone protein degradation ameliorate neurodegenerative pathology, but conversely autophagy inhibition enhances the toxicity of these proteins [184].

7. Autophagy modulators for treatment of other diseases

Similar to the details outlined above for neurodegenerative disorders, autophagy upregulation may enhance the clearance of a range of infectious agents, including multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis.* In some cases, mouse models and preclinical data have strengthened the protective role of autophagy against microbial infections, as summarized in **Table 5**.

AR-12 induces autophagic clearance of *Francisella tularensis* from the human leukemic cell line THP-1 macrophages [188] and *Salmonella enterica serovar Typhimurium* in murine macrophages, both in vitro and in vivo [189]. Additionally, experimental findings underscore the importance of host autophagy in orchestrating successful antimicrobial responses to *Mycobacterium tuberculosis* during chemotherapy with Isoniazid and Pyrazinamide [190]. Likewise, the most active form of vitamin D 1,25D3 may inhibit replication of human immunodeficiency virus (HIV) in human macrophages through autophagy activation [191].

Based on these preclinical findings, researchers have raised the possibility that some antimycobacterial chemotherapies already used in clinical for the treatment of infectious diseases anti-infection effects, at least partially, via inducing autophagy. However, it is still unclear whether those findings can be translated into the clinical treatment of certain infections [11]. Stimulation of autophagy with Rapamycin reduces intracellular survival of mycobacteria in macrophages [192].

Drugs	Effects
1,25D3	1α,25-dihydroxycholecalciferol inhibits HIV replication and mycobacterial growth [191].
AR-12	2-amino-*N*-4-5-(2 phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl phenyl-acetamide inhibits activity of phosphoinositide-dependent kinase-1 and promotes autophagic clearance of bacteria in human and murine macrophages [188, 189].
Carbamazepine	Induces antimicrobial autophagy through a mTOR-independent pathway controlled by cellular depletion of myo-inositol [193].
Fluoxetine	A selective serotonin reuptake inhibitor, enhances secretion of proinflammatory cytokine TNF-α and induces autophagy in *Mycobacterium tuberculosis*–infected macrophages [194].
Gefitinib	An inhibitor of the Epidermal Growth Factor Receptor (EGFR), activates autophagy, and restricts growth of *Mycobacterium tuberculosis* in the lungs of infected mice [194].
Isoniazid or pyrazinamide	Reduces *Mycobacterium tuberculosis (Mtb)*–induced proinflammatory responses by promoting autophagy activation and phagosomal maturation in *Mtb*-infected host cells [190].
Nitozoxanide	Nitazoxanide and its active metabolite Tizoxanide strongly stimulate autophagy and inhibit mTORC1 signaling and intracellular proliferation of *Mycobacterium tuberculosis* [195].
Nortriptyline	Induces the formation of autophagosomes that progressively acidify over time and become competent for *Mycobacterium tuberculosis* degradation in infected macrophages [196].
Prochlorperazine edisylate	Modulates autophagy that correlates with delivery of *Mycobacterium tuberculosis* to lysosomes leading to mycobacterial degradation [196].
Rapamycin	Induces autophagy and suppresses intracellular survival of *M. tuberculosis* [192].
Statins	Enhances autophagy and phagosome maturation leading to reduction the *Mycobacterium tuberculosis* burden in human macrophages and in mice [197].
Valproic acid	Stimulates autophagic killing of intracellular *Mycobacterium tuberculosis* within primary human macrophages [193].

Table 5. Preclinical studies of the effects of autophagy activators on infectious diseases.

Remarkably, several FDA-approved drugs counter *M. tuberculosis* infection, possibly through autophagy, which disrupts the host-pathogen equilibrium in favor of the host (see **Table 5**). The antidepressants Fluoxetine [194] and Nortriptyline [196], the anticonvulsants Carbamazepine and Valproic acid, and the antipsychotic Prochlorperazine edisylate reveal relevant antimycobacterial properties by targeting autophagy in the host (i.e., infected macrophages). Notably, in mice infected with a highly virulent MDR-strain of *Mycobacterium tuberculosis*, Carbamazepine reduces bacterial burden, improve lung pathology, and stimulate adaptive immunity [193]. Furthermore, the tyrosine kinase inhibitor (Gefitinib) also activates autophagy and suppress *Mycobacterium tuberculosis* in macrophages and, to some extent, in infected mice [194]. Other autophagy-inducing candidate drugs attempting to Antituberculosis Host-

Directed Therapy (HDT) include antiprotozoal drug Nitozoxanide [195] and cholesterol-lowering drugs, i.e., Statin [197]. Together these findings support that autophagy enhancement by repurposed drugs provides an easily implementable potential therapy for the treatment of multidrug-resistant mycobacterial infection.

8. Promising future therapeutic strategies

8.1. The case of the pentacyclic triterpenoids, the working hypothesis

After decades of scientific discoveries and discussions [10, 198] the general agreement is that autophagy associated cell-death is commonly linked to failure in either the fusion of autophagosomes with lysosomes or in the digestion activity of autolysosomes [27, 198]. Whereas the understanding of this process at the molecular level needs a deeper knowledge of the competition between its activation and inhibition pathways, autophagy has been explored as a potential therapeutically target for treating several diseases [11, 14]. Consequently, the impact of activating mitophagy on the condition of autophagy impairment is a noteworthy subject to explore. A recent work has proposed that by modulating parallel damage in membranes of mitochondria and lysosome, autophagy turns into a destructive process [12]. Comparative analysis of the biological effects of two chemical isomers, i.e., pentacyclic triterpenoids Betulinic and Oleanolic acids (BA and OA, respectively), Martins and colleagues showed that the main differences between the activity of BA and OA is due to their efficiency in interacting and damaging membranes [12] (see **Figure 1**). These triterpenoids are new promising drugs with various pharmacological actions (anti-inflammatory, antiviral, antifungal, antimalarial, among others), being easily extracted from plants [199]. So far, about 2167 patents for AB and 1018 for OA have been deposited.

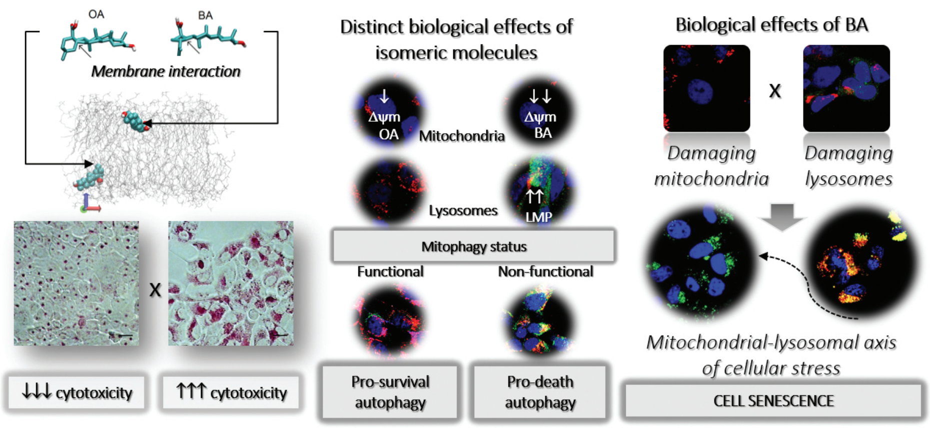

Figure 1. Modulation of membrane damage provided insights into biological effects of triterpenoids in vitro (Figure kindly supplied by Martins WK).

By comparing these triterpenoids, it was realized that the fate of autophagy may depend on the extent of lysosomal and mitochondrial membrane damage. In case of OA, there is marked

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cytoplasmic vacuolization and mitochondria shrinkage with remarkable cellular recovery that was intrinsically associated with autophagy activation. However, cell recovery failed upon concomitant lysosome inhibition with CQ or Bafilomycin A_1 [12]. Of note, the lysosomal damage BA-mediated is per se capable of compromising autophagy, without any incremental damage when lysosomal function was deeply altered by lysosomal inhibitors, such as CQ and Bafilomycin A_1.

BA and OA differ significantly on their ability to penetrate membranes, which appears to be mainly related to the twisted backbone structure of OA, in contrast to the fully planar structure of BA. Interestingly, this stronger efficiency in interacting and damaging membrane mimics ascribed to BA correlates with a higher ability of disturbing mitochondrial and lysosomal membranes of human keratinocytes [12]. The ability of BA to disturb the mitochondrial membrane is in agreement with other published results [106, 200, 201]. For example, by inhibiting the activity of steroyl-CoA-desaturase (SCD-1) BA may also directly and rapidly impact on the saturation level of cardiolipin (CL), a specific mitochondrial phospholipid lipid that has important structural and metabolic functions, and at the same time regulates mitochondria-dependent cell death [202]. Interestingly, thermodynamic analyses of Langmuir monolayers and AFM study of Langmuir-Blodgett monolayers provide insights into the ability of BA interacting with CL-enriched membranes. BA may orient nearly perpendicularly with hydroxyl group toward water, which causes phase separation and changes the permeability of CL film [203]. BA was also shown to disrupt membranes of human red blood cells (RBC) in vitro, with release of calcein from the RBC ghosts in a way similar to Digitonin in membrane permeabilization experiments [204].

Of note, the damage in lysosomal function caused by BA may not be explained by traditional justifications (lack of lysosome acidification or neutralization of its internal pH). Otherwise, BA disturbs lysosome's membrane integrity that dramatically jeopardizes the lysosomal function, leading to a lysosomal-mitochondrial axis of cellular stress that causes autophagy-associated cell death [12]. Remarkably, in the survival of BA-challenged cells occurs sustained formation of reactive oxygen species (ROS) inside nonfunctional lysosomes, which in the long-term response leads to lipofuscinogenesis, genomic instability, and cell senescence [13]. Thus, promotion of concomitant damage in mitochondrial and lysosomal membranes seems to be an efficient strategy for inducing autophagy-associated cell death and cell aging.

The AB's ability to promote parallel damage in lysosomes and mitochondria could be the explication for the positive synergistic action of BA in different antitumor protocols including radiation [205, 206], chemotherapy drugs, such as Cisplatin [207] and Vincristine [208]. Therefore, the possible increase of cell death potentially relates to the AB ability of suppressing prosurvival autophagy. The knowledge of this premise at molecular level may contribute to the development of new autophagy modulators.

Since 1995, BA has been considered as a highly promising anticancer drug showing remarkable antitumor effects against several human tumors [106, 208–222]. In addition, in the last decade, many studies have shown further effects that justify the expectation that triterpenes and synthetic analogs are useful to treat cancer by several modes of action [223]. For example, BA acid derivatives are under evaluation as chemotherapeutic agent against several types of human tumors in vitro and in vivo [152, 199, 223–235]. The synthetic analog of OA [2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO)] is currently under clinical Phase I study (NCT00322140) for treating solid tumors and lymphoma [101]. Introduced in 2009, the new semisynthetic candidate drug designated NVX-207 (3-acetyl-betulinic 2-amino-3-hydroxy-2-hydroxymethyl-hi-ethyl propanoate) enhanced apoptosis-inducing activity and dramatically enhanced solubility over BA [230]. However, limited solubility and often ultimately modest efficacy have hampered the development of this class of compounds [230]. Thus, scientific efforts focused on the elucidation of molecular mechanisms triggered by these triterpenoids, attending the interests of the scientific community as well as of the pharmaceutical industry.

9. Perspectives for drug development

During the last decade, important progress was made in understanding the molecular basis of autophagy uncovering its potential in anticancer therapies [105, 236, 237]. Because the abrogation of autophagy via knockdown of autophagy-related molecules increases the sensibility of therapy-resistant cancer cells to conventional cancer therapies, there has been great interest in developing clinically relevant autophagy inhibitors [238]. As reviewed by Yang and colleagues, multiple studies have shown that genetic knockdown of autophagy-related genes (Atgs) or pharmacological inhibition of autophagy can effectively enhance tumor cell death induced by diverse anticancer drugs in preclinical models [64].

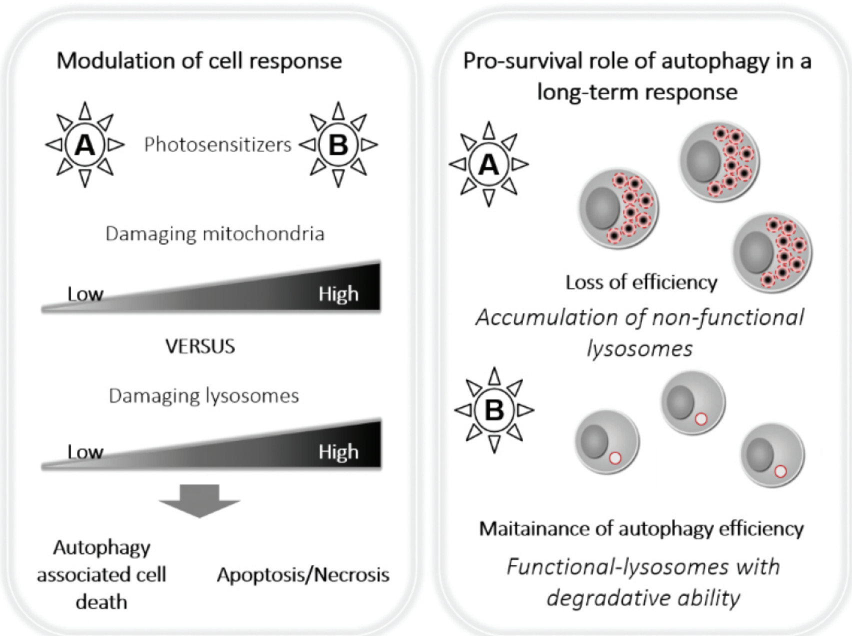

Figure 2. Modulation of membrane damage provided insights into biological effects after PDT (Figure kindly supplied by Martins WK).

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Photodynamic therapy (PDT) is a procedure that has applications in the selective eradication of cancer where sites of tumor lesions are clearly delineated. It is a two- step process whereby cells are first incubated with photosensitizers and then photoirradiated. This results in the formation of singlet molecular oxygen and other reactive oxygen species (ROS) that can cause photodamage at sites where the photosensitizing agent has localized [239]. Photosensitizers found to be clinically useful, showing affinity for the endoplasmic reticulum, mitochondria, lysosomes, or combinations of these sites [240]. The induction of cell death triggered by apoptosis and/or autophagy in photosensitized cells is a common outcome of PDT [239–242]. Therefore, the photosensitizers are drugs that are used to treat a series of different diseases. Our group has addressed the concept of parallel photodamage in mitochondria and lysosome with the consequent induction of cell death and senescence after PDT (**Figure 2**). In near future, we will exploit the possible use of this concept in the development of new photosensitizers targeting autophagy as cell death mechanism.

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