



Therapeutic Targeting of Autophagy in Pancreatic Ductal Adenocarcinoma

Alexander G. Raufi^{1,2,3,4*}, Nicholas R. Liguori^{1,5}, Lindsey Carlsen^{1,3,4,6}, Cassandra Parker^{1,7}, Liz Hernandez Borrero^{1,6}, Shengliang Zhang^{1,3,4,8}, Xiaobing Tian^{1,3,4,8}, Anna Louie^{1,7}, Lanlan Zhou^{1,3,4,8}, Attila A. Seyhan^{1,3,4,8} and Wafik S. El-Deiry^{1,2,3,4,6,8*}

¹Laboratory of Translational Oncology and Experimental Cancer Therapeutics, Warren Alpert Medical School, Brown University, Providence, RI, United States, ²Hematology/Oncology Division, Department of Medicine, Lifespan Health System and Brown University, Providence, RI, United States, ³Joint Program in Cancer Biology, Lifespan Health System and Brown University, Providence, RI, United States, ⁴Cancer Center at Brown University, Providence, RI, United States, ⁵Temple University, Lewis Katz School of Medicine, Philadelphia, PA, United States, ⁶Pathobiology Graduate Program, Warren Alpert Medical School, Brown University, Providence, RI, United States, ⁷Department of Surgery, Warren Alpert Medical School, Brown University, Providence, RI, United States, ⁸Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, United States

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Edited by:

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*Correspondence:

Wafik S. El-Deiry
wafik@brown.edu
Alexander G. Raufi
alexander_raufi@brown.edu

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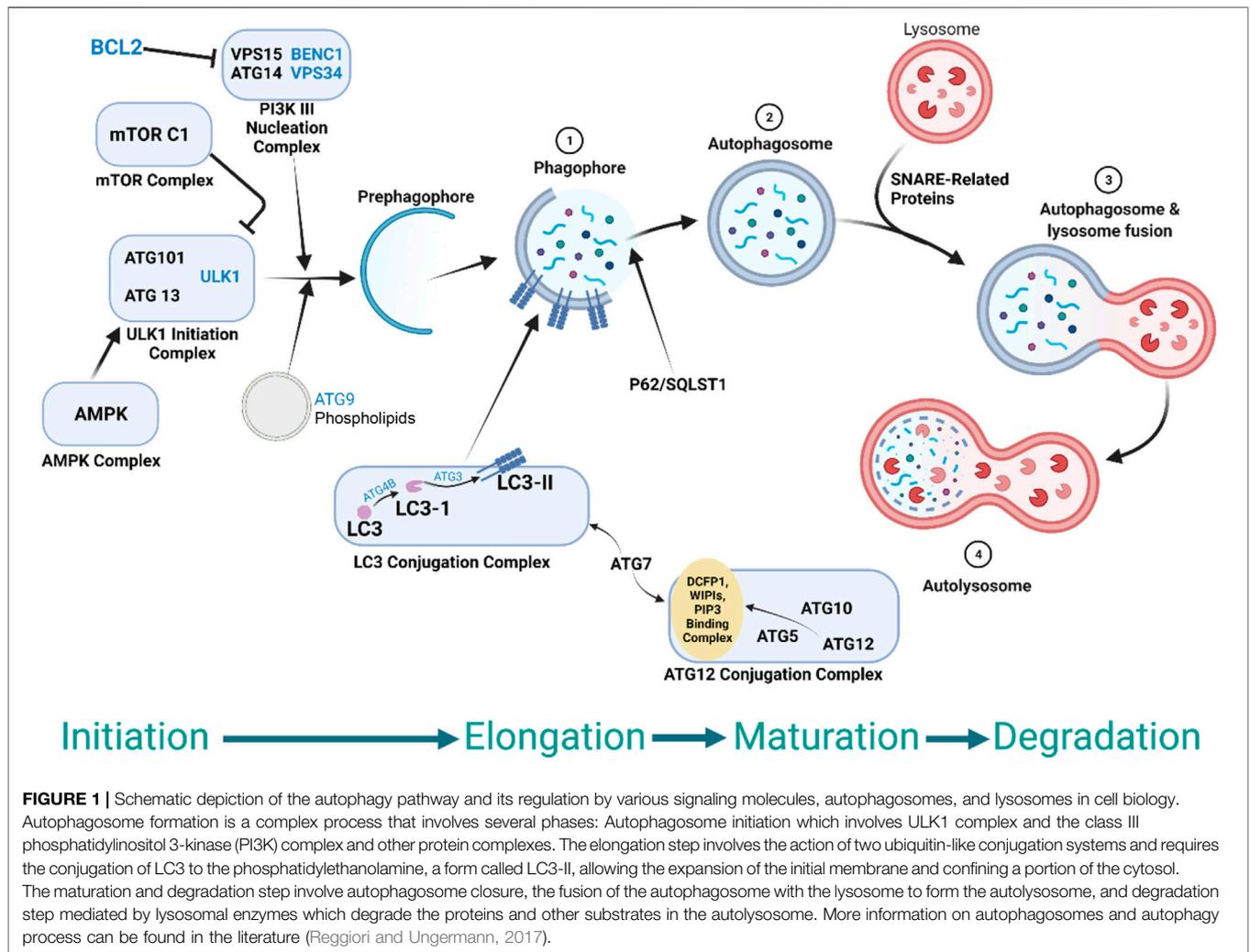
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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease characterized by early metastasis, late detection, and poor prognosis. Progress towards effective therapy has been slow despite significant efforts. Novel treatment approaches are desperately needed and autophagy, an evolutionary conserved process through which proteins and organelles are recycled for use as alternative energy sources, may represent one such target. Although incompletely understood, there is growing evidence suggesting that autophagy may play a role in PDAC carcinogenesis, metastasis, and survival. Early clinical trials involving autophagy inhibiting agents, either alone or in combination with chemotherapy, have been disappointing. Recently, evidence has demonstrated synergy between the MAPK pathway and autophagy inhibitors in PDAC, suggesting a promising therapeutic intervention. In addition, novel agents, such as ONC212, have preclinical activity in pancreatic cancer, in part through autophagy inhibition. We discuss autophagy in PDAC tumorigenesis, metabolism, modulation of the immune response, and preclinical and clinical data with selected autophagy modulators as therapeutics.

Keywords: autophagy, pancreatic cancer, MEK inhibitors, ONC212, chloroquine, Atg5, LC3, beclin 1

PANCREATIC CANCER

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease characterized by early metastasis, late detection, and little progress towards effective treatment or cure. The vast majority of patients present with incurable unresectable or metastatic disease. Even in the 15–20% of patients who are candidates for, and ultimately undergo resection, recurrence ultimately occurs in 80%. Presently, the mortality-to-incidence ratio for PDAC remains amongst the highest of all malignancies and by 2030 PDAC is projected to be the second leading cause of cancer-related death in the United States (Miller et al., 2016). For individuals diagnosed with unresectable or metastatic PDAC, combination chemotherapy with mFOLFIRINOX or gemcitabine/nab-paclitaxel remains the standard of care. These regimens provide modest benefit, improving quality of life and median overall survival by several months, however, the 5-years overall survival is only 10% (Siegel et al., 2021). In light of this, identifying novel therapeutic agents to treat PDAC has become a major focus of research.



Although several mutations (e.g., *KRAS*, *TP53*, *SMAD4*, *CDKN2A*) are commonly identified in PDAC, the disease is genetically complex and development of targeted therapy has been slow. Only two targeted therapies have been approved to date: erlotinib, an EGFR inhibitor which improves in overall survival by approximately 2 weeks, and olaparib, a PARP inhibitor, which improves progression free survival by several months in germline BRCA2-mutated metastatic PDAC that has not progressed after 4 months of platinum containing chemotherapy (Moore et al., 2007; Golan et al., 2019). Unfortunately, aside from rare cases of microsatellite instability, immune checkpoint blockade has also had little to no impact on outcomes for patients diagnosed with PDAC. The lack of effective therapies has served as an impetus to further improve our understanding of pancreatic tumor biology in order to identify alternative treatment strategies.

Autophagy is a complex, evolutionarily conserved process through which proteins and organelles are recycled for use as alternative energy sources. Although typically upregulated during states of cellular stress or starvation, tumor cells can also take advantage of this process to maintain homeostasis. In this review

we will focus on macro-autophagy, which refers to the removal of cytoplasmic components through autophagosome-delivery of organelles to lysosomes through degradation (Figure 1) (Mizushima et al., 2011). This process is required for cell survival, homeostasis, and can be upregulated through multiple cell signaling pathways. In cancer, it is thought to play a role in tumor cell survival and resistance to chemotherapy, and hence represents an area of therapeutic development.

We will discuss the role autophagy plays in PDAC tumorigenesis and metabolism, modulation of the immune response, as well as both preclinical and clinical data with select autophagy modulators.

AUTOPHAGY IS UPREGULATED IN PDAC

Although the precise role of autophagy in PDAC is incompletely understood, increased basal levels of autophagy have been reported. Using GFP-LC3 puncta as an indicator of cells undergoing autophagy, Yang et al. demonstrated increased

autophagic flux in eight PDAC cell lines (Yang et al., 2011). The authors further supported these findings by measuring levels of microtubule-associated protein 1 light chain 3 (LC3), more specifically the conversion of LC3-I to LC3-II. Previous work established an interaction between LC3 and autophagosomal membranes, notably in PDAC (Fujii et al., 2008). Yang et al. noted increased levels of LC3-II in PDAC cell lines, relative to control normal pancreatic ductal cells, a finding that was not reproducible in select lung or breast cancer cell lines, suggesting that this may be a unique feature of PDAC. Given that autophagy is a dynamic process, elevations in LC3-II could suggest a block in later stages of autophagy, such as impaired autophagosomal degradation, and not exclusively upregulation. Therefore, an analysis of long-term protein degradation using a GFP-Neo fusion protein was performed (Klionsky et al., 2008). Over a 2-day period, 8988T PDAC cells were examined and were noted to have a significant reduction in levels, further supporting increased autophagic flux. The authors were also able to restore GFP-Neo levels with the autophagy inhibitor chloroquine. Finally, they showed that chloroquine also reduces PDAC cell proliferation *in vitro*, suggesting a possible novel approach to therapy.

Several additional components of the autophagy pathway have been identified as key mediators in governing PDAC cell proliferation. ATG5, for example, is a ubiquitin-related protein shown to be necessary for autophagosomal expansion and completion (Figure 1) (Levine and Kroemer, 2008). Selective siRNA-mediated knockdown of ATG5 notably reduced 8988T PDAC cell proliferation by greater than 50% (Yang et al., 2011). The MiTF family of transcription factors (MiTF, TFE3, and TFE8) have also been implicated as drivers of autophagy in PDAC cells (Rouschop et al., 2010). Upon nuclear import, these transcription factors drive increased expression of catabolic lysosomal genes and gene set enrichment analyses indicate a strong relationship between expression of MiTF/TFE factors and autophagy in PDAC (Rouschop et al., 2010). Furthermore, MiTF, TFE3, or TFE8 knockout leads to downregulation of CLEAR (Coordinated Lysosomal Expression and Regulation)-carrying genes in PDAC cells, leading to reduction in proliferation and growth of PDAC tumor cells (Rouschop et al., 2010).

In the setting of amino acid starvation, unc5-like autophagy activating kinase 1 (ULK1) is known to play an indispensable role in driving autophagy. It is primarily regulated by nutrient-sensing kinases such as mammalian target of rapamycin (mTOR) complex-1 (mTORC1) and AMPK (Kim et al., 2011; Shang et al., 2011; Wong et al., 2015). When starvation-levels of amino acids are detected, mTORC1 is suppressed and ULK1 is phosphorylated inducing autophagy (Kim et al., 2011; Shang et al., 2011; Wong et al., 2015). Interestingly, starvation appears to be a more profound driver of autophagy than direct inhibition of mTORC1, suggesting that alternative pathways also play a role (Wong et al., 2015). Furthermore, cells with high levels of autophagy also have increased phosphatase activity, including phosphatase PPA2. This enzyme dephosphorylates ULK1 at S637 reducing levels of autophagy (Wong et al., 2015).

Given that the vast majority of PDAC cases have constitutive activation of KRAS, the effects of the MAPK pathway on autophagic flux is of particular interest. Although poorly defined, it is unlikely that constitutive MAPK signaling is solely responsible for driving increased basal levels of autophagy in PDAC. In fact, several studies have reported that inhibition of the MAPK cascade leads to increased autophagy which will be described in further detail later (Bryant et al., 2019; Kinsey et al., 2019). As described above, activation of the MAPK pathway is expected to promote phosphorylation and cytoplasmic retention of the transcription factors TFE3 and TFE8, and hence a reduce expression of autophagy promoting genes.

In summary, high rates of basal autophagy in PDAC is regulated by multiple mechanisms and each of these processes represents a unique target for further investigation.

AUTOPHAGY AND PDAC CARCINOGENESIS

The precise role autophagy plays in PDAC tumorigenesis is complicated by several conflicting studies that have shown that autophagy can lead to both promotion and inhibition of tumor development. A tumor-promoting mechanism of autophagy has been described in mice with heterozygous deletions of mammalian Beclin1. Deletion of this key autophagy promoting enzyme results in the development of malignant neoplasms in various organs in mice (Qu et al., 2003; Yue et al., 2003). Another partial autophagy phenotype, ATG5^{+/-}, leads to increased tumor formation and metastasis but this is not observed in mice completely deficient of autophagy (ATG5^{-/-}) which spontaneously developed only benign liver tumors and increased acinar-to-ductal metaplasia (Takamura et al., 2011; Görgülü et al., 2019). It has been suggested that autophagy is a relatively weak tumor suppressor yet at the same time it is necessary for the progression of benign tumors to malignancy (Takamura et al., 2011). There is also evidence suggesting that defects in autophagy lead to increased dysfunctional or damaged mitochondria in tumor cells and impaired tumorigenesis (White, 2015). This implies that autophagy may induce tumorigenesis and disease by preserving the integrity and quality of mitochondria and also by supplementing essential substrates for mitochondrial metabolism (White, 2015). Autophagy may also promote tumorigenesis by suppressing induction of the p53 tumor suppressor protein and maintaining metabolic function of mitochondria, enabling cancer cells to survive environmental stresses (White, 2015). Further study is required to bring clarity to our understanding of autophagic recycling of substrates, the identity of specific substrates, and the metabolic pathways and functions that they are used for.

Using a KRAS-driven lung cancer model, Guo et al. found that homozygous deletion of ATG7 reduced tumor burden and proliferation of tumor cells (Guo et al., 2016). ATG5, another member of the ATG family, was shown to increase PanIN but not PDAC formation in a genetically engineered PDAC mouse model

with mutant KRAS and a single Trp53 allele. Chloroquine or hydroxychloroquine treated PDAC cell lines and patient derived xenograft models led to decreased proliferation, increased DNA damage and apoptosis (Yang et al., 2014). Interestingly, ATG7 deletion in a similar KRAS mutant/Trp53null model of lung cancer showed reduced tumor burden (Karsli-Uzunbas et al., 2014). These studies support the role that autophagy plays in carcinogenesis and in maintaining tumor growth and proliferation.

AUTOPHAGY AND METABOLOMICS

As discussed in a recent review, cellular metabolism and autophagy are two interconnected cellular processes (Piffoux et al., 2021). A hallmark of tumor metabolism is the preferred use of aerobic glycolysis over oxidation of glycolytic pyruvate to produce both energy and lactate, the latter of which serves as a substrate for nucleic acid, protein, and lipid production. While aerobic glycolysis is inefficient in terms of energetics, it serves as a mechanism to promote growth, survival, and proliferation in tumor cells. This phenomenon of increased glucose uptake and fermentation of glucose to lactate is observed even in the presence of completely functioning mitochondria and is known as the Warburg Effect (Vander Heiden et al., 2009; Liberti and Locasale, 2016). Because autophagy degrades proteins and organelles to create new substrates it is integrally connected with tumor metabolism (Vander Heiden et al., 2009). It has been reported that oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function and that resistance to KRAS-targeted therapy might be mediated by a subset of tumor cells that depend on oxidative phosphorylation for survival instead of the classic Warburg effect (Viale et al., 2014). Oxidative phosphorylation is highly dependent on mitochondrial respiration, and genes involved in this process, as well as autophagy- and lysosome-related genes, were found to be upregulated in surviving cells. However, upregulation of autophagy in surviving cells is likely only one side of a transcriptional program which supplies tumor cells with nutrients (Perera et al., 2015). Collectively, autophagy has a role in maintaining sufficient supplies of energy and nutrient to tumors *via* tumor-cell-autonomous, stromal and systemic autophagy.

Autophagy induction is not only triggered by nutrient deficiency but also by low oxygen levels. Cellular adaptation to hypoxic conditions involves multiple mechanisms, such as upregulation of the unfolded protein response (UPR) (Rouschop et al., 2010). Hypoxia has been shown to increase transcription of the essential autophagy genes MAP1LC3B and ATG5 *via* the transcription factors ATF4 and CHOP, respectively. Notably, MAP1LC3B and ATG5 are not required for initiation of autophagy but are involved in phagophore expansion and autophagosome formation. Furthermore, autophagy and MAP1LC3B induction have been shown to mostly occur in hypoxic regions of tumor xenografts. Pharmacological inhibition of autophagy sensitizes human tumor cells to hypoxia and decreases the proportion of viable hypoxic tumor cells and sensitizes tumor xenografts to

irradiation. Collectively, these data suggest that the UPR is an important mediator of the hypoxic tumor microenvironment and that it contributes to resistance to treatment through its ability to facilitate autophagy.

Hypoxia is involved in tumorigenesis, associated with altered metabolism, abnormal vascularization, resistance to chemo/radiotherapy, and increased cancer cell stemness and may even promote metastasis (Wilson and Hay, 2011; Yun and Lin, 2014; Horsman and Overgaard, 2016; Minassian et al., 2019). In response to hypoxia, the transcription factor hypoxia-inducible factor 1 α (HIF1 α), activates a variety of target genes that are involved in altered metabolism, cell survival and tumor progression (Kaelin, 2011; Masson and Ratcliffe, 2014; Chen and Sang, 2016). Both hypoxia and anoxia, with oxygen concentrations <3% and <0.1%, respectively, cause autophagy through a variety of different mechanisms (Kroemer et al., 2010). Hypoxia-induced autophagy depends on hypoxia-inducible factor, HIF, while anoxia-induced autophagy is HIF-independent (Majmundar et al., 2010; Mazure and Pouyssegur, 2010). HIF is a heterodimer of a constitutive β subunit and an oxygen-regulated α subunit that only becomes stabilized (and hence expressed) when oxygen concentration declines below a threshold of ~5%. Under moderate hypoxia (1–3% oxygen), HIF activates the transcription of *BNIP3* and *BNIP3L* (NIX), two BH3-only proteins that can disrupt the inhibitory interaction between Beclin 1 and Bcl-2 (Bellot et al., 2009). Moreover, *BNIP3L*, which often is present at the outer surface of mitochondria, possesses a WXXL motif that binds to LC3 and its homolog GABARAP (Novak et al., 2010), thereby targeting mitochondria for autophagic destruction. The transcription of *BNIP3* is also upregulated by the transcription factor FOXO3, on condition that it is deacetylated by Sirt1 (Kume et al., 2010).

Under severe hypoxia or anoxia, additional pathways including the protein DJ-1, the autocrine stimulation of a PDGFR-dependent pathway, the stimulation of AMPK through metabolic stress, and the UPR of the ER have been demonstrated to play role in hypoxia-induced autophagy (Mazure and Pouyssegur, 2010). Hypoxia-mediated upregulation of autophagy also requires phosphorylation of eIF2 α mediated by PERK (see below), further highlighting the significance of the phosphorylation of eIF2 α as a universal autophagy regulator (Rouschop et al., 2010). Lastly, hypoxia has been shown to upregulate the transcription of the key autophagy genes, *LC3* and *Atg5*, *via* ATF4 and CHOP transcription factors, respectively, which are both regulated by PERK (Rouschop et al., 2010).

AUTOPHAGY AND THE INTEGRATED STRESS RESPONSE

The integrated stress response (ISR) is an evolutionarily conserved cellular stress response in eukaryotic organisms that inhibits global protein biosynthesis and activates the expression of specific genes in response to extrinsic environmental factors and intrinsic pathophysiological stresses (Pakos-Zebrucka et al.,

2016). Extrinsic stress factors include hypoxia, starvation (e.g., amino acid deprivation, glucose deprivation), viral infection, and presence of oxidants. One of the primary intrinsic factors is endoplasmic reticulum (ER) stress which results from increased levels of unfolded proteins and polypeptides in the ER. It is now well established that oncogene activation can also activate the ISR. Activation of the ISR will either stimulate the expression of specific genes to restore cellular homeostasis by resolving cellular damage caused by these stressors, or, if unable to restore homeostasis, activate programmed cell death (apoptosis) (Pakos-Zebrucka et al., 2016).

Many of the stress signaling pathways converge on eIF2 α . Phosphorylation of this transcription factor subsequently initiates the ISR, but outcome of ISR activation can be quite different and depends not only by the type of the stressor but also its extent and severity. This influences the duration of the phosphorylation of eIF2 α as well as translation of ATF4 and other bZIP transcription factors (Dey et al., 2010; Guan et al., 2014). For example, a short duration of ISR activity appears to be an adaptive, pro-survival response to various stresses aimed at overcoming the stress and restoring homeostasis, whereas activation of ISR for an extended period can induce the cell to programmed cell death (Rutkowski et al., 2006). However, this dual action of eIF2 α phosphorylation requires further elucidation.

It has been widely accepted that the ISR can regulate cell survival and cell death pathway *via* induction of autophagy which facilitates the degradation of unfolded proteins, polypeptides or protein aggregates, and damaged organelles. As a result, autophagy restores depleted amino acids pool for protein synthesis and reenergizes a starved cell restoring homeostasis. Although mechanisms by which phosphorylated eIF2 α induces autophagy are still being explored, similar extrinsic and intrinsic stress signals leading to phosphorylation of eIF2 α have been shown to activate autophagy. For example, ER stress-induced phosphorylation of eIF2 α phosphorylation has been shown to upregulate a number of autophagy receptors such as SQSTM1, NBR1, and BNIP3L *via* PERK (Deegan et al., 2013). Furthermore, pharmacologic suppression of PERK represses transcriptional upregulation of these autophagy receptors (Deegan et al., 2015). Likewise, eIF2 α phosphorylation-mediated by PERK upregulates the conversion of ATG12 and LC3 as a result of expression of polyQ72 aggregates, which is an important phase for the formation of autophagy (Kouroku et al., 2007). Consequently, the PERK-driven Unfolded Protein Response (UPR) regulates autophagy process from induction, to vesicle nucleation, phagophore elongation, and maturation (Deegan et al., 2013). The UPR, which is initiated in the setting of accumulation of misfolded proteins in the ER, is predominantly an adaptive response to the activation of the ISR. UPR protects cancer cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5 (Rouschop et al., 2010) and this is facilitated by PERK phosphorylation of eIF2 α . On the other hand, elimination of PERK signaling or expression of mutant eIF2 α S51A which cannot be phosphorylated under hypoxia decreases the transcription of MAP1LC3B and ATG5 (Rouschop et al., 2010).

Amino acid deprivation in cancer cells also promotes the phosphorylation of eIF2 α *via* GCN2, a protein essential for the activation of autophagy (Ye et al., 2010). GCN2 knockout cells exhibit decreased LC3 expression, whereas cells with mutant the eIF2 α S51A cannot induce LC3 processing (Ye et al., 2010). Similarly, phosphorylation of eIF2 α at S51 was found to be essential for regulation of autophagy induced by amino acid starvation in yeast and mouse embryonic fibroblasts (MEFs) (Tallóczy et al., 2002).

Critically, ATF4, which is essential for activation of autophagy, is downstream of eIF2 α (Kroemer et al., 2010). ATF4 activation in response to stress signals induced by amino acid deprivation upregulates several autophagy genes transcriptionally including *Atg3*, *Atg5*, *Atg7*, *Atg10*, *Atg12*, *Atg16*, *Becn1*, *Gabarap*, *Gabarapl2*, *Map1lc3b*, and *Sqstm1* (B'Chir et al., 2013). In addition, ATF4 mediates REDD1, which represses the activity of mTORC1 under conditions of ER stress or amino acid deprivation, subsequently inducing autophagy (Whitney et al., 2009; Rzymiski et al., 2010; B'Chir et al., 2013; Dennis et al., 2013; Deegan et al., 2015). Notably, several autophagy genes may have a varying magnitude of dependence on ATF4 and CHOP signaling and the transcriptional activation of these genes is controlled by the ratio of ATF4 and CHOP proteins that are bound to a particular promoter suggesting that the level of expression of autophagy genes depend on the needs of the cell (B'Chir et al., 2013).

Notably, a conditionally active form of the eIF2 α kinase PKR functions upstream of PI3K and activates the Akt/PKB-FRAP/mTOR pathway leading to the phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and that stimulation of PI3K signaling antagonizes the apoptotic and protein synthesis suppressive effects of the conditionally active PKR (Kazemi et al., 2007; Showkat et al., 2014). Furthermore, pharmacologic suppression of proteasome function with antineoplastic agent bortezomib results in depletion of amino acids in the ER required for protein synthesis leading to the activation of the ISR *via* GCN2 stress sensor (Suraweera et al., 2012). These findings suggest that proteasome inhibition has a role on survival signaling by the ISR. Moreover, amino acid depletion mediated by proteasome inhibition also induces autophagy through mTOR in an attempt to restore amino acid homeostasis (Suraweera et al., 2012), whereas, supplementation of essential amino acids depleted by the inhibition of proteasome function impairs the phosphorylation of eIF2 α and down-regulates autophagy (Suraweera et al., 2012). Thus, depletion of amino acids by proteasome inhibition forms a connection between ISR activation and activation of autophagy to sustain cell survival.

Therefore, PERK, which facilitates the phosphorylation of eIF2 α and inducing the ISR, acts alongside the different components of the UPR, IRE1, and ATF6 to suppress proteotoxicity induced by misfolded proteins and polypeptides. This is accomplished by upregulating the transcription of genes that stimulate proper protein folding and increase degradation of misfolded or aggregated proteins (Harding et al., 2000; Liu et al., 2000), as such, the cross talk between the various components of

the UPR regulates the cellular outcome (Szegezdi et al., 2006). The ISR-mediated cell survival during ER stress indicates that ATF4 acts as a hub connecting PERK-mediated translational control with IRE1- and ATF6-mediated gene expression (Ron, 2002). Strikingly, the relative extent of PERK and IRE1 signaling appears to be critical for determining the cell fate, with the constant stimulation of PERK leading to activation of programmed cell death (i.e., apoptosis) and extended duration of activation of IRE1 leading to cell survival (Lin et al., 2007; Lin et al., 2009).

AUTOPHAGY AS A MECHANISM OF RESISTANCE TO ANTICANCER THERAPY

Tumor cell activation of autophagy has been described as a potential mechanism of resistance to anticancer therapy. This is supported by several *in vitro* studies demonstrating that further augmentation of autophagic flux results in increased resistance to chemotherapy, resistance that can be overcome with inhibition of autophagy (Sotelo et al., 2006; Carew et al., 2007; Firat et al., 2012; Hu et al., 2012; Zou et al., 2012). In pancreatic cancer, inducing autophagy through upregulation of receptor for advanced glycation end products (RAGE) increases resistance to chemotherapy *in vivo* (Kang et al., 2010). Although further studies are necessary to elucidate precise mechanisms of resistance, autophagy-induced activation of several common cell signaling pathways have been described. These include epidermal growth factor receptor (EGFR), PI3K/AKT/mTOR, MAPK, and p53 pathways. Han et al. demonstrated that inhibiting EGFR with either gefitinib or erlotinib not only activates autophagy but also serves as a cytoprotective mechanism in human lung cancer. They further combined these tyrosine kinase inhibitors with various autophagy inhibitors or siRNAs targeting ATG5/7 and demonstrated enhanced cell killing (Han et al., 2011). As described earlier in this review, inhibition of the MAPK pathway also leads to upregulation of autophagy and has been proposed as a mechanism of drug resistance. Furthermore, PI3K/mTOR inhibitors have been shown to induce protective autophagy in malignant peripheral nerve sheath tumor (MPNST) cells; however, pretreatment with chloroquine or bafilomycin consistently reverses this, potentially representing a treatment strategy in this difficult to treat sarcoma subtype (Ghadimi et al., 2012). The reciprocal interaction between autophagy and p53 may also have important implications for cancer therapy. Autophagic flux increases suppression of p53 while p53 activates autophagy (White, 2016). Autophagy inhibition alone is unlikely sufficient to overcome autophagy-induced resistance to anticancer therapy, however, a deeper understanding of autophagy in this setting may lead to new therapeutic approaches.

ONC212, AUTOPHAGY AND PDAC

Our work unraveling cell death pathways (Carneiro and El-Deiry, 2020) as an approach to understand and therapeutically target human cancer led us to discover TRAIL receptor DR5 as a p53

target gene (Wu et al., 1997). We discovered that the Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), the ligand for DR5 in the extrinsic cell death pathway is also a p53-regulated gene (Kuribayashi et al., 2008). We performed screening for TRAIL-inducing compounds in 2007 and discovered TRAIL-Inducing Compound #10 (TIC10), later published in 2013 (Allen et al., 2013). TIC10 activated the TRAIL gene in a p53-independent manner that involved dual inhibition of ERK and Akt and nuclear translocation of Foxo3a to bind and transactivate the TRAIL gene (Allen et al., 2013). TIC10 was advanced to clinical trials as ONC201 (Stein et al., 2017). We discovered that ONC201/TIC10 activates the integrated stress response (ISR) through kinases HRI and PKR leading to eIF2- α phosphorylation, activation of ATF4, CHOP, and DR5 (Kline et al., 2016). We found that ONC201 targets cancer stem cells (Prabhu et al., 2015) and activates an immune response involving natural killer (NK) cells (Wagner et al., 2018). We collaborated with Provid and Oncocotics to synthesize and test ONC201/TIC10 analogues and uncovered ONC212 as a potent analogue (Wagner et al., 2017).

ONC212 appeared to have efficacy against PDAC cells and xenografted tumors *in vivo* (Lev et al., 2017). ONC212 was found to target the integrated stress response and activate the TRAIL pathway. Moreover, the compound appears to act through a mechanism involving mitochondrial caseolytic protease ClpP which targets degradation of multiple mitochondrial proteins including respiratory chain proteins involved in oxidative phosphorylation (Ferrarini, 2021). The mitochondrial stress signals the integrated stress response leading to cell death and also inhibits autophagy in pancreatic cancer (Ferrarini, 2021). As efforts are underway to bring ONC212 to clinical trials, we have been exploring combinations with ONC212 in pancreatic cancer (Jhaveri, 2020; Raufi, 2021). In particular, ONC212 appears to synergize with MEK inhibitors against PDAC cell lines, in part through effects involving autophagy inhibition (Raufi, 2021).

AUTOPHAGY, IMMUNE CELL FUNCTION AND RESPONSE TO IMMUNE CHECKPOINT BLOCKADE

PDAC is characterized by a unique and complex tumor immune microenvironment comprised of distinct stromal and tumor compartments. The stromal compartment contains cancer associated fibroblasts (CAFs), as well as both innate and adaptive immune cells. Autophagy is necessary for immune cell function, differentiation, and survival and therefore a thorough understanding of the impact of autophagy modulating agents on these cells is essential to developing new therapies.

Autophagy is required for pluripotent hematopoietic stem cell (HSC) survival and differentiation (Mortensen et al., 2011). HSCs give rise to monocytes, which differentiate into macrophages with phagocytic and cytokine production capabilities. Autophagy has been shown to be essential for monocyte survival as well as their differentiation into macrophages (Jacquel et al., 2012; Zhang et al., 2012). In mature macrophages, autophagy plays a role

in LC3-mediated phagocytosis, a form of non-canonical autophagy that promotes immune tolerance (Cunha et al., 2018). The breakdown of biomolecules during autophagy also mediates antigen presentation by dendritic cells (Li et al., 2012; Germic et al., 2019). Interestingly, autophagy inhibition-mediated tumor regression can be hindered by macrophage depletion in an autochthonous mouse model of PDAC, suggesting an essential role of the innate immune system in tumor cell killing (Yang et al., 2018).

Autophagy is also essential for adaptive immune cell function, as it supports T cell renewal, differentiation, and homeostasis. In the thymus, negative selection of CD4⁺ T cells is at least partially directed by autophagy and the transition of CD4⁺CD8⁻ cells to CD4⁺CD8⁺ cells is associated with maximum activation of autophagy, though its explicit role in this transition is incompletely understood. Autophagy also mediates T cell survival and differentiation outside the thymus. Upon autophagy inhibition, T cells accumulate organelles and shift their metabolism from oxidative phosphorylation to glycolysis. Cells that generate energy predominantly through oxidative phosphorylation [memory T cells, T regulatory cells (T-regs)] are particularly vulnerable to autophagy inhibition. The vulnerability of T-regs to autophagy inhibition is further enhanced due to their dependence on high levels of autophagy (Clarke and Simon, 2019). However, autophagy inhibition also degrades extracellular ATP and attracts T-regs. This mechanism likely plays an important role *in vivo*, as triggering autophagy in lung tumor-bearing mice improved the efficacy of chemotherapy and this was at least partially mediated by a reduction of tumor-infiltrating T-regs (Pietrocola et al., 2016).

Interest in immune checkpoint blockade (ICB) has increased in recent years following clinical success in treating various malignancies. Single agent ICB has had little to no impact on outcomes in patients with PDAC. This may be partly due to the immunosuppressive components of the tumor immune microenvironment therefore there is much interest in identifying combination treatments that improve responses to ICB (Bian and Almhanna, 2021). A recent compelling study reported that autophagy promotes immune evasion of PDAC *via* MHC-I degradation, and that autophagy inhibition and ICB synergize in mice to reduce tumor burden (Yamamoto et al., 2020). Similar observations in other cancer types support these findings. For example, mice with metastatic liver tumors experience an enhanced response to high dose IL-2 when combined with an autophagy inhibitor (Liang et al., 2012), and impairment of autophagy in mice with colon or breast tumors improved response to ICB therapy (Young et al., 2020). Together, these findings suggest a role of autophagy in limiting the response of immunotherapies such as ICB across cancer types and provide an exciting new direction for investigating combination treatments for PDAC and other cancers.

PRECLINICAL STUDIES IN PDAC

The relationship between autophagy and tumor progression is complex. First, autophagy has been shown to suppress cancer

initiation in many models. As described above, Rosenthal et al. showed that genetically modified mice with loss of autophagy genes Atg5 or Atg7 showed increased benign pancreatic cell tumor formation, but with lack of progression to malignant disease. Other genetically-modified mouse models have shown similar results in liver (Takamura et al., 2011) and lung (Strohecker et al., 2013) tumors. Additionally, there is evidence that once the growth of a malignant tumor has been initiated, autophagy promotes tumor progression. Degenhardt et al. explored the impact of autophagy on the tumor immune microenvironment and showed that autophagic activity is increased in the hypoxic tumor microenvironment, which ultimately leads to increased degradation of waste products resulting in decreased inflammation and increased tumor cell survival. This was further supported by Guo et al. who found that autophagy knock-out xenografts in a KRAS-activated mouse model showed reduced tumor growth and also exhibited an increased immune response, leading to the development of immune-driven pathologies, such as pneumonia (Guo et al., 2013). Levy et al. explored the potential role of this hyperactivated immune response in reduced tumor induction and growth in an autophagy knock-out model, and postulated that reduced induced autophagy in T cells may lead to more T cell-induced tumor cell killing (Mulcahy Levy and Thorburn, 2020). Lastly, evidence suggesting that autophagy is important for malignant cell growth can also be found at the genetic level. Transcriptome analysis has shown that core autophagy proteins highly conserved in cancer (Lebovitz et al., 2015) and that many of the transcription factors that promote autophagy are oncogenes (Rocznik-Ferguson et al., 2012).

Early pre-clinical investigations focused on the use of hydroxychloroquine and chloroquine, which act through inhibiting lysosomes, which in part leads to degradation of autophagosomes and endosomes (Dolgin, 2019). PDAC is an attractive solid tumor for autophagy inhibition, as autophagy is known to be increased in pancreatic cancer, and has been shown to correlate to poorer patient outcomes (Fujii et al., 2008). Friboes et al. showed that treatment of a malignant pancreatic cancer line with chloroquine lead to decreased cell viability and decreased levels of autophagy (Friboes et al., 2014). Yang et al. showed decreased tumor progression in an *in vitro* model when cells grown from pancreatic cancer tumors grown in genetically modified mice were treated with chloroquine (Yang and Kimmelman, 2014). Because the mechanism of action of chloroquine and hydroxychloroquine is targeted at lysosomes, and therefore not specific to the inhibition of autophagy, it is difficult to determine to what degree autophagy inhibition actually contributes to their overall mechanism of action in cancer therapy. In 2014 Maes et al. examined the use of hydroxychloroquine and chloroquine against melanoma tumor cells in an *in vivo* model and showed that treatment with these drugs leads to a normalization of the organization of tumor vessel and function, thereby decreasing hypoxia and increasing delivery of other drugs, which could certainly contribute to their antitumor effect (Maes et al., 2014). Another small molecule target for autophagy inhibition is the molecule of the PI3K class III that is known to be important in the promotion of autophagy,

and has been shown to be effective at blocking autophagy *in vivo* (Dowdle et al., 2014). Ronan et al. developed an inhibitor specific to this molecule that was shown to act synergistically with everolimus in lung and renal cancer *in vitro* (Ronan et al., 2014), and Honda et al. discovered an inhibitor shown to be effective against colorectal cancer as monotherapy in an *in vivo* model (Honda et al., 2016). This molecule also has issues with specificity. In addition to contributing to the activation of autophagosomes, it is also involved in endocytic and vesicular function, and therefore has produced concern for toxic off-target effects (Dolgin, 2019). Alternatively, many investigators are focusing in on the autophagy activating kinase ULK1. Lazarus et al. performed a structural activity relationship analysis of ULK1 in order to identify binding sites of the molecule most ideal structure of a drug to bind to and inhibit these sites (Lazarus et al., 2015; Lazarus and Shokat, 2015). Egan et al. went further to discover a specific substrate that exhibits potent and highly selective inhibition of ULK1 in an *in vitro* model, and showed that it induced increased cell death in glioblastoma and lung cancer cells when used in concert with mTOR inhibition (Tang et al., 2017).

As autophagy has been shown to be important in both blocking the initiation of tumor formation as well as potentiating the spread of tumors when growth has already been initiated, there has been interest in studying autophagy-activating drugs to treat PDAC. mTOR inhibitors have therefore been studied in several but have been shown to only lead to a cytostatic effect. In a review from 2019, Tian et al. (2019) postulate that this result is due to the ability of mTOR inhibitors to lead to optimization of the tumor microenvironment, and that this could be inhibited with the addition of an autophagy inhibitor, which could explain synergy seen in pre-clinical models that have examined dual therapy with mTOR inhibitors and autophagy inhibitors, such as the results that Ronan et al. saw when combining everolimus with and VSP34 inhibitor.

As previously mentioned, ONC212 is a novel potent imipridone analogue with preclinical activity against PDAC in multiple *in vivo* models, biochemical evidence of autophagy inhibition, and synergistic activity when combined with MEK inhibitors (Lev et al., 2017; Wagner et al., 2017; Ferrarini, 2021; Rauffi, 2021). As p53 mutations are common in human cancer, including PDAC, we have pursued therapeutic targeting of tumors with mutant p53 (Wang et al., 2006; Bassett et al., 2008; Hernández Borrero and El-Deiry, 2021). We previously reported that a p53 pathway restoring small molecule, CB002, induces morphological changes of autophagy and modulates LC3B expression in a manner that requires pro-apoptotic Noxa induction (Richardson et al., 2017; Hernandez-Borrero et al., 2018). Our recent results suggest that in addition to partial restoration of a p53 transcriptome, CB002 and other xanthine analogues impact on an S-phase cell cycle checkpoint (Hernandez Borrero et al., 2021). These small molecular weight compounds and others such as PG3-Oc and NSC59984 that restore p53 pathway responses merit further investigation as potential therapeutics in PDAC (Zhang et al., 2015; Prabhu et al., 2016; Zhang, 2017a;

Zhang, 2017b; Zhang, 2018; Hernandez Borrero et al., 2021; Tian et al., 2021).

Mutations of the oncoprotein KRAS are very common in pancreatic cancer, and therefore there has always been a great deal of interest in targeting the MAPK pathway in the treatment of pancreatic cancer, but while there have been some promising pre-clinical results, KRAS inhibitors have shown to be relatively ineffective at treating pancreatic cancer in humans. Kinsey et al. established that inhibition of the MAPK pathway also leads to up-regulation of autophagy, which has been postulated as serving as a mechanism of drug resistance (Kinsey et al., 2019). Therefore, dual inhibition of the MAPK pathway and autophagy could theoretically lead to synergistic cell death. The combination proved synergistic in PDAC cell lines *in vitro* as well as in patient-derived xenografts grown in a murine model, as well as in melanoma and colorectal cancer models. Bryant et al. also examined the relationship between the MAPK pathway and autophagy and showed not only that dual inhibition of these pathways leads to increased cell death in PDAC cell lines, but also shed light on the mechanism of this synergy (Bryant et al., 2019) by showing that inhibition of two key members of the MAPK pathway—KRAS and ERK—lead to decreased metabolic functions, and would therefore lead to an increased dependence on autophagy to avoid cell death.

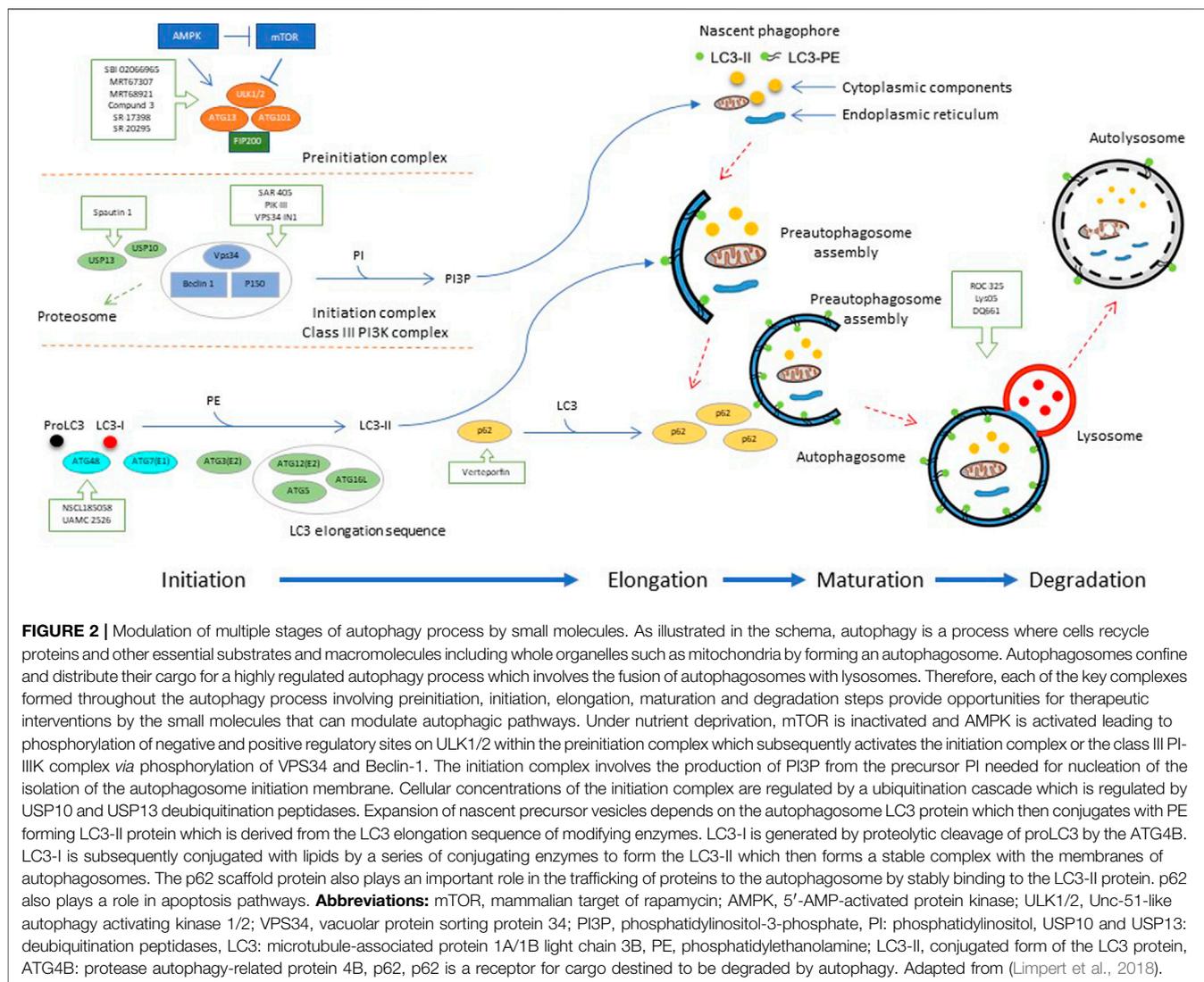
In summary, there is a breadth of literature examining the impact of autophagy on cancer initiation and growth. These studies have shown that the relationship between tumorigenesis and metastasis is complex, providing both pro- and anti-tumor effects. With this knowledge, various researchers have focused on both the inhibition and activation of autophagy. Harnessing the anti-tumor effect of autophagy inhibition has been attempted both *via* the use of existing drugs with broad mechanisms of action, such as chloroquine or hydroxychloroquine, as well as through the development of new targets to inhibit autophagy, such as VSP34 and ULK1 inhibitors. Likewise, other researchers have focused on promoters of autophagy, and have shown good effect with dual therapy with autophagy inhibitors. Lastly, it has been shown that dual targeting of the MAP kinase pathway and the autophagy pathway—especially in cancer with a high prevalence of KRAS mutation, such as pancreatic cancer—may result in increased tumor killing by inhibitors of the MAP kinase pathway by blocking autophagy, which could serve as a key mechanism of resistance.

A diagrammatic representation depicting modulation of the autophagy pathway by small molecules is shown in **Figure 2**. A list of compounds with activity as autophagy inhibitors is shown in **Table 1**.

Clinical Trials in PDAC

Various modulators of autophagy have been tested either alone or in combination with other agents in clinical trials for patients with PDAC. Chloroquine, and its less toxic derivative, hydroxychloroquine, are the among the best studied inhibitors of autophagy.

Hydroxychloroquine has been evaluated as a single agent in a phase II study published in 2014. In this study, 20 patients with previously treated metastatic PDAC received twice daily hydroxychloroquine, either 400 mg or 600 mg. Unfortunately,



no patient demonstrated a response (Wolpin et al., 2014). In 2017, the results of a phase I trial combining chloroquine with standard of care gemcitabine were published. Although three out of nine enrolled patients had partial responses and a median overall survival (OS) of 7.6 months was reported, this did not outperform historical data with gemcitabine alone (Samaras et al., 2017). More recently, the results of a randomized phase II study of the combination of standard of care gemcitabine and nab-paclitaxel with or without hydroxychloroquine were published in 2019. In total, 112 patients with previously untreated metastatic or advanced PDAC were enrolled and were randomized 1:1. The primary endpoint was OS at 1 year. The addition of hydroxychloroquine resulted in a 12 months OS rate of 41% (95% CI, 27–53%) compared with 49% (95% CI, 35–61%) with chemotherapy alone. Furthermore, the authors reported no increase in progression free survival and there was a higher rate of toxicity, visual and gastrointestinal, in the hydroxychloroquine treatment group. Interestingly, the authors did report an improvement in overall response rate, 38.2% (n =

21) in the hydroxychloroquine group versus 21.1% (n = 12) in the non-hydroxychloroquine group, which was statically significant ($p = 0.047$) (Karasic et al., 2019).

Several studies have also investigated the role of autophagy promoting agents. The oral mTOR inhibitor everolimus has been studied in a phase II study in patients with gemcitabine-refractory metastatic pancreatic cancer. No complete or partial treatment responses were noted in this trial and the median progression-free survival and OS were 1.8 and 4.5 months, respectively. One patient (3%) had a biochemical response, defined as greater than or equal to 50% reduction in serum CA19-9 (Wolpin et al., 2009). Additional studies investigating single agent mTOR inhibitors have also been disappointing (Javle et al., 2010).

There are a number of ongoing clinical trials investigating novel autophagy-modulating agents and novel combinations of agents. For example, one trial is currently investigating newer combinations of chemotherapy (e.g., paclitaxel protein bound plus gemcitabine plus cisplatin) together with hydrochloroquine (NCT04669197). Hydroxychloroquine is also being combined

TABLE 1 | Selected compounds that modulate different phases of autophagy. Adapted from (Limpert et al., 2018).

Compound	Target	Novel features	Potency/Selectivity	Refs
SBI-0206965	ULK1 and ULK2	Selective inhibitor Pyrimidine scaffold Suppresses ULK1 downstream phosphorylation of VPS34 and Beclin-1 Induces apoptosis in NSCLC cells by destabilizing Bcl2 and Bclxl	ULK1: IC ₅₀ of 108 nM; ULK2: IC ₅₀ of 711 nM	Egan et al. (2015); Tang et al. (2017)
MRT67307	ULK1 and ULK2	<i>In vitro</i> inhibitor Pyrimidine scaffold Also targets TBK1 and AMPK-related kinases	ULK1: IC ₅₀ of 45 nM; ULK2: IC ₅₀ of 38 nM	Petherick et al. (2015)
MRT68921	ULK1 and ULK2	<i>In vitro</i> inhibitor Pyrimidine scaffold Also targets TBK1 and AMPK-related kinases	ULK1: IC ₅₀ of 2.9 nM; ULK2: IC ₅₀ of 1.1 nM	Petherick et al. (2015)
Compound 1	ULK1 and ULK2	Inhibitor Pyrazole amino quinazoline scaffold Crystal structure obtained with ULK1	ULK1: IC ₅₀ of 5.3 nM; ULK2: IC ₅₀ of 13 nM; PDPK1: IC ₅₀ of 420 nM	Lazarus and Shokat, (2015)
BX-795	PDK1	Inhibitor of PDK1 Also shown to inhibit ULK1, ULK2 and IKKε Pyrimidine scaffold	ULK1: IC ₅₀ of 87 nM; ULK2: IC ₅₀ of 310 nM; PDPK1: IC ₅₀ of 65 nM	Lazarus and Shokat, (2015)
Compound 3	ULK1	Inhibitor Pyrimidine scaffold Crystal structure obtained with ULK1	ULK1: IC ₅₀ of 120 nM; ULK2: IC ₅₀ of 360 nM; PDPK1: IC ₅₀ of 710 nM	Lazarus and Shokat, (2015)
SR-17398	ULK1	Indazole-derived inhibitor Mixture of four stereoisomers	ULK1: IC ₅₀ of 22 μM	Wood et al. (2017)
SR-20295	ULK1	Indazole-derived inhibitor	ULK1: IC ₅₀ of 45 nM	Wood et al. (2017)
NSC185058	ATG4B	Inhibitor/antagonist Targets autophagosome formation, and suppresses activation and lipidation of LC3	ATG4B IC ₅₀ of 51 μM	Akin et al. (2014); Huang et al. (2017)
UAMC-2526	ATG4B	Inhibitor Benzotropolone scaffold Targets autophagosome formation Inhibits starvation-induced autophagy <i>in vivo</i>	Plasma half-life of 126 min, and 70% metabolization after 30 min	Kurdi et al. (2017)
SAR405	VPS34	Selective inhibitor Tetrahydropyrimido-pyrimidinone scaffold Dose-dependent inhibition Targets autophagosome formation Crystal structure obtained with VPS34	VPS34: IC ₅₀ of 1.2 nM and K _D of 1.5 nM	Ronan et al. (2014); Young et al. (2015); Hong et al. (2017)
PIK-III	VPS34	Selective and orally bioavailable inhibitor of VPS34 Pyrimidine scaffold	VPS34: IC ₅₀ of 18 nM; mTOR: IC ₅₀ of >9.1 μM	Dowdle et al. (2014); Honda et al. (2016)
VPS34-IN1	VPS34	Inhibits autophagy and LC3 lipidation Selective cell-permeable inhibitor Pyrimidine scaffold Selectively inhibits class III PI3K	VPS34: IC ₅₀ of 25 nM <i>in vitro</i>	Bago et al. (2014)
Verteporfin	ATG	Concentration-dependent inhibition Benzoporphyrin scaffold Targets autophagosome formation and accumulation when co-treated with CQ Targets p62: prevents autophagy-induced degradation of p62 in nutrient-deprived conditions	CQ-verteporfin EGFP-LC3 cell IC ₅₀ of 1 μM Plasma concentrations after single intraperitoneal dose of 45 mg/kg: 122 μM at 2 h, 3.9 μM at 24 h	Donohue et al. (2011); Donohue et al. (2013); Donohue et al. (2014)
Spautin-1	ATG	Autophagy inhibitor Fluoroquinazoline scaffold USP10 and USP13 inhibitor: promotes ubiquitination and decreases levels of Beclin-1 Targets autophagosome formation when co-treated with imatinib mesylate Spautin-1 alone has no activity	Co-treatment with Spautin-1 improved imatinib mesylate-induced cytotoxicity of K562 leukemia cells: IC ₅₀ from 1.03 to 0.45 μM	Shao et al. (2014)
ROC-325	ATG	Orally bioavailable inhibitor Chloroquinoline scaffold Targets lysosomal function and autophagosome accumulation ~10-fold more potent than HCQ Exhibits significant anticancer activity against range of tumor types	Acute myeloid leukemia cell IC ₅₀ range: 0.7–2.2 μM; A498 renal cell: IC ₅₀ of 4.9 μM	Nawrocki et al. (2016); Carew et al. (2017); Carew and Nawrocki, (2017)

(Continued on following page)

TABLE 1 | (Continued) Selected compounds that modulate different phases of autophagy. Adapted from (Limpert et al., 2018).

Compound	Target	Novel features	Potency/Selectivity	Refs
Lys05	ATG	Autophagy inhibitor Dimeric chloroquinoline scaffold Targets lysosomal function	LN229 (glioma), 1205Lu (melanoma), c8161 (melanoma), HT-29 (colon) cell: IC ₅₀ range 4–8 μM	Amaravadi and Winkler, (2012); McAfee et al. (2012)
DQ661	ATG	Inhibitor of autophagy and mTOR by targeting PPT1 Dimeric quinacrine scaffold <i>In vivo</i> activity against melanoma, pancreatic cancer, and colorectal cancer tumor growth in mice Can be used in combination with chemotherapy	Estimated A375P melanoma cell IC ₅₀ of ~0.1 μM	Rebecca et al. (2017); Nicastri et al. (2018)

TABLE 2 | Clinical trials of autophagy inhibitors of pancreatic cancer. Source: clinicaltrials.gov.

Title	Status	Interventions	Url	NCT number
A phase I/II/Pharmacodynamic Study of Hydroxychloroquine in Combination With Gemcitabine/Abraxane to Inhibit Autophagy in Pancreatic Cancer	Active, not recruiting	Drug: Hydroxychloroquine (HCQ) Drug: Gemcitabine Drug: Abraxane	https://clinicaltrials.gov/ct2/show/NCT01506973	NCT01506973
LY3214996 ^{+/−} HCQ in Pancreatic Cancer	Recruiting	Drug: Hydroxychloroquine Sulfate Drug: LY3214996	https://clinicaltrials.gov/ct2/show/NCT04386057	NCT04386057
Binimetinib and Hydroxychloroquine in Treating Patients With KRAS Mutant Metastatic Pancreatic Cancer	Recruiting	Drug: binimetinib Drug: Hydroxychloroquine	https://clinicaltrials.gov/ct2/show/NCT04132505	NCT04132505
Paricalcitol and Hydroxychloroquine in Combination With Gemcitabine and Nab-Paclitaxel for the Treatment of Advanced or Metastatic Pancreatic Cancer	Recruiting	Drug: Gemcitabine Drug: Hydroxychloroquine Drug: Nab-paclitaxel Drug: Paricalcitol	https://clinicaltrials.gov/ct2/show/NCT04524702	NCT04524702
Randomized phase II Trial of Pre-Operative Gemcitabine and Nab Paclitaxel With or With Out Hydroxychloroquine	Completed	Drug: gemcitabine Drug: abraxane Drug: hydroxychloroquine	https://clinicaltrials.gov/ct2/show/NCT01978184	NCT01978184
Short Course Radiation Therapy With Proton or Photon Beam Capecitabine and Hydroxychloroquine for Resectable Pancreatic Cancer	Active, not recruiting	Drug: Capecitabine Drug: Hydroxychloroquine Radiation: Proton or Photon Radiation Therapy	https://clinicaltrials.gov/ct2/show/NCT01494155	NCT01494155
Study of Combination Therapy With the MEK Inhibitor, cobimetinib, Immune Checkpoint Blockade, atezolizumab, and the AUTOphagy Inhibitor, Hydroxychloroquine in KRAS-mutated Advanced Malignancies	Recruiting	Drug: cobimetinib Drug: Hydroxychloroquine Drug: atezolizumab Drug: Hydroxychloroquine Drug: atezolizumab	https://clinicaltrials.gov/ct2/show/NCT04214418	NCT04214418
Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer	Recruiting	Drug: Hydroxychloroquine Drug: trametinib	https://clinicaltrials.gov/ct2/show/NCT03825289	NCT03825289
Phase II Study of Paclitaxel Protein Bound + Gemcitabine + Cisplatin + Hydrochloroquine as Treatment in Untreated Pancreas Cancer	Recruiting	Drug: Paclitaxel protein bound Drug: Gemcitabine Drug: Cisplatin Drug: Hydroxychloroquine	https://clinicaltrials.gov/ct2/show/NCT04669197	NCT04669197

with the vitamin D analogue, paricalcitol, and chemotherapy in a phase II trial (NCT04524702).

As discussed above, there is also interest in combining autophagy inhibitors with agents targeting the MAPK pathway. For example, two ongoing trials with two different MEK inhibitors, trametinib or binimetinib, combined with hydroxychloroquine are currently being tested in patients with PDAC (NCT03825289, NCT04132505). LY3214996, an ERK inhibitor, is currently being tested alone and in combination with hydroxychloroquine in a small phase two study (NCT04386057). The combination of the MEK inhibitor

cobimetinib and hydroxychloroquine are also being tested in combination with immune checkpoint blockade in a phase I/II trial KRAS-mutated PDAC (NCT04214418).

A listing of clinical trials employing autophagy inhibitors is listed in **Table 2**.

DISCUSSION

Recent advances in our understanding of autophagy and evidence suggesting that it may be necessary for PDAC tumorigenesis,

maintenance, and metastasis has rekindled enthusiasm to target this process for therapeutic benefit. Development of effective therapies has been slow, in part due to the tremendous complexity and dynamic roles autophagy plays in both cell survival and cell death. No agent to date has demonstrated clear clinical benefit but ongoing trials will hopefully shed light on biological effects and emerging resistance pathways.

Traditionally, autophagy has been described as an adaptive mechanism through which cells facing stress or starvation are able to maintain viability. The role of autophagy in tumorigenesis is less clear, but we do know that established PDAC tumors rely on chronically elevated levels of basal autophagy. Furthermore, there is evidence that autophagy may also be required for metastasis. The unique PDAC tumor immune microenvironment represents a hypoxic, acidic, nutrient-poor setting in which autophagy has been repeatedly demonstrated to be upregulated. Adding further complexity is the fact that autophagy also plays a role in immune cell function and therefore, it is possible that modulating this process may impact immune response to cancer.

Several potential predictive biomarkers, such as ATG5 and LC3-II, are currently being studied and may help to ensure adequate dosing of autophagy targeting agents. Incorporation of biomarker studies into future clinical trials will be necessary to confirm utility.

With the identification of novel autophagy pathway components and the development of more specific pharmacologic agents, future trials will likely hold more promise. Recent preclinical data supporting combinatory therapy with MAPK pathway and autophagy inhibition with chloroquine has led to the activation of multiple clinical trials with these agents. Additional novel agents with preclinical activity such as ONC212, with the ability to inhibit autophagy, may be well-suited for further study in combination with MEK inhibitors or other agents in pancreatic cancer. Therapeutics targeting other molecular drivers in PDAC, such as mutant p53, may have future use in this disease. Further investigation

with improved preclinical models and biomarker directed clinical trials is warranted to further our understanding of autophagy modulation and ultimately improve outcomes in PDAC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Figure 1 created with BioRender.com.

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Conflict of Interest: WE-D is a co-founder of Oncocetics, Inc., a subsidiary of Chimerix, and a Founder of p53-Therapeutics. WE-D has disclosed his relationship with these companies and potential conflict of interest to his academic institution/employer and is fully compliant with NIH and institutional policy that is managing this potential conflict of interest.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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