Published in final edited form as:

Pharmacol Ther. 2011 July ; 131(1): 130–141. doi:10.1016/j.pharmthera.2011.03.009.

Targeting Autophagy During Cancer Therapy to Improve Clinical Outcomes

Jean M Mulcahy Levy¹ and Andrew Thorburn²

¹Department of Pediatrics University of Colorado 12801 E 17th Ave RC-1 South, Rm 6400D Mail Stop 8303 Aurora, CO, USA, 80045

²Department of Pharmacology University of Colorado 12801 E 17th Ave RC-1 South, Rm 6400D Mail Stop 8303 Aurora, CO, USA, 80045

Abstract

Autophagy is a catabolic process that turns over long-lived proteins and organelles and contributes to cell and organism survival in times of stress. Current cancer therapies including chemotherapy and radiation are known to induce autophagy within tumor cells. This is therefore an attractive process to target during cancer therapy as there are safe, clinically available drugs known to both inhibit and stimulate autophagy. However, there are conflicting positive and negative effects of autophagy and no current consensus on how to manipulate autophagy to improve clinical outcomes. Careful and rigorous evaluation of autophagy with a focus on how to translate laboratory findings into relevant clinical therapies remains an important aspect of improving clinical outcomes in patients with malignant disease.

Keywords

Autophagy; Cancer; Cancer therapy; Cell death; Clinical trial; Chloroquine

1. Introduction

Macroautophagy (hereafter referred to as autophagy) is a catabolic process that turns over long-lived proteins and organelles and contributes to cell and organism survival during nutrient deprivation and other stresses. It was first described as an important cellular process almost 50 years ago (Deter & De Duve, 1967), however there has only been a rapid increase in our understanding of autophagy in the last decade (Klionsky, 2007). Autophagy has been reported to be a tumor suppressor mechanism and it has been discovered that many anti-cancer treatments in clinical use today, as well as various therapies that are under investigation, also induce autophagy in tumor cells (Table 1). Therefore, there is great interest in manipulating autophagy to improve cancer treatment. However, as discussed below, conflicting positive and negative effects of autophagy have led to considerable disagreement about how to influence the process for improved clinical outcomes (Hippert, O'Toole, & Thorburn, 2006; Kondo, Kanzawa, Sawaya, & Kondo, 2005; Levine & Kroemer, 2008; Maiuri, et al., 2009). For example, many publications report autophagy as a tumor cell killing mechanism by diverse anti-cancer agents (Garcia-Escudero & Gargini, 2008; M. H. Lin, Liu, & Liu, 2008; Turcotte, Sutphin, & Giaccia, 2008). However, autophagy during tumor cell treatment has also been reported to inhibit tumor cell killing (Amaravadi, et al., 2007; Carew, et al., 2007; Park, et al., 2008; Thorburn, et al., 2009; H.

Wu, Yang, Jin, Zhang, & Hait, 2006). Thus, because there is evidence that autophagy can prevent or promote cancer and kill or protect cancer cells following treatment with anti-cancer drugs and radiation, we still have much to learn if we are to be able to rationally interfere with this process to improve outcomes for cancer patients.

This problem of not really knowing what we should do is especially acute right now because clinical trials modulating autophagy with FDA approved drugs in adult and pediatric cancer patients are already active (Table 2). Trials combining chemotherapy agents with the autophagy inhibitors chloroquine (CQ) or hydroxychloroquine (HCQ), are recruiting patients with breast cancer, multiple myeloma, prostate cancer and other advanced tumors with the underlying hypothesis that blocking autophagy will result in increased tumor cell kill. Conversely, several trials are currently using drugs shown to induce autophagy, particularly rapamycin and its analogues, although in most of those trials autophagy is not being assessed. Determining the role (or roles) of autophagy during cancer therapy is vital to developing trials that will result in improved long-term patient survival.

2. The process of autophagy

Autophagy is ubiquitous in eukaryotic cells and is a multi-step process involving initiation, autophagosome formation, maturation, and degradation controlled by a set of autophagy-related genes (ATG) (Figure 1) (Mizushima, 2007). The process starts with the activation of the unc-51-like kinase (ULK) serine/threonine kinase complex that includes ATG13 and FIP200. This complex is regulated by the mammalian target of rapamycin (mTOR) which senses nutrient levels in the environment and under high-nutrient conditions inhibits autophagy by phosphorylation of ULK1/2; during periods of nutrient deprivation mTOR dissociates from the ULK1/2 complex (Rosenfeldt & Ryan, 2009; Roy & Debnath, 2010; Xie & Klionsky, 2007). This allows dephosphorylation of ULK1/2 which, in turn, phosphorylates and activates ATG13 and FIP200. The initiation of autophagy is completed with the accumulation of the ULK1/2-ATG13-FIP200 complex, resulting in a site for development of the isolation membrane, also known as the phagophore (Jung, et al., 2009).

Like mTOR, AMP activated protein kinase (AMPK) is also a key sensor for cellular energy that is involved in the initiation of autophagy. During glucose starvation, AMPK inhibits mTOR, but it also directly interacts with ULK1, phosphorylating serines to activate autophagy. Interestingly, under amino acid starvation or pharmacologic mTOR inhibition with agents such as rapamycin, ULK1 is activated in an AMPK-independent manner (J. Kim, Kundu, Viollet, & Guan, 2011). AMPK phosphorylation of ULK1 and ULK2 is also associated with cellular mitophagy (autophagy that specifically targets mitochondria) and a loss of AMPK or ULK1 results in impaired autophagy and defective mitophagy (Egan, et al., 2011). Recent evidence has also identified a direct substrate of mTOR that negatively regulates autophagy. Death-associated protein 1 (DAP1) is inhibited by mTOR phosphorylation in nutrient rich conditions. Under starvation stress and loss of mTOR function, DAP1 is released from phosphorylation inhibition and suppresses autophagy. The exact mechanism through which DAP1 is able to suppress autophagy is unclear, though early evidence points towards an effect occurring between the mTOR complex and the LC3 conjugation systems. By activating and suppressing autophagy at the same time, this may create a counter-balance, or “gas and brake” method, to prevent over-activation of autophagy during starvation stress (Koren, Reem, & Kimchi, 2010a, 2010b). Since mTOR is a key negative regulator of autophagy, mTOR inhibitors (Rapamycin, Temsirolimus etc.) are potent activators of autophagy. Because these agents are being widely tested in cancer therapy, we are presumably routinely activating autophagy (both in the tumor and in normal tissues) in patients treated with these drugs.

The development of the autophagosome is dependent on a class III phosphoinositide 3-kinase (PI3K) complex involving the proteins Vps-34, beclin1 (itself a proposed tumor suppressor), and p150 (Simonsen & Tooze, 2009). This complex localizes to the phagophore and recruits further ATGs to allow for elongation and completion of the autophagosome. Studies have found that positive regulators binding to beclin1 include ATG14 (Itakura, Kishi, Inoue, & Mizushima, 2008), ultraviolet radiation resistance-associated gene (UVRAG) (C. Liang, et al., 2006) and Ambra (Fimia, et al., 2007). The Rubicon molecule has been identified as a negative regulator, binding to beclin1 and reducing Vps34 activity and impairing autophagosomes formation (Zhong, et al., 2009). Another significant negative regulator of autophagy is Bcl-2, which works by binding to the beclin1 BH3 domain. While the beclin1/Bcl-2 interaction does not necessarily alter the anti-apoptotic function of Bcl-2 (although this may vary depending upon the sub-cellular location of the Bcl-2 protein), it does influence the ability of beclin1 to stimulate autophagy (R. Kang, Zeh, Lotze, & Tang, 2011). Bcl-2 binds beclin1, stabilizing it in a dimer, blocking its ability to interact with Vps-34 and decreasing beclin1-associated class III PI3K activity (Noble, Dong, Manser, & Song, 2008; Pattingre, et al., 2005). Numerous Bcl-2 inhibitors are available, some in clinical trials including ABT-737 and (-)-gossypol (M. H. Kang & Reynolds, 2009) and although these drugs were developed with an eye to inducing tumor cell apoptosis, they have also been shown to induce autophagy through their ability to alter the Bcl-2 interaction with beclin1 (Lian, et al., 2011).

Once the PI3K complex is activated, elongation of the phagophore is controlled by two ubiquitin-like conjugation systems involving ATG12 and ATG8 (Ohsumi & Mizushima, 2004). In the first system, the E1-like ATG7 and E2-like ATG10 conjugate ATG12 to ATG5. This allows ATG12-ATG5 to bind with ATG-16, creating a complex that localizes to the outer surface of the membrane. The second system involves ATG8 or its mammalian ortholog microtubule-associated protein light chain 3 (LC3). ATG8 is cleaved at the C-terminus by ATG4 (LC3-I) and then conjugated to phosphatidylethanolamine (PE) via ATG7 and E2-like ATG3. The ATG8-PE complex (LC3-II) binds to both the inner and outer membrane of the autophagosomes (Geng & Klionsky, 2008). This is the basis for the most commonly used assay to measure autophagy, using Western blot analysis to identify changes in LC3-II levels between treatment groups or by monitoring GFP-LC3 translocation to foci in cells (Klionsky, et al., 2008; Mizushima, Yoshimori, & Levine, 2010). Interaction between the two conjugation systems is essential to autophagy as the ATG16L complex targets LC3-1 to the phagophore membrane and accelerates its conjugation to PE (Fujita, et al., 2008).

Once the autophagosome is created, maturation is completed by fusion with a lysosome to form an autophagolysosome. This process involves lysosomal-associated membrane proteins (LAMP) LAMP1 and LAMP2 as well as UVRAG and the GTPase Rab7. In addition to its binding to beclin1 to up-regulate phagophore formation, UVRAG interacts with class C Vps proteins and stimulates Rab7 and autophagosomes/lysosome fusion and delivery of cargo for degradation (C. Liang, et al., 2008). The final autophagolysosome is a single membrane acidic vesicle wherein lysosomal hydrolases such as cathepsins degrade intravesicular material into amino acids and other components, which are released and used for energy and as building blocks for cellular macromolecules.

3. Autophagy as a tumor promotion and survival mechanism

Within a normal cell, autophagy's primary role is thought to be to act as a cellular survival mechanism, providing energy and substrates for cellular processes during times of stress including starvation. It also maintains overall cell homeostasis with turnover of organelles and long-lived proteins. Studies have shown that a loss of autophagy can cause detrimental

effects in the cell leading to clinically relevant diseases. For example, loss of either ATG5 or ATG7 in the brain results in the accumulation of ubiquitinated proteins in the central nervous system resulting in neurodegeneration (Hara, et al., 2006; Komatsu, et al., 2006). Defects in autophagy have also been shown to be involved in premature aging (Marino, Fernandez, & Lopez-Otin, 2010), muscular disease (Tolkovsky, 2010), Alzheimer's disease (Pickford, et al., 2008), and a possible target mechanism for adjuvant therapy of lysosome storage disorders (Raben, et al., 2010). However, autophagy's role in cancer is less clear with conflicting evidence showing autophagy can both promote tumor progression, e.g. by helping tumor cells survive or act as a tumor suppressor mechanism.

Oncogenic transformation of cells, the first step in tumorigenesis, is associated with aberrant signaling from both growth factors and constitutively active pathways known to regulate autophagy. Under normal conditions, the class I PI3K pathway senses insulin and growth factors and negatively regulates autophagy through AKT signaling, allowing activation of mTOR. The ULK1/2 complex is repressed by mTOR, blocking the initiation of phagophores (Wang & Levine, 2010). Constitutive activation of this pathway should result in a block of autophagy by mTOR, leading to metabolically stressed cells. But recent evidence has shown increased autophagy activation in nutrient poor regions of a tumor that is not seen in highly vascularized areas, which have an advantageous nutrient microenvironment (Karantza-Wadsworth, et al., 2007). Activation of autophagy in nutrient poor regions generates needed energy and substrates for growth of the tumor, providing an advantage to these cells.

Autophagy has also been shown to facilitate glycolysis, oxidative metabolism and adhesion-independent growth in cells with active Ras mutations, increasing tumorigenic potential. Cells with an H-Ras^{V12} mutation can proliferate after the loss of cell-matrix contact, and the Ras mutation promotes aerobic glycolysis, the Warburg effect, a metabolic shift in a cell that drives tumorigenesis (Lock, et al., 2011; Vander Heiden, Cantley, & Thompson, 2009). Autophagy deficient cells, with either knockout of ATG5 or knockdown of ATG7 by shRNA and transformed with H-Ras^{V12} have decreased proliferation potential and reduced glucose metabolism (Lock, et al., 2011). Furthermore, cells with activated Ras mutations require autophagy for oxidative metabolism and tumorigenesis. Cells with H-Ras^{V12} or K-Ras^{V12} and impaired autophagy accumulate abnormal mitochondria and have tricarboxylic acid cycle ATP depletion during starvation conditions (Guo, et al., 2011). These studies suggest that Ras-mediated transformation requires autophagy. Such autophagy-dependence of cells with activated Ras mutations may therefore identify a particular subset of tumors susceptible to autophagy inhibition during therapy.

Poor vascularization of tumors leads to hypoxia which selects for cells resistant to apoptosis, producing more aggressive tumors with higher metastatic potential and poorer prognosis (Butterworth, et al., 2008). The hypoxic core of tumors can induce autophagy and promote survival of these more aggressive cells (Degenhardt, et al., 2006), leading to chemotherapy and radiation resistance. This is related to hypoxia-inducible factor 1 α (HIF-1 α), a positive regulator of autophagy. HIF-1 α works through the Bcl-2/adenovirus E1B 19 kDa-interacting protein (BNIP3), a BH3-only protein (Bellot, et al., 2009). Under normal conditions, beclin1 complexes with Bcl-X_L and Bcl-2 at the BH3 domain; in hypoxic conditions, HIF-1 α activation leads BNIP3 to displace beclin1 from Bcl-X_L and Bcl-2, allowing beclin1 to induce autophagy. When HIF-1 α is knocked down and autophagy is inhibited, hypoxia-induced resistance can be overcome (Liu, et al., 2010; Martinez-Outschoorn, et al., 2010). The AMPK signaling pathway is also implicated in hypoxia-induced autophagy, independent of HIF-1 α (Papandreou, Lim, Laderoute, & Denko, 2008), inactivating mTOR during periods of nutrient depletion or hypoxia (Wullschleger, Loewith, & Hall, 2006).

4. Autophagy as a tumor suppressor mechanism

In contrast to the above studies, where autophagy would appear to promote tumor progression, autophagy is also thought to be a tumor suppression mechanism because genetic deficiencies in various autophagy regulators leads to increased cancer while many oncogenes inhibit autophagy and tumor suppressors increase autophagy (Maiuri, et al., 2009). For example, loss of beclin1 gene function has been associated with various solid tumors including breast, ovarian and prostate tumors (X. H. Liang, et al., 1999; Qu, et al., 2003; Yue, Jin, Yang, Levine, & Heintz, 2003). Mice with heterozygous loss of beclin1 have an increased frequency of spontaneous tumor formation as well as more rapid development of hepatitis B virus-induced lesions (Qu, et al., 2003). Conversely, restoration of beclin1 in breast cancer cells with a loss of gene function resulted in a decreased ability for these cells to proliferate and produce tumors when implanted into nude mice (X. H. Liang, et al., 1999). Beclin1 also works with endophilin B1 (Bif-1) to act as a tumor suppressor mechanism. Bif-1 interacts with beclin1 via UVRAG and promotes Vps34 activation leading to autophagosomes formation. Loss of Bif-1 is associated with multiple human tumors including gastric carcinoma (Lee, et al., 2006), colorectal adenocarcinoma (Coppola, et al., 2008), and urinary and gallbladder cancers (S. Y. Kim, et al., 2008). Bif-1 $-/-$ mice also have a higher rate of tumor development compared to wild type controls (Takahashi, et al., 2007). Other mouse models have also shown an increase in tumorigenesis with the loss of autophagy genes, despite no apparent effect on normal embryogenesis and development. For example, mice deficient for ATG4 show an increased development of fibrosarcomas when exposed to chemical carcinogens (Marino, et al., 2007). In contrast, UVRAG mediated activation of autophagy suppresses the proliferation and tumorigenicity of human colon cancer cells when implanted in nude mice (C. Liang, et al., 2006).

Genetic regulators of autophagy and sequestosome 1 (also known as p62), a selective autophagy substrate, have been proposed as regulators that explain the tumor suppressive effect of autophagy. While its relatively easy to see how autophagy's known functions for cell protection in response to stress might promote tumor progression, for example by protecting tumor cells while they are subjected to nutritional stress, the molecular mechanisms through which autophagy's tumor suppressor activities could be mediated are less obvious. The best-determined mechanisms by which this occurs is through autophagy's ability to protect cells against damage that could lead to increased mutations and other oncogenic events. The accumulation of abnormal proteins when autophagy is suppressed is associated with the build up of reactive oxygen species (ROS) and resultant DNA damage (Mathew, Karantza-Wadsworth, & White, 2007). A tissue specific loss of ATG7 leads to accumulation of polyubiquitinated proteins due to a lack of autophagic clearance (Komatsu, et al., 2006) and subsequently, ROS increase. Cells with functional autophagy are able to clear these abnormal proteins and prevent ROS mediated DNA damage. In addition, loss of beclin1 was shown to promote chromosome instability and be associated with increased DNA damage, gene amplifications, and aneuploidy (Mathew, Kongara, et al., 2007). Cells with increased DNA damage secondary to loss of autophagy and increased ROS are not only more susceptible to cell death, but also to further DNA damage and future tumorigenic potential (Karantza-Wadsworth, et al., 2007; Mathew, Kongara, et al., 2007).

P62 is an LC3 interacting protein that binds ubiquitinated proteins via their C-terminal domains and directs these proteins to autophagosomes for selective autophagic degradation (Kirkin, McEwan, Novak, & Dikic, 2009). In addition to the increase in ROS, the aberrant accumulation of aggregated proteins leads to an accumulation of p62 that can directly cause oxidative damage. This results in further buildup of ROS, creating a positive feedback loop and further DNA damage (Mathew, et al., 2009; Moscat & Diaz-Meco, 2009). The accumulation of p62 sequesters cancer-relevant proteins such as p53 and prevents their

degradation by delaying their delivery to the ubiquitin-proteasome system (Korolchuk, Mansilla, Menzies, & Rubinshtein, 2009). P62 is also associated with regulation of proliferation and survival of cells through interaction with signaling proteins such as the nuclear factor- κ B (NF- κ B). NF- κ B is induced by the Ras oncogene to promote cell survival. A p62 increase in human tumors may be required for Ras/NF- κ B induced survival and transformation and p62^{-/-} mice are resistant to Ras-induced lung adenocarcinomas (Duran, et al., 2008). Other signaling pathways are also in part regulated by p62 sequestration of involved proteins such as the extra-cellular signal regulated kinase (Rodriguez, et al., 2006), tumor necrosis factor receptor-associated factor 6 (Duran, et al., 2008), and MEK5, a member of the mitogen-activated protein kinase group (Lamark, et al., 2003).

5. Autophagy Inhibition in Cancer Therapy

Although the process of autophagy is complex and how it should be manipulated when treating patients is not fully defined (Dalby, Tekedereli, Lopez-Berestein, & Ozpolat, 2010), there are a number of pharmacologic mediators of autophagy in clinical use today. As of February 2011, a search for autophagy on the clinical trials database ClinicalTrials.gov identifies at least twenty clinical trials looking specifically at the modulation of autophagy in cancer therapy (Table 2). The majority of these phase I and II trials use CQ or HCQ as an autophagy inhibitor based on the belief that autophagy is a tumor survival mechanism and by blocking the process, it is possible to increase tumor cell kill with chemotherapy, radiation, or other therapies. There is some reason to think that such autophagy inhibitors improve outcome. For example, a small single institution study of CQ added to conventional chemotherapy for the treatment of glioblastoma found a prolonged median survival and decreased rate of death in the CQ treated group. The results of this study were not statistically significant, likely due to a small sample size, but it does prove the safety of adding CQ to current chemotherapeutic regimens and that CQ has the potential to improve patient survival (Sotelo, Briceno, & Lopez-Gonzalez, 2006).

CQ and HCQ are widely used as anti-malarial and anti-rheumatic treatments and work by inhibiting lysosome acidification, therefore blocking the late stages of autophagy. Interestingly, long before autophagy was thought of as a process that affects cancer, evidence of an anti-cancer effect of CQ was obtained. A malaria suppression program utilizing CQ and carried out in Tanzania from 1977 to 1982 found that in addition to a decrease prevalence of malaria, there was a significant decline in the rate of Burkitt's lymphoma in the same region. Rate of Burkitt's returned to baseline levels two years after the CQ distribution program was discontinued. Although not definitive proof, this suggested that CQ may have been a factor in the decrease in Burkitt's seen during the study years (Geser, Brubaker, & Draper, 1989). Recent follow up studies in mice found that intermittent dosing of CQ suppressed Myc-induced lymphomagenesis in a p53 dependent manner in mouse models for Burkitt's as well as ataxia telangiectasia, a known cancer predisposition disorder (Maclean, Dorsey, Cleveland, & Kastan, 2008). Further mouse studies using lymphoma cells with p53-induced apoptosis have demonstrated a subset of surviving cells with autophagy, thought to be associated with their survival. Blocking autophagy with CQ resulted in increased apoptosis and tumor regression, as well as increased time to recurrence (Amaravadi, et al., 2007).

CQ has also been studied as an adjuvant therapy in non-Myc induced tumors. Tyrosine kinase inhibitors (TKI) against the Src family kinases (SFK) in prostate cancer cause G1 growth arrest and diminished invasiveness, but do not appear to induce apoptosis. Blocking Src activity induces a high level of autophagy via the PI3K/AKT/mTOR pathway, protecting the cells from apoptotic cell death. Preventing autophagy induction by SFK inhibitors using either genetic inhibition of ATG7 or pharmacologic inhibition with CQ or 3-methyladenine

(3-MA) in vitro resulted in increased cell death. This effect was preserved in vivo when a SFK inhibitor was combined with CQ, reducing prostate cancer growth in a mouse model (Z. Wu, et al., 2010). Other TKIs are also potentiated with concomitant autophagy inhibition, in particular those drugs that target the BCR-ABL fusion protein. The TKI imatinib mesylate is a direct competitor with the constitutively active BCR/ABL kinase and is first line therapy in BCR/ABL positive chronic myeloid leukemia (CML). Removal of the BCR/ABL signal induces apoptosis in these cells, but has also been shown to induce autophagy. In vitro and in vivo studies found increased levels of imatinib induced cell death when combined with CQ inhibition of autophagy (Bellodi, et al., 2009). CML cells with high levels of BCR-ABL also have autophagy induced by DNA damaging agents such as etoposide with subsequent delayed cell death similar to the findings with imatinib, suggesting further potential targets for concomitant autophagy inhibition (Crowley, Elzinga, O'Sullivan, & McKenna, 2011).

Other drugs that could be used along with or after imatinib may also affect autophagy. For example, histone deacetylase (HDAC) inhibitors such as suberoylanilide hydroxamic acid (SAHA) are being investigated as a second-line therapy for imatinib resistant CML, and have also been shown to induce autophagy (Shao, Gao, Marks, & Jiang, 2004). In the presence of CQ, there is an increase in death in SAHA treated, imatinib resistant cells. This is in part mediated by increased SAHA-induced ROS generation in the presence of CQ (Carew, et al., 2007). The induction of autophagy by SAHA and other drugs such as sorafenib, a multiple kinase inhibitor, is sometimes dependent on generation of secondary signaling molecules. For example, cancer cells treated with or SAHA or sorafenib demonstrated increased autophagy only when ceramide was generated allowing parallel activation of the CD95 death receptor and pro-caspase 8 and the CD95-promoted phosphorylation of PDR-like endoplasmic reticulum kinase resulting in autophagy (Park, et al., 2008). This was shown to be particularly important in glioma cells, where melanoma differentiation associated gene-7 IL-24 therapy induces endoplasmic reticulum stress and ceramide production, ultimately leading to glioma cell autophagy and death (Yacoub, et al., 2010). Similar findings were made in prostate and gastrointestinal tumor models as well (Bhutia, et al., 2010; Park, et al., 2008).

Inhibition of autophagy has also been studied as a method to sensitize cells to radiation treatment. Cancer stem cells, a sub-population of cells with independent tumor initiation capabilities, have been shown to be highly resistant to radiation and other anti-cancer treatments (Pajonk, Vlashi, & McBride, 2010). There are some suggestions that at least part of this therapy resistance may be due to increased autophagy in this "stem cell" subpopulation compared with the bulk of the tumor. For example, in malignant gliomas, the cancer stem cells can be identified by CD133 positivity and have higher levels of autophagy-related proteins including LC3, ATG5, and ATG12 and can induce higher levels of autophagy following radiation than CD133-cells (Lomonaco, et al., 2009). It was also shown that glioma cells treated with autophagy inhibitors had more extensive DNA double-strand breaks than cells treated with radiation alone (Ito, Daido, Kanzawa, Kondo, & Kondo, 2005). This effect is not limited to glioma. In vitro treatment of TE-1 esophageal cancer cells with radiation induces accumulation of autophagosomes and up-regulation of beclin-1 and LC3 II expression. Blocking autophagy with 3-MA increased cell death by apoptosis (Chen, et al., 2010). A broader evaluation of a number of cell lines representing radiosensitive and radioresistant cancers including breast, pharyngeal, cervical, lung, and rectal cancer found all cell lines accumulated autophagosomes following irradiation. Genetic down-regulation of autophagy by siRNA knockdown of various autophagy related proteins including beclin1, ATG2, ATG5, ATG4, and ATG12 resulted in a decreased accumulation of autophagosomes. Autophagy inhibition in the radio-resistant cell lines resulted in enhanced cytotoxicity following irradiation, whereas there was not a significant change in

survival in cells that were already radiosensitive (Apel, Herr, Schwarz, Rodemann, & Mayer, 2008). Thus although this issue is far from being definitively answered, different levels of autophagy within a population of tumor cells may partly explain the different behavior including the chemo- and radio-resistance of cancer stem cells. Further testing of this hypothesis is clearly needed, however if this proves to be a general attribute of cancer stem cells that represent the tumor initiating cells in a tumor, then specific targeting of autophagy during cancer therapy could be viewed as a way to selectively disable this subpopulation.

Although CQ and HCQ are the most commonly utilized autophagy inhibitors in the lab and in clinical use, there are other drugs and compounds being investigated. For example, verteporfin, a benzoporphyrin derivative, was identified as a potential clinical autophagy inhibitor. An automated microscopy assay found that verteporfin inhibited autophagosome accumulation. Follow up studies localized its effect to inhibiting autophagic degradation and sequestration of material into autophagosomes while not changing LC3 processing (Donohue, et al., 2010). The small molecule lucanthone, currently used as an anti-schistosome agent, inhibits autophagy by allowing LC3 processing, but impairing autophagic degradation. It also induces apoptosis by the induction of cathepsin D and enhanced HDAC induced cell death in breast cancer models (Carew, Espitia, et al., 2010). Finally, cells treated with 2-phenylethanesulfonamide, a small molecule inhibitor of heat shock protein 70, demonstrated cytotoxicity due to increased p62 aggregation, inhibited lysosomal function, and overall impaired autophagy (Leu, Pimkina, Frank, Murphy, & George, 2009). All of these compounds, as well as a myriad of others that are starting to be identified, require additional pre-clinical studies to define their roles in autophagy inhibition before widespread use in clinical trials.

6. Autophagy Stimulation in Cancer Therapy

For each tumor subset being studied in clinical trials inhibiting autophagy with CQ or HCQ, there are alternative trials evaluating the effectiveness of drug regimes shown to induce autophagy (Table 3). Numerous anti-cancer therapies are known to induce autophagy including as mentioned above, radiation (Zois & Koukourakis, 2009) and diverse chemotherapy treatments (Table 1). In most cases, the clinical trials that use these agents are not deliberately trying to induce tumor autophagy and no attempt is made to determine if tumor autophagy is even altered in patients treated with these agents, raising the issue of whether drugs that inadvertently increase autophagy would be more effective if the autophagy was blocked. However, it has also been suggested that one should actively try to increase autophagy in order to kill tumor cells. Programmed cell death is a regulated process, the most common being apoptotic, or type I, cell death. There is also some evidence for type II cell death, or autophagy-dependent cell death. Unlike with apoptosis, in type II cell death there is no chromatin condensation and no role for caspase activation. Instead, cells have a large number of autophagosomes with extensive degradation of cytoplasmic material. However, there is robust ongoing debate if the autophagic process really drives tumor cell death in this case, or if what has been called autophagic death is actually a secondary response to a failed survival mechanism and better viewed as “death with autophagy” rather than “death by autophagy” (Kroemer & Levine, 2008).

Notwithstanding this ongoing controversy, multiple studies have reported autophagy-dependent cell death, often in conjunction with apoptosis and sometimes in response to the exact same treatments that are reported to display autophagy-mediated protection. In glioma cells in vitro and in vivo, Δ^9 -tetrahydrocannabinol (THC), the active component of marijuana, induces ER stress and subsequent AKT/mTOR inhibition leading to autophagy and cell death. Blocking autophagy by either genetic or pharmacologic inhibition prevented

the cell death. Although controlled by autophagy, the cell death was dependent on apoptosis as caspase inhibitors provided the same degree of resistance as blocking autophagy (Salazar, et al., 2009). Pancreatic cancer cell lines also show autophagy-dependent cell death through apoptosis when treated with gemcitabine, which activates vacuole membrane protein-1 induced autophagy. Features of apoptosis were lost with vacuole membrane protein-1 knockdown as well as when autophagy was blocked pharmacologically with 3-MA (Pardo, et al., 2010).

Autophagy-dependent cell death can occur through modulation of the Bcl-2 family as well. Using small-molecule inhibitors such as (-)-gossypol, a BH3-mimetic, or direct down-regulation of Bcl-2 to allow release of beclin1 and autophagy induction, cells will undergo type II programmed cell death without features of apoptosis (Lian, et al., 2011; Shimizu, et al., 2004; Voss, et al., 2010). Cells with specific genetic mutations can also be targeted for autophagy induction and increased cell death. STF-62247 is a small molecule that selectively induces cell death in renal cell carcinoma cells with a loss of function of the von-Hippel-Lindau tumor suppressor gene that is inactivated in 75% of renal cell carcinomas. STF-62247 toxicity is due to induction of autophagy and can be reduced by autophagy inhibition (Turcotte, Chan, et al., 2008).

Other studies have claimed autophagy-dependent cell death and sensitization to radiation in, for example, prostate (Cao, et al., 2006) and lung cancer (K. W. Kim, Moretti, Mitchell, Jung, & Lu, 2009). Treatment of multiple pancreatic cancer cells lines with radiation found that the autophagy induced by therapy contributed to cell death, enhancing cytotoxicity (Mukubou, Tsujimura, Sasaki, & Ku, 2010). Autophagy induction in papillary thyroid cancer through mTOR inhibition has also been shown to increase cytotoxicity to chemotherapy and radiation (C. I. Lin, et al., 2010). Furthermore, it has been suggested that breast cancer can have improved radiosensitivity by promoting autophagic cell death through a vitamin D analog (Gewirtz, Hilliker, & Wilson, 2009). Even the results in glioma cell lines are contradictory. While the studies discussed earlier found inhibition of autophagy improved cell death in glioma cancer stem cells, a study of three glioma cells lines with varying resistance to radiation therapy pre-treated with pitavastatin, a NF- κ B inhibitor, increased type II autophagic cell death in all the cell lines, regardless of their initiation radiation resistance (C. I. Lin, et al., 2010). Use of an Akt inhibitor, 1L-6-hydroxymethyl-chiro-inositol 2(R)-2-O-methyl-3-O-octadecylcarbonate, increased radiosensitivity in both radiosensitive and radioresistant glioma cells by the induction of autophagy (Fujiwara, et al., 2007). Clearly, we need to work out the basis for such fundamental differences where the same treatment may be able to induce autophagy dependent tumor cell death in one series of experiments and protect against cell death in another. There are a number of potential explanations for the confusion. Different studies often use different tumor cell lines and we may in fact be uncovering a real difference whereby autophagy is protective in some tumors but toxic in others – if this is the case then it should be possible to identify markers that underlie these differences and which could be used to select which tumors should be targeted for autophagy inhibition and which should be targeted for autophagy induction. One starting place for this kind of analysis would be the direct comparison of Ras-transformed tumor cells with cells that have not undergone Ras-dependent transformation since there may be something special about Ras that leads to a kind of “autophagy addiction” (Lock, et al., 2011)(Guo, et al., 2011). However it is also quite possible that much of the confusion in the field is due to misinterpretation of the experiments. For example, in many experiments in the literature apparent protection of cells (e.g. after autophagy inhibition, which would presumably indicate an example of autophagy-dependent death) does not exclude the possibility that the cell death response was merely altered in terms of its kinetics – i.e. that the cells that are scored as alive were in fact destined to die but at a later time after the experiment was terminated. Additionally, one aspect that is often not adequately considered

in chemosensitivity studies of autophagy manipulation is that the treatments used to manipulate autophagy can also have other effects. This problem applies both to pharmacological treatments and genetic interventions such as knockdown of ATGs, many of which have been shown to participate in other cellular activities. One thing that is sorely lacking in this field is the direct side-by-side comparison of the effect of simultaneously targeting autophagy (both positively and negatively) by multiple independent methods and directly testing if this leads to altered chemosensitivity in different contexts (e.g. cell lines with different genetic backgrounds or in response to different anti-cancer treatments). We believe it is likely that such analysis (which would however be technically demanding) will allow us to clarify some of the confusion and hopefully work out whether or not autophagy really is a tumor cell killer in some contexts but a tumor cell protector in others.

The most common method for clinical induction of autophagy is through the PI3K/Akt/mTOR pathway, with multiple pharmacologic agents available for clinical use. A direct PI3K inhibitor, LY294002, activates autophagy through p53 and caspase-3 activation in gastric cancer cells in vitro (Xing, Zhu, Liu, Yao, & Zhang, 2008). Akt inhibition induces autophagy and increased cell death when combined with radiation and chemotherapy as well (Degtyarev, et al., 2008; Fujiwara, et al., 2007). Direct mTOR inhibition with rapamycin or one of its analogues is perhaps the most widely used clinical method where we would expect robust autophagy induction in tumors, with numerous clinical trials evaluating the effectiveness of mTOR inhibition alone and in combination with other therapies (Table 3). mTOR inhibition shows activity in numerous cell types including T and B cell leukemia (Evangelisti, et al., 2011; Saunders, Cisterne, Weiss, Bradstock, & Bendall, 2011), advanced pancreatic tumors (Yao, et al., 2010), subependymal giant-cell astrocytomas (Krueger, et al., 2010), and numerous other tumors (Dancey, 2010)– it is quite unclear how much, if any, of this activity is due to increased autophagy.

Research into the effectiveness of autophagy inducers found in natural foods is also active. Diets high in phenethyl isothiocyanate (PEITC), found in cruciferous vegetables such as broccoli, cauliflower, and green leafy vegetables, have been associated with decreased cancer rates. When transgenic mice with prostate adenocarcinoma fed a normal diet were compared to matched mice fed a diet enriched with PEITC, there was a significant decrease in prostate tumor development in the PEITC fed mice. Interestingly, these mice also had induction of autophagy shown by electron microscopy and decreased p62 levels but no evidence of apoptosis induction or inhibition of neoangiogenesis (Powolny, et al., 2011).

7. Clinical Monitoring and Measurement of Autophagy

Determining the role of autophagy manipulation in clinical therapy will require methods of evaluating changes in autophagy in patients and their tumors. Potential plasma biomarkers would be the most clinically useful method of monitoring changes in autophagy with treatment, although there has been very limited research in this area. One potential plasma biomarker is the clusterin protein. In mice fed a diet high in PEITC, clusterin protein levels were significantly suppressed as autophagy was induced, and a dose dependent decrease in clusterin was reproducible in vitro (Powolny, et al., 2011). Radiotracers for the non-invasive monitoring of changes in cell proliferation have also been suggested as a potential method for monitoring autophagy. Methods using caspase targeted, small molecule, and Annexin V based probes are already in development to image apoptosis during therapy. Although there are no current probes for imaging autophagy, it has been suggested that the development of a radiotracer specific for LC3-II or another autophagy specific protein may be possible (Kapte, Murray, & Mercer, 2010).

It is possible to measure levels of autophagy related proteins in tumor samples for use as potential predictive markers. One study of high-grade gliomas found it was possible to classify tumor samples according to their cytoplasmic expression of beclin1, and that a higher level of beclin1 was associated with a higher Karnofski performance score and with completing optimal postoperative therapy. It was also significantly correlated with survival (Pirtoli, et al., 2009). The measurement of beclin1 and the presence of LC3-positive autophagosomes were inversely correlated with the expression of Bcl-2 in non-Hodgkin lymphoma, and tumors with up-regulated autophagy (>20% of cells with high levels of beclin1) were determined to be more responsive to chemotherapy (Nicotra, et al., 2010). Conversely, in a study of 125 breast cancer tissue samples, beclin1 expression was also inversely correlated with Bcl-2 expression by tissue microarray, but there was no correlation between beclin1 expression and cumulative survival (Won, Kim, Kim, Song, & Lim, 2010). Levels of tumor cell autophagy, measured by electron microscopy, could predict melanoma invasiveness and resistance to therapy. Patients with a high number of autophagosomes per cell had less response to therapy and shorter overall survival when compared to patients with a low autophagic index. These findings were replicated in vitro with aggressive melanoma cell lines grown in three-dimensional culture showing more autophagy than indolent cell lines (Ma, et al., 2011).

Using LC3 immunohistochemical staining as a predictive marker has also been suggested. A study of 71 pancreatic tumors found that strong LC3 expression was correlated with increased tumor size, poor clinical outcome and shorter time to disease progression (Fujii, et al., 2008). Caution must be used when interpreting results of LC3 immunostaining as LC3 expression can vary greatly between cell types and in response to various stresses and such staining is one “snapshot” in time and may not represent the overall level of autophagic flux present in a tumor (Barth, Glick, & Macleod, 2010). Finally, in vivo labeling of autophagosomes can be achieved with the use of monodansylcadaverine. This compound auto-fluoresces and integrates into the membranes of autophagosomes and autophagolysosomes (Vazquez & Colombo, 2009).

Another potential avenue for predicting which patients may benefit from autophagy manipulation is gene expression patterns. Some early studies have found differences in autophagy related gene expression as well as gene methylation. An RT-PCR study of the transcriptional regulation of 26 human ATG genes found a ubiquitous expression pattern for all genes studied except ATG2A, ATG9B, and WIPI2. ATG2A specifically was up-regulated in cells treated with etoposide and ATG2A mRNA was up-regulated following treatment with doxorubicin (Kusama, et al., 2009). Gene methylation studies of adult CML patients found that ATG16L2 had a higher frequency of epigenetic inactivation by methylation than other genes studied during both chronic and blast phases of the disease. ATG16L2 also had increased methylation in kidney tumor cell lines, although was not in colorectal lung, breast, prostate, or glioma cell lines. This is significant because patients with hypermethylated ATG16L2 had a significantly decreased rate of major molecular response to imatinib therapy compared to patients without hypermethylation (Dunwell, et al., 2010). Interestingly, DAPI has also been shown to be silenced by methylation leading to autophagy inhibition (Gozuacik & Kimchi, 2006). However, once again we need to be cautious in interpreting these kinds of data. Although there may indeed be differences in expression of genes that regulate autophagy that are correlated with different clinical response, this does not necessarily mean that different levels of autophagy were responsible for the different response. Indeed, a result that finds altered levels of one autophagy-related gene is associated with altered clinical response while others are not may be indicating that it is some other autophagy-independent function of that gene that is important.

8. Conclusion

The study of autophagy is a rapidly changing field that holds great potential for improving the treatment and cure of malignant disease. However, despite this potential, there are still numerous questions that need to be answered, most importantly, whether autophagy should be inhibited or stimulated to improve clinical outcomes in patients or whether some people should have autophagy induced while others should have it inhibited. In fact, this question may also be too simple as autophagy is a complex multi-step process that can be influenced by a number of cellular pathways as well as altered by the tumor microenvironment and there may also be differences when we block autophagy at the start of the process compared to half way through or at the end. It is clear from current research that it may also not be possible to have a unified treatment strategy for autophagy as there is conflicting evidence not only between tumor types, but also within the same tumor type when evaluating different treatment modalities and as noted above, direct comparison between different treatments and different tumor cells has been, for the most part, lacking in the field.

Addressing these questions and conflicts is vital in the future planning of clinical trials manipulating autophagy. Ongoing trials of autophagy inhibition must be closely monitored and those results directly compared to completed and current studies in the same tumor types utilizing drugs known to induce autophagy. New and reliable methods for monitoring and measuring autophagy in patients will also be important in understanding the results of these clinical trials, as the changes in autophagy seen in vitro and in xenograft models may not directly translate into real clinical situations. They may also be beneficial in determining which patients will most likely benefit from autophagy manipulation and whether they should be treated with autophagy inhibitors or stimulators. Keeping these difficulties in mind, careful and rigorous evaluation of autophagy with a focus on how to translate laboratory findings into relevant clinical therapies remains an important aspect of improving clinical outcomes in patients with malignant disease.

Acknowledgments

JM Mulcahy Levy is supported by a St. Baldrick's Foundation Fellowship. Additional support provided by The Morgan Adams Foundation and NIH grants CA150925 and CA111421.

Abbreviations

AMPK	AMP activated protein kinase
ATG	autophagy-related genes
Bif-1	endophilin B1
BNIP3	Bcl-2/adenovirus E1B 19 kDa-interacting protein
CML	chronic myeloid leukemia
CQ	chloroquine
DAPI	death-associated protein 1
HCQ	hydroxychloroquine
HDAC	histone deacetylase
HIF-1α	hypoxia-inducible factor 1 α
LAMP	lysosomal-associated membrane proteins
LC3	microtubule-associated protein light chain 3

mTOR	mammalian target of rapamycin
NF-κB	nuclear factor- κ B
PE	phosphatidylethanolamine
PEITC	phenethyl isothiocyanate
PI3K	phosphoinositide 3-kinase
ROS	reactive oxygen species
SAHA	suberoylanilide hydroxamic acid
SFK	Src family kinases
THC	Δ^9 -tetrahydrocannabinol
TKI	tyrosine kinase inhibitors
ULK	unc-51-like kinase
UVRAG	ultraviolet radiation resistance-associated gene
3-MA	3-methyladenine

References

- Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, et al. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J Clin Invest.* 2007; 117(2):326–336. [PubMed: 17235397]
- Anandappa G, Hollingdale A, Eisen T. Everolimus - a new approach in the treatment of renal cell carcinoma. *Cancer Manag Res.* 2010; 2:61–70. [PubMed: 21188097]
- Aoki H, Takada Y, Kondo S, Sawaya R, Aggarwal BB, Kondo Y. Evidence that curcumin suppresses the growth of malignant gliomas in vitro and in vivo through induction of autophagy: role of Akt and extracellular signal-regulated kinase signaling pathways. *Mol Pharmacol.* 2007; 72(1):29–39. [PubMed: 17395690]
- Apel A, Herr I, Schwarz H, Rodemann HP, Mayer A. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. *Cancer Res.* 2008; 68(5):1485–1494. [PubMed: 18316613]
- Barth S, Glick D, Macleod KF. Autophagy: assays and artifacts. *J Pathol.* 2010; 221(2):117–124. [PubMed: 20225337]
- Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, et al. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *J Clin Invest.* 2009; 119(5):1109–1123. [PubMed: 19363292]
- Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouyssegur J, et al. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol.* 2009; 29(10):2570–2581. [PubMed: 19273585]
- Bhutia SK, Dash R, Das SK, Azab B, Su ZZ, Lee SG, et al. Mechanism of autophagy to apoptosis switch triggered in prostate cancer cells by antitumor cytokine melanoma differentiation-associated gene 7/interleukin-24. *Cancer Res.* 2010; 70(9):3667–3676. [PubMed: 20406981]
- Bianco R, Garofalo S, Rosa R, Damiano V, Gelardi T, Daniele G, et al. Inhibition of mTOR pathway by everolimus cooperates with EGFR inhibitors in human tumours sensitive and resistant to anti-EGFR drugs. *Br J Cancer.* 2008; 98(5):923–930. [PubMed: 18319715]
- Butterworth KT, McCarthy HO, Devlin A, Ming L, Robson T, McKeown SR, et al. Hypoxia selects for androgen independent LNCaP cells with a more malignant geno- and phenotype. *Int J Cancer.* 2008; 123(4):760–768. [PubMed: 18512241]

- Cao C, Subhawong T, Albert JM, Kim KW, Geng L, Sekhar KR, et al. Inhibition of mammalian target of rapamycin or apoptotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells. *Cancer Res.* 2006; 66(20):10040–10047. [PubMed: 17047067]
- Carew JS, Espitia CM, Esquivel JA 2nd, Mahalingam D, Kelly KR, Reddy G, et al. Lucanthone: A novel inhibitor of autophagy that induces cathepsin D-mediated apoptosis. *J Biol Chem.* 2010
- Carew JS, Medina EC, Esquivel JA 2nd, Mahalingam D, Swords R, Kelly K, et al. Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. *J Cell Mol Med.* 2010; 14(10):2448–2459. [PubMed: 19583815]
- Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, et al. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. *Blood.* 2007; 110(1):313–322. [PubMed: 17363733]
- Chen YS, Song HX, Lu Y, Li X, Chen T, Zhang Y, et al. Autophagy inhibition contributes to radiation sensitization of esophageal squamous carcinoma cells. *Dis Esophagus.* 2010
- Ciuffreda L, Di Sanza C, Incani UC, Milella M. The mTOR pathway: a new target in cancer therapy. *Curr Cancer Drug Targets.* 2010; 10(5):484–495. [PubMed: 20384580]
- Coiffier B, Ribrag V. Exploring mammalian target of rapamycin (mTOR) inhibition for treatment of mantle cell lymphoma and other hematologic malignancies. *Leuk Lymphoma.* 2009; 50(12):1916–1930. [PubMed: 19757306]
- Coppola D, Khalil F, Eschrich SA, Boulware D, Yeatman T, Wang HG. Down-regulation of Bax-interacting factor-1 in colorectal adenocarcinoma. *Cancer.* 2008; 113(10):2665–2670. [PubMed: 18833585]
- Crowley LC, Elzinga BM, O'Sullivan GC, McKenna SL. Autophagy induction by Bcr-Abl-expressing cells facilitates their recovery from a targeted or nontargeted treatment. *Am J Hematol.* 2011; 86(1):38–47. [PubMed: 21132731]
- Dalby KN, Tekedereli I, Lopez-Berestein G, Ozpolat B. Targeting the prodeath and prosurvival functions of autophagy as novel therapeutic strategies in cancer. *Autophagy.* 2010; 6(3):322–329. [PubMed: 20224296]
- Dancey J. mTOR signaling and drug development in cancer. *Nat Rev Clin Oncol.* 2010; 7(4):209–219. [PubMed: 20234352]
- Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell.* 2006; 10(1):51–64. [PubMed: 16843265]
- Degtyarev M, De Maziere A, Orr C, Lin J, Lee BB, Tien JY, et al. Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents. *J Cell Biol.* 2008; 183(1):101–116. [PubMed: 18838554]
- Deter RL, De Duve C. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *J Cell Biol.* 1967; 33(2):437–449. [PubMed: 4292315]
- Dhillon T, Mauri FA, Bellezza G, Cagini L, Barbareschi M, North BV, et al. Overexpression of the mammalian target of rapamycin: a novel biomarker for poor survival in resected early stage non-small cell lung cancer. *J Thorac Oncol.* 2010; 5(3):314–319. [PubMed: 20093977]
- Donohue E, Tovey A, Vogl AW, Arns S, Sternberg E, Young RN, et al. Inhibition of autophagosome formation by the benzoporphyrin derivative verteporfin. *J Biol Chem.* 2010
- Dunwell T, Hesson L, Rauch TA, Wang L, Clark RE, Dallol A, et al. A genomewide screen identifies frequently methylated genes in haematological and epithelial cancers. *Mol Cancer.* 2010; 9:44. [PubMed: 20184741]
- Duran A, Linares JF, Galvez AS, Wikenheiser K, Flores JM, Diaz-Meco MT, et al. The signaling adaptor p62 is an important NF-kappaB mediator in tumorigenesis. *Cancer Cell.* 2008; 13(4):343–354. [PubMed: 18394557]
- Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science.* 2011; 331(6016):456–461. [PubMed: 21205641]
- Eum KH, Lee M. Crosstalk between autophagy and apoptosis in the regulation of paclitaxel-induced cell death in v-Ha-ras-transformed fibroblasts. *Mol Cell Biochem.* 2011; 348(1-2):61–68. [PubMed: 21069434]

- Evangelisti C, Ricci F, Tazzari P, Tabellini G, Battistelli M, Falcieri E, et al. Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia. *Leukemia*. 2011
- Fabre C, Carvalho G, Tasdemir E, Braun T, Ades L, Grosjean J, et al. NF-kappaB inhibition sensitizes to starvation-induced cell death in high-risk myelodysplastic syndrome and acute myeloid leukemia. *Oncogene*. 2007; 26(28):4071–4083. [PubMed: 17213804]
- Fanzani A, Zanola A, Rovetta F, Rossi S, Aleo MF. Cisplatin triggers atrophy of skeletal C2C12 myotubes via impairment of Akt signalling pathway and subsequent increment activity of proteasome and autophagy systems. *Toxicol Appl Pharmacol*. 2011; 250(3):312–321. [PubMed: 21074548]
- Farag SS, Zhang S, Jansak BS, Wang X, Kraut E, Chan K, et al. Phase II trial of temsirolimus in patients with relapsed or refractory multiple myeloma. *Leuk Res*. 2009; 33(11):1475–1480. [PubMed: 19261329]
- Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, et al. Ambra1 regulates autophagy and development of the nervous system. *Nature*. 2007; 447(7148):1121–1125. [PubMed: 17589504]
- Fujii S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, et al. Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. *Cancer Sci*. 2008; 99(9):1813–1819. [PubMed: 18616529]
- Fujishita T, Aoki K, Lane HA, Aoki M, Taketo MM. Inhibition of the mTORC1 pathway suppresses intestinal polyp formation and reduces mortality in ApcDelta716 mice. *Proc Natl Acad Sci U S A*. 2008; 105(36):13544–13549. [PubMed: 18768809]
- Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell*. 2008; 19(5):2092–2100. [PubMed: 18321988]
- Fujiwara K, Iwado E, Mills GB, Sawaya R, Kondo S, Kondo Y. Akt inhibitor shows anticancer and radiosensitizing effects in malignant glioma cells by inducing autophagy. *Int J Oncol*. 2007; 31(4):753–760. [PubMed: 17786305]
- Fung AS, Wu L, Tannock IF. Concurrent and sequential administration of chemotherapy and the Mammalian target of rapamycin inhibitor temsirolimus in human cancer cells and xenografts. *Clin Cancer Res*. 2009; 15(17):5389–5395. [PubMed: 19706800]
- Garcia JA, Danielpour D. Mammalian target of rapamycin inhibition as a therapeutic strategy in the management of urologic malignancies. *Mol Cancer Ther*. 2008; 7(6):1347–1354. [PubMed: 18566209]
- Garcia-Escudero V, Gargini R. Autophagy induction as an efficient strategy to eradicate tumors. *Autophagy*. 2008; 4(7):923–925. [PubMed: 18716458]
- Geng J, Klionsky DJ. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. ‘Protein modifications: beyond the usual suspects’ review series. *EMBO Rep*. 2008; 9(9):859–864. [PubMed: 18704115]
- Geser A, Brubaker G, Draper CC. Effect of a malaria suppression program on the incidence of African Burkitt’s lymphoma. *Am J Epidemiol*. 1989; 129(4):740–752. [PubMed: 2923122]
- Gewirtz DA, Hilliker ML, Wilson EN. Promotion of autophagy as a mechanism for radiation sensitization of breast tumor cells. *Radiother Oncol*. 2009; 92(3):323–328. [PubMed: 19541381]
- Gills JJ, Lopiccolo J, Dennis PA. Nelfinavir, a new anti-cancer drug with pleiotropic effects and many paths to autophagy. *Autophagy*. 2008; 4(1):107–109. [PubMed: 18000394]
- Gills JJ, Lopiccolo J, Tsurutani J, Shoemaker RH, Best CJ, Abu-Asab MS, et al. Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis in vitro and in vivo. *Clin Cancer Res*. 2007; 13(17):5183–5194. [PubMed: 17785575]
- Gossner G, Choi M, Tan L, Fogoros S, Griffith KA, Kuenker M, et al. Genistein-induced apoptosis and autophagocytosis in ovarian cancer cells. *Gynecol Oncol*. 2007; 105(1):23–30. [PubMed: 17234261]
- Gozuacik D, Kimchi A. DAPk protein family and cancer. *Autophagy*. 2006; 2(2):74–79. [PubMed: 17139808]

- Grander D, Kharaziha P, Laane E, Pokrovskaja K, Panaretakis T. Autophagy as the main means of cytotoxicity by glucocorticoids in hematological malignancies. *Autophagy*. 2009; 5(8):1198–1200. [PubMed: 19855186]
- Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev*. 2011
- Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*. 2006; 441(7095):885–889. [PubMed: 16625204]
- Heist RS, Fain J, Chinnasami B, Khan W, Molina JR, Sequist LV, et al. Phase I/II study of AT-101 with topotecan in relapsed and refractory small cell lung cancer. *J Thorac Oncol*. 2010; 5(10): 1637–1643. [PubMed: 20808253]
- Herman-Antosiewicz A, Johnson DE, Singh SV. Sulforaphane causes autophagy to inhibit release of cytochrome C and apoptosis in human prostate cancer cells. *Cancer Res*. 2006; 66(11):5828–5835. [PubMed: 16740722]
- Hersey P, Bastholt L, Chiarion-Sileni V, Cinat G, Dummer R, Eggermont AM, et al. Small molecules and targeted therapies in distant metastatic disease. *Ann Oncol*. 2009; 20(Suppl 6):vi35–40. [PubMed: 19617296]
- Hippert MM, O'Toole PS, Thorburn A. Autophagy in cancer: good, bad, or both? *Cancer Res*. 2006; 66(19):9349–9351. [PubMed: 17018585]
- Hoyer-Hansen M, Bastholm L, Mathiasen IS, Elling F, Jaattela M. Vitamin D analog EB1089 triggers dramatic lysosomal changes and Beclin 1-mediated autophagic cell death. *Cell Death Differ*. 2005; 12(10):1297–1309. [PubMed: 15905882]
- Itakura E, Kishi C, Inoue K, Mizushima N. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell*. 2008; 19(12):5360–5372. [PubMed: 18843052]
- Ito H, Daido S, Kanzawa T, Kondo S, Kondo Y. Radiation-induced autophagy is associated with LC3 and its inhibition sensitizes malignant glioma cells. *Int J Oncol*. 2005; 26(5):1401–1410. [PubMed: 15809734]
- Jerusalem G, Fasolo A, Dieras V, Cardoso F, Bergh J, Vittori L, et al. Phase I trial of oral mTOR inhibitor everolimus in combination with trastuzumab and vinorelbine in pre-treated patients with HER2-overexpressing metastatic breast cancer. *Breast Cancer Res Treat*. 2011; 125(2):447–455. [PubMed: 21107682]
- Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell*. 2009; 20(7):1992–2003. [PubMed: 19225151]
- Kang MH, Reynolds CP. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. *Clin Cancer Res*. 2009; 15(4):1126–1132. [PubMed: 19228717]
- Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ*. 2011
- Kanzawa T, Kondo Y, Ito H, Kondo S, Germano I. Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. *Cancer Res*. 2003; 63(9):2103–2108. [PubMed: 12727826]
- Kapty J, Murray D, Mercer J. Radiotracers for noninvasive molecular imaging of tumor cell death. *Cancer Biother Radiopharm*. 2010; 25(6):615–628. [PubMed: 21204755]
- Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev*. 2007; 21(13):1621–1635. [PubMed: 17606641]
- Karpathiou G, Sivridis E, Koukourakis M, Mikroulis D, Bouros D, Froudarakis M, et al. LC3A autophagic activity and prognostic significance in non-small cell lung carcinomas. *Chest*. 2010
- Kawaguchi T, Miyazawa K, Moriya S, Ohtomo T, Che XF, Naito M, et al. Combined treatment with bortezomib plus bafilomycin A1 enhances the cytotoxic effect and induces endoplasmic reticulum stress in U266 myeloma cells: Crosstalk among proteasome, autophagy-lysosome and ER stress. *Int J Oncol*. 2011; 38(3):643–654. [PubMed: 21174067]
- Kessel D, Vicente MG, Reiners JJ Jr. Initiation of apoptosis and autophagy by photodynamic therapy. *Autophagy*. 2006; 2(4):289–290. [PubMed: 16921269]

- Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* 2011; 13(2):132–141. [PubMed: 21258367]
- Kim KW, Moretti L, Mitchell LR, Jung DK, Lu B. Combined Bcl-2/mammalian target of rapamycin inhibition leads to enhanced radiosensitization via induction of apoptosis and autophagy in non-small cell lung tumor xenograft model. *Clin Cancer Res.* 2009; 15(19):6096–6105. [PubMed: 19773376]
- Kim RH, Bold RJ, Kung HJ. ADI, autophagy and apoptosis: metabolic stress as a therapeutic option for prostate cancer. *Autophagy.* 2009; 5(4):567–568. [PubMed: 19276647]
- Kim SY, Oh YL, Kim KM, Jeong EG, Kim MS, Yoo NJ, et al. Decreased expression of Bax-interacting factor-1 (Bif-1) in invasive urinary bladder and gallbladder cancers. *Pathology.* 2008; 40(6):553–557. [PubMed: 18752120]
- Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. *Mol Cell.* 2009; 34(3):259–269. [PubMed: 19450525]
- Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol.* 2007; 8(11):931–937. [PubMed: 17712358]
- Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy.* 2008; 4(2):151–175. [PubMed: 18188003]
- Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature.* 2006; 441(7095):880–884. [PubMed: 16625205]
- Kondo Y, Kanzawa T, Sawaya R, Kondo S. The role of autophagy in cancer development and response to therapy. *Nat Rev Cancer.* 2005; 5(9):726–734. [PubMed: 16148885]
- Koren I, Reem E, Kimchi A. Autophagy gets a brake: DAP1, a novel mTOR substrate, is activated to suppress the autophagic process. *Autophagy.* 2010a; 6(8):1179–1180. [PubMed: 20818178]
- Koren I, Reem E, Kimchi A. DAP1, a Novel Substrate of mTOR, Negatively Regulates Autophagy. *Curr Biol.* 2010b
- Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. *Mol Cell.* 2009; 33(4):517–527. [PubMed: 19250912]
- Koukourakis MI, Giatromanolaki A, Sivridis E, Pitiakoudis M, Gatter KC, Harris AL. Beclin 1 over- and underexpression in colorectal cancer: distinct patterns relate to prognosis and tumour hypoxia. *Br J Cancer.* 2010; 103(8):1209–1214. [PubMed: 20842118]
- Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol.* 2008; 9(12):1004–1010. [PubMed: 18971948]
- Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med.* 2010; 363(19):1801–1811. [PubMed: 21047224]
- Kusama Y, Sato K, Kimura N, Mitamura J, Ohdaira H, Yoshida K. Comprehensive analysis of expression pattern and promoter regulation of human autophagy-related genes. *Apoptosis.* 2009; 14(10):1165–1175. [PubMed: 19657746]
- Lamark T, Perander M, Outzen H, Kristiansen K, Overvatn A, Michaelsen E, et al. Interaction codes within the family of mammalian Phox and Bem1p domaincontaining proteins. *J Biol Chem.* 2003; 278(36):34568–34581. [PubMed: 12813044]
- Lazova R, Klump V, Pawelek J. Autophagy in cutaneous malignant melanoma. *J Cutan Pathol.* 2010; 37(2):256–268. [PubMed: 19615007]
- Lee JW, Jeong EG, Soung YH, Nam SW, Lee JY, Yoo NJ, et al. Decreased expression of tumour suppressor Bax-interacting factor-1 (Bif-1), a Bax activator, in gastric carcinomas. *Pathology.* 2006; 38(4):312–315. [PubMed: 16916719]
- Lei HY, Chang CP. Induction of autophagy by concanavalin A and its application in anti-tumor therapy. *Autophagy.* 2007; 3(4):402–404. [PubMed: 17471013]
- Leu JJ, Pimkina J, Frank A, Murphy ME, George DL. A small molecule inhibitor of inducible heat shock protein 70. *Mol Cell.* 2009; 36(1):15–27. [PubMed: 19818706]

- Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008; 132(1):27–42. [PubMed: 18191218]
- Lian J, Wu X, He F, Karnak D, Tang W, Meng Y, et al. A natural BH3 mimetic induces autophagy in apoptosis-resistant prostate cancer via modulating Bcl-2-Beclin1 interaction at endoplasmic reticulum. *Cell Death Differ*. 2011; 18(1):60–71. [PubMed: 20577262]
- Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, et al. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol*. 2006; 8(7):688–699. [PubMed: 16799551]
- Liang C, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, et al. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol*. 2008; 10(7):776–787. [PubMed: 18552835]
- Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*. 1999; 402(6762):672–676. [PubMed: 10604474]
- Lin CI, Whang EE, Donner DB, Du J, Lorch J, He F, et al. Autophagy induction with RAD001 enhances chemosensitivity and radiosensitivity through Met inhibition in papillary thyroid cancer. *Mol Cancer Res*. 2010; 8(9):1217–1226. [PubMed: 20736296]
- Lin MH, Liu SY, Liu YC. Autophagy induction by a natural ingredient of areca nut. *Autophagy*. 2008; 4(7):967–968. [PubMed: 18758233]
- Liu XW, Su Y, Zhu H, Cao J, Ding WJ, Zhao YC, et al. HIF-1 α -dependent autophagy protects HeLa cells from fenretinide (4-HPR)-induced apoptosis in hypoxia. *Pharmacol Res*. 2010; 62(5):416–425. [PubMed: 20637870]
- Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, et al. Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Mol Biol Cell*. 2011; 22(2):165–178. [PubMed: 21119005]
- Lomonaco SL, Finniss S, Xiang C, Decarvalho A, Umansky F, Kalkanis SN, et al. The induction of autophagy by gamma-radiation contributes to the radioresistance of glioma stem cells. *Int J Cancer*. 2009; 125(3):717–722. [PubMed: 19431142]
- Lopez G, Torres K, Lev D. Autophagy blockade enhances HDAC inhibitors' proapoptotic effects: Potential implications for the treatment of a therapeutic-resistant malignancy. *Autophagy*. 2011; 7(4)
- Ma X, Piao S, Wang DW, McAfee QW, Nathanson KL, Lum JJ, et al. Measurements of tumor cell autophagy predict invasiveness, resistance to chemotherapy, and survival in melanoma. *Clin Cancer Res*. 2011
- Maclean KH, Dorsey FC, Cleveland JL, Kastan MB. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. *J Clin Invest*. 2008; 118(1):79–88. [PubMed: 18097482]
- Maiuri MC, Tasdemir E, Criollo A, Morselli E, Vicencio JM, Carnuccio R, et al. Control of autophagy by oncogenes and tumor suppressor genes. *Cell Death Differ*. 2009; 16(1):87–93. [PubMed: 18806760]
- Margolin K, Longmate J, Baratta T, Synold T, Christensen S, Weber J, et al. CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. *Cancer*. 2005; 104(5):1045–1048. [PubMed: 16007689]
- Marino G, Fernandez AF, Lopez-Otin C. Autophagy and aging: lessons from progeria models. *Adv Exp Med Biol*. 2010; 694:61–68. [PubMed: 20886757]
- Marino G, Salvador-Montoliu N, Fueyo A, Knecht E, Mizushima N, Lopez-Otin C. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *J Biol Chem*. 2007; 282(25):18573–18583. [PubMed: 17442669]
- Marinov M, Ziogas A, Pardo OE, Tan LT, Dhillon T, Mauri FA, et al. AKT/mTOR pathway activation and BCL-2 family proteins modulate the sensitivity of human small cell lung cancer cells to RAD001. *Clin Cancer Res*. 2009; 15(4):1277–1287. [PubMed: 19228731]
- Martinez-Outschoorn UE, Trimmer C, Lin Z, Whitaker-Menezes D, Chiavarina B, Zhou J, et al. Autophagy in cancer associated fibroblasts promotes tumor cell survival: Role of hypoxia, HIF1

- induction and NFkappaB activation in the tumor stromal microenvironment. *Cell Cycle*. 2010; 9(17):3515–3533. [PubMed: 20855962]
- Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. *Nat Rev Cancer*. 2007; 7(12):961–967. [PubMed: 17972889]
- Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. *Cell*. 2009; 137(6):1062–1075. [PubMed: 19524509]
- Mathew R, Kongara S, Beaudoin B, Karp CM, Bray K, Degenhardt K, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev*. 2007; 21(11):1367–1381. [PubMed: 17510285]
- Miracco C, Cevenini G, Franchi A, Luzi P, Cosci E, Mourmouras V, et al. Beclin 1 and LC3 autophagic gene expression in cutaneous melanocytic lesions. *Hum Pathol*. 2010; 41(4):503–512. [PubMed: 20004946]
- Mizushima N. Autophagy: process and function. *Genes Dev*. 2007; 21(22):2861–2873. [PubMed: 18006683]
- Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. *Cell*. 2010; 140(3):313–326. [PubMed: 20144757]
- Morgan TM, Pitts TE, Gross TS, Poliachik SL, Vessella RL, Corey E. RAD001 (Everolimus) inhibits growth of prostate cancer in the bone and the inhibitory effects are increased by combination with docetaxel and zoledronic acid. *Prostate*. 2008; 68(8):861–871. [PubMed: 18361409]
- Moscat J, Diaz-Meco MT. p62 at the crossroads of autophagy, apoptosis, and cancer. *Cell*. 2009; 137(6):1001–1004. [PubMed: 19524504]
- Mukoubou H, Tsujimura T, Sasaki R, Ku Y. The role of autophagy in the treatment of pancreatic cancer with gemcitabine and ionizing radiation. *Int J Oncol*. 2010; 37(4):821–828. [PubMed: 20811703]
- Nahta R, O'Regan RM. Evolving Strategies for Overcoming Resistance to HER2-Directed Therapy: Targeting the PI3K/Akt/mTOR Pathway. *Clin Breast Cancer*. 2010; 10:S72–78. [PubMed: 21115425]
- Natsumeda M, Aoki H, Miyahara H, Yajima N, Uzuka T, Toyoshima Y, et al. Induction of autophagy in temozolomide treated malignant gliomas. *Neuropathology*. 2011
- Nicotra G, Mercalli F, Peracchio C, Castino R, Follo C, Valente G, et al. Autophagy-active beclin-1 correlates with favourable clinical outcome in non-Hodgkin lymphomas. *Mod Pathol*. 2010; 23(7):937–950. [PubMed: 20473282]
- Noble CG, Dong JM, Manser E, Song H. Bcl-xL and UVRAG cause a monomer-dimer switch in Beclin1. *J Biol Chem*. 2008; 283(38):26274–26282. [PubMed: 18641390]
- O'Donovan TR, O'Sullivan GC, McKenna S. Induction of autophagy by drug-resistant esophageal cancer cells promotes their survival and recovery following treatment with chemotherapeutics. *Autophagy*. 2011; 7(6)
- Ohsumi Y, Mizushima N. Two ubiquitin-like conjugation systems essential for autophagy. *Semin Cell Dev Biol*. 2004; 15(2):231–236. [PubMed: 15209383]
- Pajonk F, Vlashi E, McBride WH. Radiation resistance of cancer stem cells: the 4 R's of radiobiology revisited. *Stem Cells*. 2010; 28(4):639–648. [PubMed: 20135685]
- Papandreou I, Lim AL, Laderoute K, Denko NC. Hypoxia signals autophagy in tumor cells via AMPK activity, independent of HIF-1, BNIP3, and BNIP3L. *Cell Death Differ*. 2008; 15(10):1572–1581. [PubMed: 18551130]
- Pardo R, Lo Re A, Archange C, Ropolo A, Papademetrio DL, Gonzalez CD, et al. Gemcitabine induces the VMP1-mediated autophagy pathway to promote apoptotic death in human pancreatic cancer cells. *Pancreatol*. 2010; 10(1):19–26. [PubMed: 20299819]
- Park MA, Zhang G, Norris J, Hylemon PB, Fisher PB, Grant S, et al. Regulation of autophagy by ceramide-CD95-PERK signaling. *Autophagy*. 2008; 4(7):929–931. [PubMed: 18719356]
- Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell*. 2005; 122(6):927–939. [PubMed: 16179260]
- Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, et al. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *J Clin Invest*. 2008; 118(6):2190–2199. [PubMed: 18497889]

- Pirtoli L, Cevenini G, Tini P, Vannini M, Oliveri G, Marsili S, et al. The prognostic role of Beclin 1 protein expression in high-grade gliomas. *Autophagy*. 2009; 5(7):930–936. [PubMed: 19556884]
- Powolny AA, Bommarreddy A, Hahm ER, Normolle DP, Beumer JH, Nelson JB, et al. Chemopreventative Potential of the Cruciferous Vegetable Constituent Phenethyl Isothiocyanate in a Mouse Model of Prostate Cancer. *J Natl Cancer Inst*. 2011
- Puissant A, Robert G, Auberger P. Targeting autophagy to fight hematopoietic malignancies. *Cell Cycle*. 2010; 9(17):3470–3478. [PubMed: 20703092]
- Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest*. 2003; 112(12):1809–1820. [PubMed: 14638851]
- Raben N, Schreiner C, Baum R, Takikita S, Xu S, Xie T, et al. Suppression of autophagy permits successful enzyme replacement therapy in a lysosomal storage disorder--murine Pompe disease. *Autophagy*. 2010; 6(8):1078–1089. [PubMed: 20861693]
- Racanelli AC, Rothbart SB, Heyer CL, Moran RG. Therapeutics by cytotoxic metabolite accumulation: pemetrexed causes ZMP accumulation, AMPK activation, and mammalian target of rapamycin inhibition. *Cancer Res*. 2009; 69(13):5467–5474. [PubMed: 19549896]
- Ramakrishnan S, Nguyen TM, Subramanian IV, Kelekar A. Autophagy and angiogenesis inhibition. *Autophagy*. 2007; 3(5):512–515. [PubMed: 17643071]
- Ren JH, He WS, Nong L, Zhu QY, Hu K, Zhang RG, et al. Acquired cisplatin resistance in human lung adenocarcinoma cells is associated with enhanced autophagy. *Cancer Biother Radiopharm*. 2010; 25(1):75–80. [PubMed: 20187799]
- Rez G, Csak J, Fellingner E, Laszlo L, Kovacs AL, Oliva O, et al. Time course of vinblastine-induced autophagocytosis and changes in the endoplasmic reticulum in murine pancreatic acinar cells: a morphometric and biochemical study. *Eur J Cell Biol*. 1996; 71(4):341–350. [PubMed: 8980904]
- Rivera E, Gomez H. Chemotherapy resistance in metastatic breast cancer: the evolving role of ixabepilone. *Breast Cancer Res*. 2010; 12(Suppl 2):S2. [PubMed: 21050423]
- Rodriguez A, Duran A, Selloum M, Champy MF, Diez-Guerra FJ, Flores JM, et al. Mature-onset obesity and insulin resistance in mice deficient in the signaling adapter p62. *Cell Metab*. 2006; 3(3):211–222. [PubMed: 16517408]
- Rosenfeldt MT, Ryan KM. The role of autophagy in tumour development and cancer therapy. *Expert Rev Mol Med*. 2009; 11:e36. [PubMed: 19951459]
- Rothbart SB, Racanelli AC, Moran RG. Pemetrexed indirectly activates the metabolic kinase AMPK in human carcinomas. *Cancer Res*. 2010; 70(24):10299–10309. [PubMed: 21159649]
- Roulin D, Cerantola Y, Dormond-Meuwly A, Demartines N, Dormond O. Targeting mTORC2 inhibits colon cancer cell proliferation in vitro and tumor formation in vivo. *Mol Cancer*. 2010; 9:57. [PubMed: 20226010]
- Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkoul K, et al. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest*. 2010; 120(1):127–141. [PubMed: 20038797]
- Roy S, Debnath J. Autophagy and tumorigenesis. *Semin Immunopathol*. 2010; 32(4):383–396. [PubMed: 20589500]
- Salazar M, Carracedo A, Salanueva IJ, Hernandez-Tiedra S, Lorente M, Egia A, et al. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *J Clin Invest*. 2009; 119(5):1359–1372. [PubMed: 19425170]
- Salomoni P, Calabretta B. Targeted therapies and autophagy: new insights from chronic myeloid leukemia. *Autophagy*. 2009; 5(7):1050–1051. [PubMed: 19713759]
- Saunders P, Cisterne A, Weiss J, Bradstock KF, Bendall LJ. The mammalian target of rapamycin inhibitor RAD001 (everolimus) synergizes with chemotherapeutic agents, ionizing radiation and proteasome inhibitors in pre-B acute lymphocytic leukemia. *Haematologica*. 2011; 96(1):69–77. [PubMed: 20952516]
- Savaraj N, You M, Wu C, Wangpaichitr M, Kuo MT, Feun LG. Arginine deprivation, autophagy, apoptosis (AAA) for the treatment of melanoma. *Curr Mol Med*. 2010; 10(4):405–412. [PubMed: 20459375]

- Shao Y, Gao Z, Marks PA, Jiang X. Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. *Proc Natl Acad Sci U S A*. 2004; 101(52):18030–18035. [PubMed: 15596714]
- Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nat Cell Biol*. 2004; 6(12):1221–1228. [PubMed: 15558033]
- Simms-Waldrup T, Rodriguez-Gonzalez A, Lin T, Ikeda AK, Fu C, Sakamoto KM. The aggresome pathway as a target for therapy in hematologic malignancies. *Mol Genet Metab*. 2008; 94(3): 283–286. [PubMed: 18472289]
- Simonsen A, Tooze SA. Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J Cell Biol*. 2009; 186(6):773–782. [PubMed: 19797076]
- Sohma I, Fujiwara Y, Sugita Y, Yoshioka A, Shirakawa M, Moon JH, et al. Parthenolide, An NF- κ B Inhibitor, Suppresses Tumor Growth and Enhances Response to Chemotherapy in Gastric Cancer. *Cancer Genomics Proteomics*. 2011; 8(1):39–47. [PubMed: 21289336]
- Sotelo J, Briceno E, Lopez-Gonzalez MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2006; 144(5):337–343. [PubMed: 16520474]
- Staehler M, Haseke N, Khoder W, Stief CG. Profile of temsirolimus in the treatment of advanced renal cell carcinoma. *Onco Targets Ther*. 2010; 3:191–196. [PubMed: 21049085]
- Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, et al. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol*. 2007; 9(10):1142–1151. [PubMed: 17891140]
- Tan EY, Campo L, Han C, Turley H, Pezzella F, Gatter KC, et al. BNIP3 as a progression marker in primary human breast cancer; opposing functions in situ versus invasive cancer. *Clin Cancer Res*. 2007; 13(2 Pt 1):467–474. [PubMed: 17255267]
- Tarhini A, Kotsakis A, Gooding W, Shuai Y, Petro D, Friedland D, et al. Phase II study of everolimus (RAD001) in previously treated small cell lung cancer. *Clin Cancer Res*. 2010; 16(23):5900–5907. [PubMed: 21045083]
- Thallinger C, Poepl W, Pratscher B, Mayerhofer M, Valent P, Tappeiner G, et al. CCI-779 plus cisplatin is highly effective against human melanoma in a SCID mouse xenotransplantation model. *Pharmacology*. 2007; 79(4):207–213. [PubMed: 17377418]
- Thorburn J, Horita H, Redzic J, Hansen K, Frankel AE, Thorburn A. Autophagy regulates selective HMGB1 release in tumor cells that are destined to die. *Cell Death Differ*. 2009; 16(1):175–183. [PubMed: 18846108]
- Tolkovsky AM. Autophagy thwarts muscle disease. *Nat Med*. 2010; 16(11):1188–1190. [PubMed: 21052066]
- Turcotte S, Chan DA, Sutphin PD, Hay MP, Denny WA, Giaccia AJ. A molecule targeting VHL-deficient renal cell carcinoma that induces autophagy. *Cancer Cell*. 2008; 14(1):90–102. [PubMed: 18598947]
- Turcotte S, Giaccia AJ. Targeting cancer cells through autophagy for anticancer therapy. *Curr Opin Cell Biol*. 2010; 22(2):246–251. [PubMed: 20056398]
- Turcotte S, Sutphin PD, Giaccia AJ. Targeted therapy for the loss of von Hippel-Lindau in renal cell carcinoma: a novel molecule that induces autophagic cell death. *Autophagy*. 2008; 4(7):944–946. [PubMed: 18769110]
- Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009; 324(5930):1029–1033. [PubMed: 19460998]
- Vazquez CL, Colombo MI. Assays to assess autophagy induction and fusion of autophagic vacuoles with a degradative compartment, using monodansylcadaverine (MDC) and DQ-BSA. *Methods Enzymol*. 2009; 452:85–95. [PubMed: 19200877]
- Voss V, Senft C, Lang V, Ronellenfisch MW, Steinbach JP, Seifert V, et al. The pan-Bcl-2 inhibitor (-)-gossypol triggers autophagic cell death in malignant glioma. *Mol Cancer Res*. 2010; 8(7): 1002–1016. [PubMed: 20587533]
- Wang RC, Levine B. Autophagy in cellular growth control. *FEBS Lett*. 2010; 584(7):1417–1426. [PubMed: 20096689]

- Won KY, Kim GY, Kim YW, Song JY, Lim SJ. Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. *Hum Pathol.* 2010; 41(1):107–112. [PubMed: 19762066]
- Wu H, Yang JM, Jin S, Zhang H, Hait WN. Elongation factor-2 kinase regulates autophagy in human glioblastoma cells. *Cancer Res.* 2006; 66(6):3015–3023. [PubMed: 16540650]
- Wu L, Birle DC, Tannock IF. Effects of the mammalian target of rapamycin inhibitor CCI-779 used alone or with chemotherapy on human prostate cancer cells and xenografts. *Cancer Res.* 2005; 65(7):2825–2831. [PubMed: 15805283]
- Wu WK, Sakamoto KM, Milani M, Aldana-Masangkay G, Fan D, Wu K, et al. Macroautophagy modulates cellular response to proteasome inhibitors in cancer therapy. *Drug Resist Updat.* 2010; 13(3):87–92. [PubMed: 20462785]
- Wu Z, Chang PC, Yang JC, Chu CY, Wang LY, Chen NT, et al. Autophagy Blockade Sensitizes Prostate Cancer Cells towards Src Family Kinase Inhibitors. *Genes Cancer.* 2010; 1(1):40–49. [PubMed: 20811583]
- Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell.* 2006; 124(3):471–484. [PubMed: 16469695]
- Xie Z, Klionsky DJ. Autophagosome formation: core machinery and adaptations. *Nat Cell Biol.* 2007; 9(10):1102–1109. [PubMed: 17909521]
- Xing C, Zhu B, Liu H, Yao H, Zhang L. Class I phosphatidylinositol 3-kinase inhibitor LY294002 activates autophagy and induces apoptosis through p53 pathway in gastric cancer cell line SGC7901. *Acta Biochim Biophys Sin (Shanghai).* 2008; 40(3):194–201. [PubMed: 18330473]
- Yacoub A, Hamed HA, Allegood J, Mitchell C, Spiegel S, Lesniak MS, et al. PERK-dependent regulation of ceramide synthase 6 and thioredoxin play a key role in mda-7/IL-24-induced killing of primary human glioblastoma multiforme cells. *Cancer Res.* 2010; 70(3):1120–1129. [PubMed: 20103619]
- Yan H, Frost P, Shi Y, Hoang B, Sharma S, Fisher M, et al. Mechanism by which mammalian target of rapamycin inhibitors sensitize multiple myeloma cells to dexamethasone-induced apoptosis. *Cancer Res.* 2006; 66(4):2305–2313. [PubMed: 16489035]
- Yang PM, Liu YL, Lin YC, Shun CT, Wu MS, Chen CC. Inhibition of autophagy enhances anticancer effects of atorvastatin in digestive malignancies. *Cancer Res.* 2010; 70(19):7699–7709. [PubMed: 20876807]
- Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruzsniewski P, et al. Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. *J Clin Oncol.* 2010; 28(1):69–76. [PubMed: 19933912]
- Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, et al. Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med.* 2011; 364(6):514–523. [PubMed: 21306238]
- Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci U S A.* 2003; 100(25):15077–15082. [PubMed: 14657337]
- Zeng X, Yan T, Schupp JE, Seo Y, Kinsella TJ. DNA mismatch repair initiates 6-thioguanine--induced autophagy through p53 activation in human tumor cells. *Clin Cancer Res.* 2007; 13(4):1315–1321. [PubMed: 17317843]
- Zhang YJ, Dai Q, Sun DF, Xiong H, Tian XQ, Gao FH, et al. mTOR signaling pathway is a target for the treatment of colorectal cancer. *Ann Surg Oncol.* 2009; 16(9):2617–2628. [PubMed: 19517193]
- Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat Cell Biol.* 2009; 11(4):468–476. [PubMed: 19270693]
- Zois CE, Koukourakis MI. Radiation-induced autophagy in normal and cancer cells: towards novel cytoprotection and radio-sensitization policies? *Autophagy.* 2009; 5(4):442–450. [PubMed: 19164950]

Fig. 1.

Table 1

Selected List of Cancer Therapies Shown to Induce Autophagy

Mechanism	Treatment	Reference
Alkylating agents	Temozolomide	(Natsumeda, et al., 2011)
	Cisplatin	(Fanzani, Zanola, Rovetta, Rossi, & Aleo, 2011)
Anti-metabolites	5-fluorouracil	(O'Donovan, O'Sullivan, & McKenna, 2011)
	6-thioguanine	(Zeng, Yan, Schupp, Seo, & Kinsella, 2007)
DNA damaging agents	Photodynamic therapy	(Kessel, Vicente, & Reiners, 2006)
	Radiation	(Ito, et al., 2005; Lomonaco, et al., 2009; Zois & Koukourakis, 2009)
Histone deacetylase inhibitors	Butyrate, suberoylanilide hydroxamic acid	(Shao, et al., 2004)
mTOR inhibitors	Rapamycin, temsirolimus, everolimus	(Ciuffreda, Di Sanza, Incani, & Milella, 2010)
Natural agents	Concanavalin A	(Lei & Chang, 2007)
	Curcumin	(Aoki, et al., 2007)
	Genistein	(Gossner, et al., 2007)
	Sulforaphane	(Herman-Antosiewicz, Johnson, & Singh, 2006)
	Vitamin D Analogues	(Hoyer-Hansen, Bastholm, Mathiasen, Elling, & Jaattela, 2005)
NF-kappaB inhibition	BAY11-7082,	(Fabre, et al., 2007)
	Parthenolide	(Sohma, et al., 2011)
Plant alkaloids	Etoposide	(Crowley, et al., 2011)
	Paclitaxel	(Eum & Lee, 2011)
	Vinblastine	(Rez, et al., 1996)
Proteasome inhibitor	Bortezomib	(W. K. Wu, et al., 2010)
Src family kinase inhibitors	saracatinib	(Z. Wu, et al., 2010)
Tyrosine kinase inhibitors	Imatinib, dasatinib	(Salomoni & Calabretta, 2009)
Miscellaneous	Arsenic trioxide	(Kanzawa, Kondo, Ito, Kondo, & Germano, 2003)
	Glucocorticoids	(Grander, Kharaziha, Laane, Pokrovskaja, & Panaretakis, 2009)
	Nelfinavir	(Gills, Lopiccolo, & Dennis, 2008)

Table 2

Clinical Trials Evaluating Autophagy

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
Breast	NCT01292408	II	HCQ	Post-biopsy, pre-surgery	Proof of principle, prevent tumor survival in hypoxic conditions	Recruiting	(Rouschop, et al., 2010)
	NCT00765765	I II	HCQ	Ixabepilone	Dose determination of HCQ, improve tumor response rate to ixabepilone, third line therapy	Recruiting	(Rivera & Gomez, 2010)
	NCT01023477	I II	CQ	Tamoxifen	Reduce ability of ductal carcinoma in situ to survive and spread prior to surgery	Recruiting	(Tan, et al., 2007)
Non-small cell lung cancer	NCT01009437	I II	None	Ritonavir Surgery	Determine effects of protease inhibitor, including autophagy biomarkers	Recruiting	(Gills, et al., 2007; W. K. Wu, et al., 2010)
	NCT00933803	I II	HCQ	Paclitaxel Carboplatin Bevacizumab	Evaluate for increased anti-tumor activity of standard therapy	Recruiting	(Karpathiou, et al., 2010)
	NCT00728845	I II	HCQ	Paclitaxel Carboplatin Bevacizumab	Dose determination of HCQ, improve tumor	Terminate d for slow accrual	(Ramakrishnan, Nguyen, Subramanian, & Kelekar, 2007)

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
					response rate to chemotherapy and anti-angiogenesis		
Small cell lung cancer	NCT00969306	I II	CQ	Cisplatin Etoposide Radiation	Dose determination of HCQ, prevent tumor survival in hypoxic conditions	Recruiting	(Rouschop, et al., 2010) (Ren, et al., 2010)
Melanoma	NCT00962845	I	HCQ	Surgery	Characterize HCQ effects on markers of autophagy (p62, Beclin1, LC3, GRp170)	Not yet recruiting	(Savaraj, et al., 2010)
	NCT01092728	II	None	Dasatinib	Assess apoptosis, autophagy, and cell proliferation markers after treatment with dasatinib	Not yet recruiting	(Lazova, Klump, & Pawelek, 2010; Miracco, et al., 2010)
Rectal/Colon Adeno-carcinoma	NCT01206530	I II	HCQ	Oxaliplatin 5-fluorouracil Bevacizumab	Compare tumor response to changes in autophagy markers (autophagosomes in PMNs, expression of autophagy proteins)	Recruiting	(Koukourakis, et al., 2010)

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
	NCT01006369	II	HCQ	Capecitabine Oxaliplatin Bevacizumab	Assess progression-free survival with combination therapy with anti-angiogenesis	Recruiting	(Yang, et al., 2010)
Renal cell carcinoma	NCT01144169	I	HCQ	Surgery	Measure biologic markers of autophagy in tumor and normal surrounding cells, measure effects on immune cells	Recruiting	(Turcotte & Giaccia, 2010; Turcotte, Sulphin, et al., 2008)
Advanced Solid Tumors	NCT00909831	I	HCQ	Temsirolimus	Dose determination of HCQ in combination with an mTOR inhibitor	Recruiting	(Ciuffreda, et al., 2010)
	NCT01023737	I	HCQ	Vorinostat	Dose determination of HCQ in combination with an HDAC inhibitor	Recruiting	(Carew, Medina, et al., 2010; Lopez, Torres, & Lev, 2011)
	NCT01266057	I	HCQ	Sirolimus Sorinostat	Dose determination of HCQ in combination with an mTOR or HDAC inhibitor	Not yet Recruiting	(Carew, Medina, et al., 2010; Carew, et al., 2007; Ciuffreda, et al., 2010; Lopez, et al., 2011)

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
Prostate	NCT00786682	II	HCQ	Docetaxel	Assess antitumor activity of combination therapy	Recruiting	(R. H. Kim, Bold, & Kung, 2009)
Multiple myeloma	NCT00568880	I II	HCQ	Bortezomib	Dose determination of HCQ in combination with a protease inhibitor, confirm synergistic effect	Recruiting	(Kawaguchi, et al., 2011; W. K. Wu, et al., 2010)
Pancreas	NCT01128296	I II	HCQ	Gemcitabine Surgery	Establish the safety and biologic activity of combination therapy	Recruiting	
Hematologic malignancies	NCT01164709	I	None	Bortezomib Nelfinavir meyleate	Determine effect of combination therapy on unfolded protein response, AKT phosphorylation, and autophagy	Recruiting	(Puissant, Robert, & Auberger, 2010; Simms-Waldrip, et al., 2008)
	NCT01210274	0	None	Azacitidine	Observational and in vitro studies of apoptosis vs. autophagy in patient samples	Not yet recruiting	(Fabre, et al., 2007; Puissant, et al., 2010)

Table 3

Representative Clinical Trials With Autophagy Stimulation

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
Breast	NCT00411788	II	Rapamycin	Trastuzumab	Evaluation of mTOR inhibition in combination with HER2/neu monoclonal antibody	Recruiting	(Nahra & O'Regan, 2010)
	NCT01088893	II	Everolimus	Pre-surgery Anthracycline and/or taxane based neoadjuvant therapy	Measure change in biomarkers in pre/post surgery samples	Recruiting	(Jerusalem, et al., 2011)
Non-small cell lung cancer	NCT00079235	II	Temsirolimus	None	Evaluate response and progression-free survival in patients treated with mTOR inhibition	Complete, results pending	(Dhillon, et al., 2010; Marinov, et al., 2009)
	NCT00923273	I II	Sirolimus	Pemetrexed	Dose determination of sirolimus in combination with a folate antimetabolite, evaluate for synergistic effect	Recruiting	(Racanelli, Rothbart, Heyer, & Moran, 2009; Rothbart, Racanelli, & Moran, 2010)
Small cell lung cancer	NCT01079481	I II	Everolimus	Taxol	Dose determination of everolimus	Recruiting	(Tarfimi, et al., 2010)

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
					s in combination with a plant alkaloid, evaluate objective response		
	NCT00773955	II	(-)-gossypol	None	Evaluate tumor responses, correlation of Bcl-2 expression with response	Complete, results pending	(Heist, et al., 2010)
Melanoma	NCT01014351	II	Everolimus	Pacitaxel Carboplatin	Evaluate progression free survival with the addition of mTOR inhibitor	Active, not recruiting	(Hersey, et al., 2009)
	NCT01166126	II	Temsirolimus	Selumetinib	Evaluation of mTOR inhibition in combination with a MEK1/2 inhibitor	Recruiting	(Margolin, et al., 2005; Thallinger, et al., 2007)
Rectal/Colon Adeno- carcinoma	NCT00849550	I II	Everolimus	Oxaliplatin Folinic acid Fluorouracil Bevacizumab	Evaluate effect on progression free survival with addition of mTOR inhibition	Recruiting	(Bianco, et al., 2008; Fujishita, Aoki, Lane, Aoki, & Taketo, 2008)
	NCT00522665	I II	Everolimus	Irinotecan Cetuximab	Dose determination of temsirolimus in combination with a chemother	Recruiting	(Roulin, Cerantola, Dormond-Meuwly, Demartines, & Dormond, 2010; Zhang, et al., 2009)

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
Renal cell carcinoma					apy, evaluate for synergistic effect		
	NCT00830895	II	Everolimus	None	Evaluate effect on progression free survival with mTOR inhibition	Recruiting	(Anandappa, Hollingdale, & Eisen, 2010)
Advanced Solid Tumor	NCT01090466	I II	Temsirolimus	Gemcitabine Cisplatin	Dose determination of temsirolimus in combination with a chemotherapy, evaluate for synergistic effect	Recruiting	(Staehler, Haseke, Khoder, & Stief, 2010)
	NCT01155258	I	Temsirolimus	Vinorelbine	Evaluation of mTOR inhibition in combination with vinorelbine	Recruiting	(Fung, Wu, & Tannock, 2009)
Prostate	NCT01020305	I II	Temsirolimus	None	Evaluation of mTOR inhibition on prostate specific antigen levels	Recruiting	(Garcia & Danielpour, 2008; L. Wu, Birle, & Tannock, 2005)
	NCT00657982	II	Everolimus	None	Evaluation of radiologic response to mTOR inhibition	Recruiting	(Morgan, et al., 2008)

Tumor Type	Identifier	Phase	Autophagy Modulator	Treatment	Purpose	Status	Ref
Multiple myeloma	NCT00693433	I	Temsirolimus	Dexamethasone	Maximum tolerated dose determination of temsirolimus, evaluation of response to treatment and change in biologic markers (p70, AKT activation, PTEN)	Complete, results pending	(Farag, et al., 2009; Yan, et al., 2006)
		I II	Everolimus	Sorafenib	Toxicity profile, evaluate tumor response	Recruiting	(Coiffier & Ribrag, 2009)
Pancreas		II	Everolimus	None	Evaluate progression-free survival following mTOR inhibition	Completed	(Yao, et al., 2010; Yao, et al., 2011)