Clinical relevance of autophagy therapy in cancer: Investigating the current trends, and future prospects

Subhadip Mukhopadhyay, Niharika Sinha, Durgesh Nandini Das, Prashanta Kumar Panda, Prajna Paramita Naik, and Sujit Kumar Bhutia

Department of Life Science, National Institute of Technology, Rourkela, Odisha, India

Abstract

Oncophagy (cancer-related autophagy) has a complex dual character at different stages of tumor progression. It remains an important clinical problem to unravel the reasons that propel the shift in the role of oncophagy from tumor inhibition to a protective mechanism that shields full-blown malignancy. Most treatment strategies emphasize curbing protective oncophagy while triggering the oncophagy that is lethal to tumor cells. In this review, we focus on the trends in current therapeutics as well as various challenges in clinical trials to address the oncophagic dilemma and evaluate the potential of these developing therapies. A detailed analysis of the clinical and pre-clinical scenario of the anticancer medicines highlights the various inducers and inhibitors of autophagy. The ways in which tumor stage, the microenvironment and combination drug treatment continue to play an important tactical role are discussed. Moreover, autophagy targets also play a crucial role in developing the best possible solution to this oncophagy paradox. In this review, we provide a comprehensive update on the current clinical impact of autophagy-based cancer therapeutic drugs and try to lessen the gap between translational medicine and clinical science.

Keywords

Autophagy, apoptosis, cancer patients, drugs, therapy, oncophagy, clinical trials, translational medicine

History

Received 15 July 2015
Revised 3 October 2015
Accepted 18 December 2015
Published online 12 February 2016

Introduction

In 1963, based on electron microscopic observation in mammalian cells, Christian de Duve coined the term autophagy (Greek for “self-eating”). Since then, autophagy has emerged as a potential therapeutic tool for cancer, protein aggregopathies, and neurodegenerative and infectious diseases, to name a few. The three major types of autophagy are microautophagy, macroautophagy and chaperone-mediated autophagy. In microautophagy, bulk impaired cytosolic cargo...
trapped by invagination of the lysosomal membrane is engulfed by the lysosome. In *chaperone-mediated autophagy*, cargo proteins are transferred across the membrane of the lysosome in a complex with chaperone proteins, viz. Hsc-70, that are recognized by lysosomal membrane receptors; then, the cargo proteins in the lysosome are unfolded and degraded. The classical concept of *macropathic autophagy* (henceforth termed autophagy) is an active catabolic pathway that is triggered by stress. Autophagy stimulates the cell to ´self-eat´ and bypass the stress to maintain homeostasis. Any cellular stress stimulates the production of a crescent-shaped phagophore (or isolation membrane) that sequesters stress-damaged portions of the cell and matures into a ring-shaped autophagosome. The autophagosome fuses with the lysosome to give rise to the autolysosome in which the cell’s long-lived proteins and damaged organelles undergo recycling to generate amino acids and fatty acids. Aside from playing a pivotal role in homeostasis and developmental processes, autophagic defects are involved in several diseases, including cancer.

Initially during tumorigenesis, autophagy engulfs defective proteins and dysfunctional organelles and plays a lethal role. However, as stress levels intensify in the later stages of tumorigenesis, cells in the core of a tumor tend to face extreme starvation and hypoxia that trigger a switching of the role of autophagy from a lethal to protective one. Various chemotherapeutic drugs often induce this effect. Protective autophagy triggers unrestrained growth in cancer cells and ensures chemoresistance and tumor recurrence. From a cytoprotective phenomenon that manages cellular stress in cells, the mechanism goes on to exhibit a multitude of highly intricate effects in cancerous cells that remain distinct from the normal cellular pattern. Therefore, it would be justified to consider the autophagic involvement in a cancer cell as a distinct therapeutic theme of ´oncophagy´ (combination of onco, referring to cancer, and autophagy). Although the dual characteristics of oncophagy appear to be paradoxical, this may prove to be the Achilles’ heel of cancer as it is also an important hallmark of cancer stem cells (CSCs). The presently prescribed anticancer medicines trigger a stress response in the tumor to trigger autophagic stimuli. It remains to be investigated whether this autophagy is a nesissisis in disguise. The fact remains that autophagy-based treatments are making a solid entry into the pharmaceutical scenario because of their capability to attack the cancer cells. The specificity of targeting the connections involving autophagic pathway in tumorigenesis and proliferation may prove to be a key therapeutic approach. Unlike the tumor suppressive nature of apoptosis, the crucial role of oncophagy remains highly convoluted as it varies across the course of tumorigenesis. Besides inducing autophagic death in apoptosis-resistant cancer cells, highly advanced malignant cells often show imperfect autophagy machinery that supports the proposition that autophagy can be used to suppress tumor growth.

We focus this review on elucidating the current molecular mechanisms of autophagy and autophagic cell death in cancer. We summarize the clinical relevance of oncophagy by highlighting the potential of drug targets that stimulate or inhibit autophagy. We describe recent data on oncophagy-based cancer clinical investigations and highlight the present status, upcoming challenges and future therapeutic prospects from a global perspective of cancer clinical trial proceedings.

**Decrypting the autophagy network: autophagy in the time of stress**

An optimum basal autophagy level is involved in the maintenance of cellular homeostasis, and stress induction above a finite threshold results in autophagy induction. The most significant breakthrough in our understanding of the molecular pathway of autophagy came from yeast genetic analysis, and today more than 37 Atg (autophagy-related) genes have been identified. It is important to note that the basic autophagy machinery is conserved throughout eukaryotes. Different kinds of cellular stresses, such as pH fluctuation, osmotic imbalance, temperature perturbation and pharmacological modulation, initiate an autophagy response by inactivation of mTOR (mammalian target of rapamycin). mTOR comprises mTORC1 and mTORC2 complexes. In brief, the phosphatidylinositol-3-kinase (PI3K)-mTOR pathway is stimulated as different growth factors bind to its respective cognate receptors on the plasma membrane, thereby phosphorylating the receptor tyrosine moieties. This results in activation of PI3K, which in turn triggers Akt to inactivate the TSC1/2 (tuberous sclerosis complex 1/2) that finally activates the mTOR complex. Any alteration in the intracellular level of the ATP/AMP ratio leads to activation of the energy-sensor AMPK (AMP-activated protein kinase), which activates TSC1/2. Induction of genotoxic stress also leads to AMPK activation. During optimal nutrient supply, mTORC1 phosphorylates Ulk1/2, Atg13, hence inhibiting Ulk1/2 kinase activity. However, stress leads to hypophosphorylation of Ulk1/2, which releases Ulk from mTORC1 inhibition and brings about phosphorylation of Atg13 and FIP200-10. Consequently, Ulk1 dynamically engages Atg9 to extract lipids from diverse sources like endosomes, the nucleus, golgi bodies and ER (endoplasmic reticulum) to initiate the nucleation of the phagophore.

The developing phagophore continues to elongate and undergoes maturation under the guidance of class III phosphoinositide-3-kinases such as Vps34 (vacuolar protein sorting 34), which binds with Beclin 1 to catalyze PI3P (phosphatidylinositol-3-phosphate) production. The nascent curvature of the isolation membrane incorporated with PI3P is sensed by the BATS domain (Barkor/Atg14(L) autophagosome targeting sequence) of Atg14. Accumulation of Atg14 at the developing autophagic membrane site essentially maintains the curvature. Under stress conditions, Atg14 recruits Vps34 at the omegasomes (PI3P-rich ω-shaped structure formed at the periphery of ER). Binding between Vps34 and Beclin 1 is enhanced by Ambra1 (activating molecule in Beclin 1 regulated autophagy protein-1), Bif1 (Bax interacting factor-1) and UVRAG (ultraviolet radiation resistance-associated gene). However, Bcl-xL, Bcl2 and Rubicon (run domain Beclin 1 interacting cysteine-rich containing protein) inhibit this interaction. WIP11/WIP12 (WD repeat protein interacting with phosphoinositides) and DFCP1 (double FYVE domain containing protein), two PI3P effectors, contribute to this nascent elongating...
membrane. Recently, it has also been reported that mTORC1 impedes autophagosome and endosome maturation via phosphorylation of UVRAG. Another stress induced transmembrane protein, VMP1 (vacuole membrane protein 1), interacts with Beclin 1 to induce autophagy. A couple of ubiquitin-like conjugation units become involved during phagophore elongation through a chain of events. Atg12 interacts with Atg7 (E1 ubiquitin-like activating enzyme), following which Atg12 binds to Atg10 (E2-like ubiquitin carrier), thus associating Atg12 to the central region of Atg5 through the C-terminal end of Atg5. The N-terminal region of Atg5 binds with Atg16L, giving rise to the Atg12–Atg5–Atg16L multimeric complex, which is known as the Atg16L complex. Both Atg16 and Atg9 are transported from the plasma membrane to the autophagosome. A second ubiquitin-like system is involved in LC3 (microtubule associated protein light chain 3) processing. Atg4 cleaves LC3 to an active intermediate LC3-I by conjugating with E1-like Atg7 through an ATP-dependent process. Subsequently, Atg3 acting as E2-like carrier molecule interacts with LC3-I. The Atg16L complex acts as the E3 ligase for conjugation of LC3 with lipids to produce LC3-I–PE (phosphatidylethanolamine)-conjugate or LC3-II. In this regard, p62/SQSTM1 (sequestosome 1), which is present on ubiquitinated proteins, acts as a cognate adaptor molecule toward LC3 recognition during engulfment of the aggregated proteins. Another example of such an adaptor protein is Atg32, which is involved in the recognition of impaired mitochondrion by the autophagosome through the mitophagy process.

Completely mature autophagosomes dock with lysosomes through several membrane fusion proteins and give rise to autolysosomes. SNARE [soluble N-ethylmaleimide-sensitive fusion (NSF) attachment protein receptors] proteins actively engage themselves to mediate fusion of the autophagosome and lysosome. Different SNARE proteins facilitate in fusion events that are essential for autophagosome biogenesis. Docking of the cargo-enriched autophagosome onto the lysosome is aided by the LAMP2 (lysosome-associated membrane protein 2) protein. The ESCRT (endosomal sorting complex required for transport) machinery controls the process of recognition of cargo and moving it into endosomes. This machinery regulates the fusion of membranes with late autophagosomes, thereby monitoring the autophagic flux. Interestingly, the HOPS (homotypic fusion and vacuole protein sorting) complex was reported to interact with Syx17 (syntaxin 17), a newly identified autophagosomal SNARE, which is involved in processing the fusion between autophagosome and lysosome.

The autolysosome is the ultimate catabolic apparatus where the low pH contributed by the lysosome results in immediate degradation of the cargo load that is brought up by the autophagosome. Lysosomal proteases, hydrolases and permeases enhance the autolysosomal activity, resulting in the production of free fatty acids and amino acids. These are then readily absorbed into the cellular pool to counter the persisting stress situation of the cell. Upon withdrawal of stress, ALR (autolyosomal to lysosome reformation) occurs through a de novo synthesis of protolysosomes that mature into a functional lysosome. Lack of induced stress reactivates mTOR; this act triggers ALR and results in clathrin-mediated protolysosomal formation from the autolysosome. Protolysosomes end as mature lysosomes. Notably, a cytoprotective mechanism like autophagy that is initially triggered as an adaptive response to stress becomes a cause of cell death when the stress continues for an extended period; beyond this period, the autophagy flux is unable to replenish cellular energy demand. Therefore, the terms autophagy and autophagic cell death should not be confused. Researchers have come up with a working definition of autophagic cell death as the process that combines an increase of autophagic vesicles with an upsurge in the autophagy level such that the cells cross a point of no return and commit themselves to undergo cell death. The concept of autophagic cell death represents a non-apoptotic form of death, typically classified as type II programmed cell death. To solve the role of autophagy in cell death, Liu et al. proposed a new form of autophagic cell death called ‘‘autosis’’. Extreme stress-mediated by autophagy-inducing drugs and starvation can lead to autotic cell death facilitated through the Na+, K+-ATPase pump with exclusive features such as nuclear membrane changes. However, the mechanism of autotic cell death in cancer patients through oncophagic drug treatment is yet to be understood. Figure 1 summarizes a schematic diagram that illustrates the different players and phases of the autophagic process.

Dual role of autophagy in tumor cells: kill, heal or die – the ultimate oncophagy paradox

In the course of a clinical trial, it becomes decisive for oncologists to identify whether a treatment evoking autophagy behaves like a loyal therapeutic aide by killing the cancer cells and healing the subject or whether it ends up in the patient’s death. The defective autophagic process involves overburdening of damaged mitochondria and mutated proteins which trigger the reactive oxygen species (ROS) accumulation in the cell. Increase in the ROS level initiates the process of tumorigenesis and establishes a potential relationship interconnecting defective autophagy, ROS and tumor development (Figure 2). The antitumor effect of Atg and other autophagy-associated genes displays an array of onco-suppressive properties that reduce oxidative stress. These genes also repair DNA lesions to prevent genomic damage; this is followed by engulfing the inflammatory or necrotic bodies to negate the cancer initiation process. In this regard, p62-mediated mitophagy response enhances antioxidant expression. In addition, inhibition of autophagy elevated p62 that accentuated tumor formation through multiple pathways like downregulation of the NF-xB transcription pathway, an increase in ROS and enhanced DNA damaging effects. The absence of autophagy triggers necrosis and inflammatory responses to nurture a microenvironment that is highly conducive to tumor proliferation. Loss of autophagy evokes genomic instability by inducing aneuploidy that further accelerates the tumorigenesis. Interestingly, activation of major oncogenes like Akt and Bcl2 ensures autophagy suppression through mTOR activation and binding with Beclin 1, respectively. On the other hand, in this initial time of tumor development, loss of PTEN is also known to downregulate autophagy by mTOR activation. However, the stress relieving AMPK signaling promotes
Figure 1. Autophagy cycle – the facts of stress: Schematic diagram highlighting the fundamental process of autophagy. The dark and light boxes show the key steps and main players involved in the respective steps of autophagy. An initial stress response induces the development of an isolation membrane which accumulates the damaged organelles as autophagic cargo and elongates as the mature autophagosome. The lysosome fuses with the autophagosome to give rise to the autolysosome in which the nutrients are returned to the cellular pool to circumvent the stress. The autolysosome-to-lysosome reformation also gives rise to protolysosomes that later develop as lysosomes. (For further details refer to section, “Decrypting the autophagy network: autophagy in the time of stress”).
mTOR-mediated lethal autophagy by utilizing MAPK (Ras/mitogen-activated protein kinase), PI3K/Akt pathways, to resist anoikis in the migrating cancer cells and offer survival opportunity despite matrix detachment. However, there exists a dichotomy over the autophagic role of tumor suppressor p53’s involvement in tumorigenesis. p53 can trigger autophagy by mTOR inactivation or stimulation of DRAM (damage-regulated autophagy modulator). However, cytoplasmic p53 was reported to be specifically involved in the suppression of basal autophagy. Furthermore, Atg7-deficient tumors triggered premature p53 expression and inhibited proliferation. It was also established that autophagy prevented p53-facilitated inhibition of tumor progression in PALB2 (partner and localizer of BRCA2) breast cancer cases.

Beclin 1, a core autophagy molecule, was found to be a haplo-insufficient tumor suppressive gene. Loss of both the copies of Atg5 and Atg7 genes exerted a stronger tumorigenic effect by amnulling autophagy as compared to the homozygous deletion of a single copy of Beclin 1. However, the depletion of autophagy in mice bearing mutated p53 and oncogenic Kras enhanced the process of tumorigenesis by metabolically fueling tumor cells. On the other hand, autophagic repression modulated pancreatic tumor proliferation independent of p53 status. The enhanced autophagic flux producing ketones and lactate in the tumor-associated fibroblasts (TAFs) provided optimum nutrients to fuel the metabolic response and served as metastasis-promoting chemo-attractants in cancer cells.

Surprisingly, as tumorigenesis tends to achieve the full-blown status of malignancy, high expression of Atg4 and HMGB1 (high mobility group box 1) triggers autophagy in response to oxidative stress. Pre-clinical strategies targeting the increase of pro-tumorigenic autophagy response culminates in the use of various autophagy inhibitors like CQ (chloroquine) and HCQ (hydroxychloroquine) along with chemotherapeutics and radiotherapy to induce apoptosis. For example, CQ enhanced temozolomide toxicity by reducing chemo-tolerance by blocking the pro-survival autophagic process, thereby helping glioma proliferation and therapy resistance. Consequently, it has established the autophagic cell death induction propensity of many anticancer drugs (discussed in later sections). Another feature is the involvement of oncogenesis-related autophagic defects that
stimulate tumor nodules after escaping from immunosurveillance. Radiation and therapy-inducing chemooquiescence obstruct malignancy progression due to damage of pro-tumorigenic autophagy that induces regression of tumor cells. A high incidence of autophagy markers like Atg5, Beclin 1, LC3, Atg9, and Ulk1 were reported in patients having non-metastatic clear cell renal carcinoma recurrence following radical nephrectomy.

In connection with cancer therapy, it is important to note that in 2012, there were 14.1 million new cancer reports, 8.2 million deaths and 32.6 million patients (within 5 years of prognosis) across the world. We surveyed the literature and analyzed the status of cancer clinical trials targeting autophagy, from the federal clinical trials network (http://clinicaltrials.gov), where the database of clinical studies on human volunteers around the world is maintained and regularly updated by the service of the U.S. National Institutes of Health. From 2007 to 2015, out of 53 internationally-registered and curated autophagy-based clinical trials, 46 studies involve cancer trials at different phases; these were filtered from http://clinicaltrials.gov using the search words “autophagy”, “cancer”. In Figure 3(A), we have depicted the variation in the number of clinical trials involving oncophagy from 2007 until the present day. The USA, Netherlands and Canada have 34, 5 and 2 oncophagy trial cases, respectively. Likewise, UK, Mexico, France, Spain and Taiwan each have a registered case of an autophagy-targeted cancer clinical trial (Figure 3B). Reports show that the combination of pro-death autophagy inducer with a pro-survival autophagy inhibitor forms a new approach to active pre-clinical testing. The evidence shows that 65% of the autophagy-targeted cancer clinical trials try to inhibit autophagy, while 22% seek to analyze the variation pattern of autophagy upon treatment (Figure 3C, Supplementary Table 2). Moreover, it is observed that only 13% of these clinical trials try to induce the pro-death form of autophagy to remove malignancy (Supplementary Table 3). Speculation about the induction of tumor resistance due to the triggering of protective autophagy may hinder clinicians from opening up this path of a therapeutic approach, which represents the unresolved gray area of autophagy. Among the autophagy inhibiting trials, 83% comprise treatment based on HCQ, while 15% represent treatment based on CQ combinations (Figure 3C). Figure 3(D) represents the organ site-specific cancers that are addressed in particular by various types of autophagy-based clinical trials.
Table 1. Pharmacological agents inducing autophagy in cancer cells.

| Class                          | Drug                   | Target                  | Inducer of autophagy type | Cancer type                             | Clinical availability status | References |
|-------------------------------|------------------------|-------------------------|---------------------------|-----------------------------------------|-----------------------------|------------|
| RTK inhibitor                 | Imatinib               | BCR-ABL                 | PD                        | Chronic myeloid lymphoma                | Approved                    | 54         |
|                               | Dasatinib              | Src/Abl                 | PD                        | Gastrointestinal                        | Approved                    | 57         |
|                               | Sorafenib              | VEGF, PDGFR, RAF, KIT   | PD                        | Ovarian                                 | Approved                    | 58         |
|                               | Laptatinib             | EGFR, HER2              | PD                        | B-cell chronic lymphocytic leukemia     | Approved                    | 59-61      |
| Estrogen receptor antagonist  | Tamoxifen              | Estrogen receptor       | PD                        | Breast                                  | Approved                    | 68         |
| Targeted antibodies           | Panitumunab            | EGFR                    | PD                        | Colorectal                              | Approved                    | 68         |
|                               | Ritisimab              | CD20                    | PD                        | Lymphoma                                | Approved                    | 71,72      |
|                               | Cetuximab              | EGFR                    | PS                        | Colorectal and head/neck cancer         | Approved                    | 74,75      |
|                               | Bevacizumab            | VEGF                    | PS                        | Brain, lung, breast, colon              | Approved                    | 77         |
| BHB mimetics                 | ABT 737                | BCI-2                   | PS                        | Prostate                                | Preclinical                 | 81,82      |
|                               | GX15-070 (Obatoclax)   | PS                      | Acute lymphoblastic leukemia | Approved                               | 86                         |
|                               | Gossypol               | PS                      | Breast, cervical           | Phase II                                | 87                         |
| HDAC inhibitor                | Vorinostat (SAHA)      | HDAC                    | PS                        | T-cell lymphoma                         | Approved                    | 90         |
|                               | Panobinostat (LBH 589) | PS                      | Triple negative breast cancer | Phase I–II                              | 91                         |
| PI3K inhibitor                | NVP BEZ235             | PI3K/Akt/mTOR            | PS                        | Renal, cholangiocarcinoma               | Phase I                     | 94,95      |
|                               | LY294002               | PI-103 hydrochloride     | PD                        | Gastric                                 | Phase I–II                  | 97         |
|                               | PI-103 hydrochloride   | Akt                     | PS                        | Chronic myelogenous leukemia            | Phase I/II                  | 101        |
| Akt inhibitor                 | Penfusine              | PS                      | Acute lymphoblastic leukemia | Phase II                                | 102                        |
|                               | Triciribine             | PS                      | Uterine leiomyoma          | Phase II                                | 103                        |
| AMPK activator                | Metformin              | AMPK                    | PD                        | B and T-lymphoma                        | Preclinical (although approved for diabetes) | 104        |
|                               | Fangchinoline          | PS                      | Liver                     | Phase II                                | 107                        |
|                               | Resveratrol (Stilbenoids) | PS                  | Non-small cell lung cancer | Phase II                                | 108,109                    |
|                               | Safingol               | PS                      | Breast, colorectal         | Phase I                                 | 110                        |
| AMPK inhibitor                | Dorsomorphin           | AMPK                    | PD                        | Glioma                                  | Preclinical                 | 116        |
|                               | Enzalutamide           | PS                      | Prostate                  | Preclinical                              | 117,118                    |
| mTOR complex inhibitor        | Sirolimus              | mTOR complex            | PD                        | Glioma                                  | Phase I–III                 | 119        |
|                               | PP24                   | PS                      | Endometrial cancer         | Preclinical                              | 120                        |
|                               | Everolimus             | PS                      | Nasopharyngeal            | Phase I                                 | 121                        |
|                               | Temsirolimus           | PS                      | Mantle cell lymphoma       | Phase II                                | 122,123                    |
|                               | AZD8055                | PS                      | Non-small cell lung cancer | Phase I                                 | 124,125                    |
| Genotoxic stress inducer      | Cisplatin              | DNA                     | PD                        | Breast                                  | Preclinical                 | 126        |
|                               | Oxaliplatin            | PS                      | Ovarian, cervical          | Approved                                | 128,130                    |
|                               | Usolic acid            | PS                      | Liver, colorectal          | Approved                                | 131,132                    |
|                               | Temozolomide           | PS                      | Cervical                  | Approved                                | 133                        |
| Topo-isomerase inhibitor      | SN-38/irinotecan       | Topoisomerase-I          | PD                        | Colon                                   | Phase II–III               | 134,135    |
|                               | (Camptothecin)         |                         |                           |                                        |                             | 137        |
Oncophagy inducing therapy: high time to terminate cancer

The data representing the complexity of autophagy-inducing oncogenes highlight the complete pathway of the autophagy map that is opening new opportunities to augment our present medical armamentarium. In this section, we describe the broad set of drugs that have clinical relevance in the induction of cellular autophagy in cancer cells (Table 1).

Receptor tyrosine kinase inhibitors

Receptor tyrosine kinases (RTK), occurring on the plasma membrane, evoke multiple signaling transductions to support malignant cell dynamics. These receptors bind with a cognate membrane-bound or soluble protein moiety that results in hyperactive and uncontrolled growth patterns of cancer cells. This class of inhibitor is mostly known to induce pro-death autophagy. They tend to prohibit the activation of the downstream signaling cascade of the tyrosine kinase receptors. Among the RTK inhibitors, the most important are imatinib, dasatinib, sorafenib and lapatinib. Imatinib, a known chemotherapeutic agent against CML (chronic myeloid lymphoma), inhibits the expression of oncogenic tyrosine kinase BCR-ABL, which otherwise leads to malignant transformation. The high efficacy of imatinib in the early phase of CML was shown to be due to induction of the pro-death form of autophagy through the overexpression of Beclin 1 and Atg554. However, in gastrointestinal stromal tumors that are associated with a high level of PDGFRA (platelet-derived growth factor receptor A), the molecule was shown to evoke the pro-survival form of autophagy; this has led to the rare hope of a cure55. Imatinib mesylate tablets were approved by the FDA (U.S. Food and Drug Administration) in 2002 for therapy in patients suffering from Kit (CD117) positive unresectable gastrointestinal stromal tumor. Miselli et al. showed that samples from surgically-resected imatinib-treated patients had a higher expression of proautophagic Beclin 1-Vps34, along with the presence of LC3-II, and a lower expression of antiautophagy Beclin 1-Bcl2 complexes56. Imatinib also received approval for use in Philadelphia-positive chronic myelogenous leukemia in adults (2001) and pediatric cases (2003).

Dasatinib, an inhibitor of Src/Abl kinase, induced a pro-death form of autophagy in ovarian cancer and pro-survival autophagy in B-cell chronic lymphocytic leukemia cells57,58. Interestingly, it was shown that dasatinib had a higher efficacy in comparison to imatinib and a lower adverse effect. Hence, the FDA gave this drug molecule approval in 2006 to be used for patients suffering from chronic phase or accelerated phase chronic myelogenous leukemia even with resistance to imatinib therapy. Sorafenib received FDA clearance in 2007 to be used as an orally-administered kinase inhibitor in unresectable advanced cases of hepatocellular carcinoma. Of note, pro-death autophagy-inducing sorafenib showed a marked cytotoxic effect in the liver, thyroid and renal cancer. Sorafenib displayed a double approach to tackling cancer. As a multi-kinase inhibitor, it inhibited the tumor signaling pathway occurring through Raf signaling, along with phosphorylation of MEK (mitogen-activated protein kinase), and ERK (extracellular signal related kinase) in different in vitro and in vivo models. On the other hand, this

| Drug | Target | Class | Inducer of autophagy type | Cancer type | Clinical availability | References |
|------|--------|-------|---------------------------|-------------|----------------------|------------|
| Doxorubicin | Topoisomerase-II | Thymidylate synthase inhibitor | PS | Breast | Approved | 138 |
| Topotecan | Topoisomerase-II | Thymidylate synthase inhibitor | PS | Colon | Approved | 140 |
| Etoposide | Topoisomerase-II | Thymidylate synthase inhibitor | PS | Lung | Approved | 141 |
| VMP-1 inducer | VMP-1 | VMP-1 inducer | PS | Prostate | Approved | 144 |
| 2-Deoxy-D-glucose | Glycolytic inhibitor | PS | Breast | Approved | 155 |
| Gene-based therapy | MDA7/IL24 | Gene-based therapy | PS | Prostate | Preclinical | 156 |
| Lonafarnib | Farnesyl-transferase inhibitor | PD | Bone | Phase I–II | 150 |
| HIV protease inhibitor | HIV protease inhibitor | PD | Breast | Phase I–II | 151 |
| Paclitaxel | Mitotic inhibitor | PD | Lung | Phase I–II | 153 |
| STF-62247 | Mevalonate inhibitor | PD | Renal | Preclinical | 154 |
| 2-Deoxy-D-glucose | Glycolytic inhibitor | PD | Breast | Preclinical | 157 |

DOI: 10.3109/10408363.2015.1135103

Clinical relevance of autophagic therapy in cancer
molecule modulated tumor angiogenesis and vasculature through inhibition of platelet-derived growth factor receptor families (PDGFR-beta, Kit) and vascular endothelial growth factor receptor families (VEGFR-2, VEGFR-3). A phase III, randomized clinical trial in thyroid cancer patients treated with sorafenib showed an increase of mean survival from 5.8 months in the placebo group to 10.8 months in the treatment group. Further study revealed that sorafenib and its derivative, SC-59 mediated autophagy in hepatoma through the SHP1-STAT3-Mcl1-Beclin 1 pathway. Silencing Beclin 1 or Atg5 reversed the combined drug-induced cytotoxicity. However, unlike sorafenib-lapatinib combined therapy, sorafenib, when combined with indium-111-labeled-girentuximab to cure renal carcinoma, showed a decline in uptake of the monoclonal antibody and blunted the therapeutic efficacy of the combination.

Lapatinib received approval by FDA in 2007 for oral use in highly metastatic breast cancers showing overexpression of HER2 (human epidermal growth factor receptor 2) with a previous history of anthracycline, taxane and trastuzumab therapy. Although earmarked as an HER2 tyrosine kinase inhibitor, lapatinib was also found to inhibit EGFR (epidermal growth factor receptor) and impede breast carcinoma progression. Closer inspection of the simultaneous autophagy and apoptosis induction by lapatinib revealed that a switchover from autophagy to apoptosis was taking place in breast cancer patients. Chen et al. recently reported a study in which they showed that repealing autophagic involvement in lapatinib-resistant cells might be a useful strategy to kill them. Their preliminary data showed that an increase in autophagosomes in lapatinib resistant cancer cells enhanced the survival chances of the resistant cells. This finding established the protective role of autophagy, in contrast to the well-reported pro-death role. Tang et al. described the activity of lapatinib, in association with obatoclax, in triggering lethal autophagy that was driven by NOXA-dependent dislodgement of Mcl1 from the crucial autophagy player, Beclin 1. Obatoclax-mediated inhibition of Mcl1 rapidly enhanced lapatinib toxicity in tumor cells via induction of lethal autophagy.

**Estrogen receptor antagonist**

Estrogens display a high propensity for stimulating cellular proliferation in cancer. Estrogen stimulation can be inhibited by the use of estrogen receptor antagonists. Tamoxifen, a drug well known as a selective estrogen receptor modulator, is widely used in breast cancer. Tamoxifen exhibits pro-death autophagy by blocking the estrogen receptors. 3-MA (3-methyl adenine) pre-treatment decreases tamoxifen-induced cell death and autophagic vacuole formation in MCF-7. Since its FDA approval in the year 1977, the widespread use of tamoxifen against highly metastatic and recurrent forms of breast carcinoma continued until recently, when reports regarding drug resistance started appearing. Nagelkerke et al. showed that inhibition of autophagy by knocking out LAMP3 resensitized the tamoxifen-resistant breast cancer cells.

**Targeted monoclonal antibody therapy**

Drugs such as panitumumab (antibody against EGFR) and rituximab (chimeric monoclonal antibody against CD20) have been described to show a pro-death form of autophagy in colorectal cancer and lymphoma, respectively. Panitumumab received FDA permission in 2007 for the use in EGFR-expressing advanced stages of colorectal cancer patients. Currently, Giannopoulou et al. showed that irrespective of K-RAS mutation and EGFR protein level, panitumumab induced autophagic cell death without affecting apoptosis and necrosis, through an increase in the level of Beclin 1. Rituximab was approved by the FDA in 1997 to treat cancer victims with recurrent low-grade or follicular B-cell non-Hodgkin’s lymphoma. In 2009, Turzanski et al. demonstrated that rituximab played a vital role, independent of the caspase-mediated killing of Burkitt lymphoma cells, through autophagy. Interestingly, obatoclax, when combined with rituximab-sensitive or resistant cell lines derived from B-cell lymphoma patients, showed a synergistic killing effect through a non-apoptotic pathway. Inhibition of caspase activity did not affect the functioning of obatoclax, suggesting the existence of caspase-independent death. This pathway occurred through the increase of LC3-II conversion along with a surge in autophagic vesicle number as detected by electron microscopy. Immunohistology of 118 tumor patient samples from 2003 to 2007 showed Beclin 1 overexpression in B-cell lymphoma patients with a previous history of rituximab treatment. These patients had a higher progression free survival chance through clinicopathological prognostic model analysis. The FDA recommended the anti-EGFR antibody, cetuximab, for use against highly metastatic colon cancer. Analyzing the advanced colon cancer reports from 2005 to 2008, Guo et al. reported that patients with lower Beclin 1 had a longer progression free survival, whereas those with higher LC3 expression had a higher objective response rate. This monoclonal antibody-based solid tumor therapy targeted against EGFR is known to induce autophagy by inhibiting the class I PI3K-Akt-mTOR pathway and stimulating the class III (hVps34)-Beclin 1 axis of signaling. Inhibiting the autophagy progression by silencing Atg genes or using lysosomal inhibitors ensured a switch from autophagy to apoptosis, highlighting the fact that treatment with cetuximab induced a protective version of autophagy. However, co-treatment of cetuximab with the mTOR inhibitor, rapamycin, induced autophagic cell death with a weak apoptotic phenomenon operating in the background. These findings open up new opportunities for unraveling EGFR-mediated cetuximab clinical trials in different combination therapies. Likewise, studies on angiogenesis inhibitors like bevacizumab were initiated to neutralize VEGF (vascular endothelial growth factor). The VEGF pathway is reported to be one of the strong proliferating signals that contribute to the development of tumor vasculature and angiogenesis promotion. In 2004, bevacizumab was approved by the FDA for use in recurrent cases of malignant colon carcinoma. Later, it was used for cancer treatment in the lung (2006), breast (2008), kidney and glioma (2009). Evidence indicates that bevacizumab treatment resulted in a concomitant rise in protective autophagy through an increase of autophagy related genes like LC3-II and Beclin 1 in hepatoma xenografts. However, suppression of autophagy by CQ showed a marked increase in apoptosis. Recently, the use of bevacizumab in combination with fluoropyrimidine–irinotecan or fluoropyrimidine–
oxaliplatin in highly advanced and progressive cases of colorectal cancer has been approved by FDA. Bevacizumab treatment in HT29 xenografted mice showed increased resistance to antiangiogenic therapies by overexpression of essential autophagic proteins like Atg5 and Beclin 1, thus indicating the pro-survival effects of the autophagy arm. However, autophagy inhibition by adding CQ showed a convincing decline in tumor progression. Similarly, Guo et al. showed that Beclin 1, LC3-mediated autophagosome formation increased with bevacizumab treatment in the xenograft hepatoma model. Inhibition of autophagy by CQ, in this case, led to ROS-mediated DNA damage, culminating in an induced apoptosis effect.

BH3-mimetics

Cancer therapy targeted against pro-survival proteins is an emerging approach. BH3-mimetic ABT-737 was reported to be an extremely effective agent in tumors with a high level of Bcl-2 but with low Mcl-1 levels. Pre-clinical findings support the idea that ABT-737 represses death resistance in glioma cells due to Bcl2L12- and temozolomide-mediated antiapoptosis and protective autophagy effects, respectively. However, ROS-mediated autophagy evoked through ABT-737 protected prostate cancer cells. Combining ABT-737 with HCQ turned out to increase cytotoxicity in vitro as well as in vivo. Besides the pre-clinical studies on ABT-737, an oral derivative of the same molecule, ABT-263 (navitoclax), is presently undergoing phase I–II clinical trials. The efficacy of ABT-737/263 was shown in malignancies displaying a high Bcl-2 level. However, from recent pre-clinical data, ABT-737-mediated autophagic treatment in small cell lung cancer had modest effect when combined with CQ.

Trisciuoglio et al. have elegantly demonstrated that Bcl-2 overexpression led to tumor progression, and that the exclusion of the BH4 domain of Bcl-2 protein activated an autophagic response impairing tumor growth. From these findings, clinical researchers have developed a pan-Bcl-2 inhibitor called GX15-070 (obatoclax) that is involved in inducing autophagic cell death in acute lymphoblastic leukemia through the Atg5-dependent pathway. Prior to the onset of cell death, there was evidence of LC3-II conversion and p62 degradation. Gossypol was showed to disrupt Beclin 1 and Bcl-2 interaction and to stimulate cell defense, and was promoted by inducing autophagy in HeLa and MCF-7 cells. This type of autophagy was shown to progress in a canonical as well as a non-canonical form. Interestingly, knocking down of essential autophagy-related genes increased the apoptotic cell population. Gossypol was used in phase II clinical trials in adult brain tumor patients and metastatic adenocortical carcinoma cases. These results unravel a novel paradigm for Bcl-2 inhibition-based autophagy-targeted therapy.

HDAC inhibitors

Altered epigenetic signatures like the acetylation pattern of histones form some of the characteristic markers of cancer cells. Emerging studies have highlighted the application of small molecules like HDAC (histone deacetylase) inhibitors, with the aim of inducing cell death in cancer cells. Interestingly, Hamai and Codogno have recently reported new targets for acetylation in autophagy. Many HDACi (HDAC inhibitors) are being actively investigated, and are being assessed in clinical trials, but the mechanism of their action is not yet fully known. Del Bufalo et al. described the potential clinical implication of pemetrexed (multi-targeted antifolate) followed by HDACi treatment through an autophagy- and apoptosis-mediated pathway in non-small cell lung carcinoma.

HDACi, like vorinostat (SAHA, suberanilohydroxamic acid) and panobinostat (LBH589), are already approved and have earned satisfactory grades from clinicians. Insights into the complexity of autophagy modulators revealed a fascinating crosstalk pattern of vorinostat treatment in U937 and SUDHL6 hematological cell lines. Vorinostat-resistant U937, SUDHL6 regained sensitivity to vorinostat upon disruption of autophagy through the knock down of Beclin 1 or LAMP2. However, strikingly autophagy suppression in non-resistant cells reduced vorinostat sensitivity. These findings highlighted the concept that autophagy functions were changing from a pro-death to the pro-survival response during drug resistance. Furthermore, panobinostat (LBH589) treatment in human estrogen/progesterone receptor and HER2 TNBC (triple-negative breast cancer) cells showed that protective autophagy defended the cells from undergoing apoptosis. A combination of panobinostat with CQ led to a lethal build-up of polyubiquitinated proteins that resulted in inhibition of TNBC cell proliferation both in vitro and in vivo. This promising drug candidate is going through active phase I–II clinical trials for the treatment in leukemia, lymphoma and breast cancer patients.

Class I PI3K inhibitors

A search for different druggable targets has identified the potential involvement of PI3K inhibitors. The PI3K-Akt-mTOR axis becomes ubiquitously hyperactive in malignant cells, and researchers are looking for potential inhibitors to modulate these target molecules as a new strategy to tackle cancer. One such key molecule is orally available imidazole NVP-BEZ235, a dual PI3K/Akt inhibitor. This molecule is in phase I clinical trials. Li et al. showed that the protective autophagy triggered by NVP-BEZ235 in renal carcinoma cells in vitro when challenged with an autophagy inhibitor led to a switch from autophagy to apoptosis. Analysis of NVP-BEZ235 treatment in cholangiocarcinoma patients showed that this molecule inhibited cell migration, metastasis progression and cell cycle arrest without inducing apoptotic stimuli. Immunohistochemistry analysis of such patients validated the autophagic response that occurred through the PI3K-Akt-mTOR pathway, mainly through loss of action of PTEN. Interestingly, when prostate cancer cells were challenged with NVP-BEZ235, cells bearing the wild type PTEN were showed to induce apoptotic cell death, whereas PTEN-null cells showed autophagic cell death. Furthermore, Mukherjee et al. illustrated that NVP-BEZ235 escalated radio-sensitization of glioma cells along with a simultaneous rise of apoptosis and fall in DNA repairing ability. NVP-BEZ235 was also characterized to inhibit the hypoxia and TGFβ1-induced epithelial to mesenchymal transition in ovarian and prostate cancer cells.

LY294002, a PI3K inhibitor, stimulated lethal autophagy by triggering caspase-3. On the other hand, it turned on...
apoptotic cell death in gastric cancer by activating PUMA (p53-up-regulated modulator of apoptosis), through the p53-mediated pathway. The present goal is to stratify the patients’ PI3K results and undertake robust trials with various PI3K inhibitor combinations to unravel the therapeutic benefits of these molecules.

A pyridofuroxypyrimidine compound like PI-103 hydrochloride is reported to inhibit the PI3K-Akt-mTOR signaling axis and induce cancer progression, G0–G1 cell cycle arrest and tumor growth regression in glioma xenografted models. Besides inhibiting PI3K, this molecule dephosphorylates important signaling molecules like Akt and mTOR that subsequently go on to regulate the downstream target molecules that are involved in controlling growth and proliferation.

Akt inhibitors

Akt kinase activation is implicated to be one of the most hyperactive signals occurring in highly invasive malignant cases. Akt inhibitors try to induce autophagy by manifesting a fall in PI3K-Akt signaling. Inhibiting the kinase action of Akt can be achieved by the implementation of perifosine, triciribine and MK2206. Perifosine- and triciribine-induced autophagies were showed to provide stress protection in cancer cells. Perifosine inhibited the PI3K-Akt-mTOR signaling pathway and was also reported to increase Atg5 expression in chronic myelogenous leukemia. Similarly, triciribine synergized with vincristine showed the ability to increase T-cell acute lymphoblastic leukemia patients survival by inhibiting Akt when autophagy was inhibited using CQ. Interestingly, MK2206 was shown to evoke a pro-death form of autophagy in uterine leiomyoma in a non-canonical manner independent of Atg5 and Atg7.

AMPK activators

AMPK is an attractive target for clinicians as this stress sensor is heavily turned on and modulates proliferation and progression through different aberrant cell cycle checkpoints in cancer cells undergoing ATP deprivation. Some major AMPK activators known to induce autophagy are metformin, fangchinoline, resveratrol, safingol, nilotinib, atorvastatin, vitamin D3 and cannabinoids. Apart from resveratrol, safingol and atorvastatin, the rest of the members are known to execute the lethal form of autophagy-mediated cell death in cancer cells. Pre-clinical findings of metformin-based studies were reported to induce a pro-death form of autophagy in B- and T- lymphoma. Metformin enhanced AMPK activation and inhibited mTORC1. A study on Taiwanese type 2 diabetes patients presented the first evidence of the reduction of thyroid cancer risk upon metformin administration. Similarly, metformin treatment reduced lung cancer risk in a dose-dependent manner in diabetic patients in a 10-year cohort study. Wang et al. showed fangchinoline to be a promising alternative in hepatocellular carcinoma through a possible mechanism of sestrin2 activation that upregulated AMPK and induced autophagic cell death, independent of the mTOR pathway. The Akt-mTOR-p70S6K-4EBP1 pathway was reported to be inhibited from inducing autophagy in this case. The involvement of resveratrol is associated with the self-defensive mechanism in cancer trials. Bishayee highlighted the status of resveratrol-based ongoing phase I–II clinical trials. Safingol, a drug, currently in a phase I clinical trial, mediates an elevation of ROS in breast and colorectal carcinoma cells. The increase in ROS-induced autophagy protects cancer cells. Another drug, nilotinib, currently under phase I–IV clinical trials, was shown to activate a pro-death form of autophagy by activating AMPK through the PP2A (protein phosphatase 2A) pathway, in liver cancer. In a novel study to elucidate the molecular mechanism of statins, Yang et al. showed that atorvastatin rendered autophagy-mediated protection in hepatocellular and colorectal carcinoma, through AMPK activation, that was enhanced through a rise in p21 and ER stress level. The NCI (National Cancer Institute, USA) clinical trial database reports that atorvastatin is under an active phase II clinical trial in renal, lymphoma and breast cancer patients. Recently findings in MCF-7 breast cancer cells have shown that vitamin D3 provides radio-sensitization by autophagy promotion in irradiated cells. Although randomized phase I clinical trials are being undertaken to understand the role of vitamin D3 in the prostate, lung and colorectal cancer, at present there is no evidence that vitamin D3 has a curative role in cancer. Vitamin D3 analog, EB 1089, treatment in combination with radiotherapy in breast cancer cells increased autophagic cell death along with obstructing the recovery of proliferative potential. Moreover, it is now known from pre-clinical evidence that cannabinoids, which are part of complementary and alternative medicine, can induce autophagic cell death through ROS-mediated AMPK activation in pancreatic carcinoma cells.

AMPK inhibitors

The AMPK inhibitors are also potent drug candidates in oncophagy therapy. In an interesting study, dorsomorphin (compound C), a known AMPK inhibitor, was shown to inhibit the proliferation of glioma cells in an AMPK-independent pathway by inhibiting the Akt-mTOR pathway. Importantly, dorsomorphin-induced autophagic cell death was restricted to normal astrocytes. An oral formulation of enzalutamide, a second-generation androgen receptor antagonist, received FDA approval for use against castration-resistant prostate cancer in patients who previously underwent docetaxel treatment. Enzalutamide was deciphered to be inducing AMPK-mediated protective autophagy in prostate cancer cells and inhibition of AMPK accentuated apoptosis event.

mTOR complex inhibitors

mTOR complex forms a vital target for oncophagy. Occurring downstream to the PI3K-Akt pathway, novel therapies are being tried to block this complex with the aim of providing sustained tumor regression. Sirolimus (rapamycin) was approved by the FDA for use in graft rejection; however, its ability to inhibit the highly aberrant level of mTOR kinase expression makes it a potential anticancer therapy. Progress was made on determining the mechanism of action by Arcella et al., who showed that sirolimus treatment resulted in autophagy induction and prevented glioblastoma cell proliferation in vitro and in vivo. Most endometrial
cases were associated with PTEN deletion that resulted in mTOR upregulation and induction of cell proliferation. In this connection, PP242, a catalytic inhibitor of mTORC1/2, led to a significant lowering of tumor growth in mice xenografted with grade 3 endometrial cancer. The combination of PP242 with a DNA-damaging agent like carboplatin further enhanced the therapeutic efficacy of the treatment. Unlike PP242, everolimus (RAD001) inhibited only mTORC1 through an allosteric mechanism. Different phase I clinical trials using everolimus alone or in combination are being undertaken in advanced cases of breast, renal, head and neck carcinoma cases. Notably, everolimus treatment played a protective role in human nasopharyngeal carcinoma and upon inhibition of autophagy there was a concomitant rise in cells undergoing apoptosis, indicating the pro-survival response evoked by the molecule in cancer. However, temsirolimus (CCI-779) (belonging to the same group of mTOR complex inhibitors), is under active phase II clinical trials in recurrent mantle cell lymphoma patients; temsirolimus is known to operate by evoking autophagy and downregulating cyclin D1 expression. The combination of temsirolimus and perifosine was investigated in a phase I study in patients suffering from non-small cell lung cancer and advanced bladder carcinoma. Temsirolimus strategically augmented cancer progression-free overall survival in mantle cell lymphoma patients. In this regard, the combination of temsirolimus to suppress malignancy by autophagic cell death along with angiogenesis inhibitors could be a useful approach. AZD8055, a phase I clinical trial candidate, was shown to induce autophagic cell death by allosterically inhibiting the kinase activity of mTOR complex, by inactivating substrates such as p70S6K, 4E-BP1, AKT and other multiple downstream signaling molecules. Also, Zheng et al. showed the autophagy and apoptosis inducing pre-clinical efficacy of AZD-2014, a dual inhibitor of mTORC1/2, in renal carcinoma cells. They showed that AZD-2014 mediated autophagy by disrupting mTORC1/2 phosphorylation and enhancing Atg5–12, Beclin 1 expression plus LC3-II puncta formation. The in vivo efficacy of AZD-2014 even surpassed RAD001 through HIF-1α/α2 or p-AKT downregulation in 786–0 xenografts. Fascinatingly, autophagy inhibition by co-administration with 3-MA or si-RNA augmented renal cell cancer apoptosis. Relating to this line of thought, Aurora kinase A inhibition was correlated with depletion of phosphorylated mTOR to induce a pro-survival form of autophagy through elevation of LC3-II and SQSTM1 levels. Aurora kinase A inhibition highlights a new strategy for sensitizing drug-resistant breast cancer cells to apoptosis. Similarly to PP242, researchers have also identified a small non-competitive inhibitor known as torin-1 that inhibits both mTORC1 and mTORC2. The torin-mediated response mechanism in cancer cells has not been entirely worked out. However, Francipane and Lagasse showed that torin-1 can induce cell death by mTOR inhibition in human colon cancer stem-like cells.

Genotoxic inducer

Modulation of malignancy progression by the use of genotoxic inducers provides an upcoming strategy to provide a cure for cancer. Genotoxic inducers induce stress in the cancer cell genome at multiple stages and through different pathways. One such drug is cisplatin, whose chemotherapeutic efficacy was shown to be diminished by the protective effects of ERK-transduced autophagy. Silencing ERK or averting autophagy induction can sensitize cisplatin-resistant ovarian cancer cells by apoptosis induction. Knocking down of Beclin 1 boosts cisplatin-mediated mitochondrial apoptosis in SKOV3/DDP human ovarian cancer cells.

Likewise, pre-treatment with 3-MA or CQ, followed by cisplatin at a low dose, was able to increase cell death in cervical carcinoma cells. Presently, cisplatin is clinically approved for use in advanced or inoperable cases of bladder, esophageal, cervical, lung, ovarian, testicular, head and neck cancer patients. Cisplatin is currently undergoing over a hundred clinical trials in various combinations. In vitro or in vivo treatment with oxaliplatin resulted in elevation of ROS levels; this provided a protective shield to hepatoma cells that was mediated through autophagy induction. However, the use of autophagy inhibitors or knocking down of essential autophagy regulating genes decreased the tumor cell survival. Oxaliplatin combinations with 5-FU (5-fluorouracil) are approved for the treatment of stage III and advanced colorectal cancer patients. However, autophagy was found to play a protective role against oxaliplatin-mediated cell death induced via ER stress and ROS outburst in colorectal cells. Studies on bioactive triterpenoids focus on the autophagic cell death-inducing potential of ursolic acid. Preclinical findings demonstrated that ursolic acid mediated the Atg5-dependent pro-death form of autophagy in TC-1 cervical carcinoma cells. Temozolomide was a groundbreaking advance in patients undergoing glioma therapy. This drug successfully impedes the migration potential and proliferation of highly metastatic gliomas and astrocytomas by promoting death-inducing autophagy.

Topoisomerase inhibitors

The high expression of topoisomerases is known to play a critical role that helps in the process of aberrant replication and transcription of cancer cells. Topoisomerase inhibitor-based anticancer therapy should be an ideal anticancer option for chemotherapy treatment. Importantly, stopping autophagy progression by ablating p38α (mitogen-activated protein kinase MAPK14) expression inhibited pro-survival autophagy triggered by SN-38 (active metabolite of irinotecan) in the HCT116 human colorectal adenocarcinoma cells. Irinotecan (camptothecin) and its derivatives that are currently going through phase II–III trials, are promising agents to target topoisomerase-mediated therapy. A topoisomerase I inhibitor, doxorubicin, at lower doses induced autophagy through elevated expression of Atg5 and Beclin 1 in breast cancer cells. Recently the same group showed that doxorubicin treatment in Beclin 1 knockdown cells led to a two-fold increase in the apoptosis level and concluded that this was a cytotoxic pattern of autophagy response. Doxorubicin derivatives belonging to the anthracycline antibiotic group displaying strong anticancer effects. Doxorubicin is used to treat acute lymphoblastic leukemia, acute myelogenous leukemia, lymphoma, breast, ovarian, thyroid, small cell lung cancer and other neoplasias. Clinical trials
with doxorubicin in single use or in different combinations are underway. Studies on colon cancer provide evidence that topotecan (topoisomerase I inhibitor) evoked pro-cancerous autophagy under the regulation of wild-type p53. p53 induces sestrin 2 to phosphorylate AMPK, which ultimately inhibits mTORC1 to promote cytoprotective autophagy. Topotecan hydrochloride was approved for phase IVB clinical trial in recurrent or persistent cervical cancer cases. Of note, etoposide (topoisomerase II inhibitor) was shown to resist treatment by autophagy induction in glioma. Knocking down of the retinoblastoma (Rb) tumor suppressor gene resulted in a fall in etoposide-mediated autophagy and promoted apoptosis in glioma cell lines, thus pioneering a new connection between Rb protein, etoposide and autophagy-apoptosis switch over. Presently, etoposide is used for the treatment of small cell lung and testicular cancer. In addition, etoposide is also indicated as a mild but effective chemo-agent against children suffering from neuroblastoma.

**Thymidylate synthase inhibitor**

Thymidylate synthase regulates a vital step of DNA synthesis. The design of thymidylate synthase inhibitors that thwart aberrant DNA synthesis in neoplasia could be a strategic model prototype of anticancer medication. 5-FU, which is metabolically converted to a active thymidylate synthase inhibitor, is part of the treatment of many cancer patients. Fluorouracil compounds, alone or in combination, are used against gastrointestinal, breast, head and neck cancers and in cancers recurrences. It was found that 5-FU elicited apoptosis as well as autophagy in non-small cell lung cancer A549 cells. However, inhibition of protective autophagy by knocking down Atg7 or using 3-MA upon 5-FU treatment stimulates ROS-mediated apoptosis in A549 cells. Liang et al. showed that the combined therapy of pre-treatment with CQ followed by 5-FU could be a successful treatment against gallbladder cancer. This antimetabolite-type antineoplastic agent is presently prescribed by clinicians for use in rectal, colon, head and neck cancers.

**Proteasome inhibitors**

Proteasome inhibitors are chemotherapeutic agents being used routinely all over the world. Ubiquitin-proteasome inhibitors hold a key to regulating the progression of many malignancies and to maintaining a balance between the growth of drug-resistant cancer cells and cell death. Emerging trends focus on targeting the ubiquitin-proteasome degradation pathway as a useful anticancer strategy. Bortezomib is the first FDA-approved proteasome inhibitor to be implemented in the treatment of malignancy, either singly or in combination with other therapies, and represents a significant landmark. Novel proteasome inhibitors such as carfilzomib and marizomib have been developed in recent times and are undergoing clinical trials. Bortezomib, which is presently used to treat multiple myeloma and mantle cell lymphoma, is reported to induce both apoptosis and autophagy. Jia et al. showed that inhibiting autophagy averts NF-κB stimulation by abrogating I-κB expression in bortezomib-treated lymphoma cells. Co-treatment of bortezomib with autophagy inhibitors highlighted the protective mechanism of autophagy in glioma cells. Combined treatment with bortezomib and HCQ in a phase I clinical trial has shown 14% partial response, 14% minor response and 45% stable disease in cases of multiple refractory myeloma. Stopping the protective arm of autophagy should provide more effective bortezomib-based therapies. Another proteasome inhibitor, NPI-0052, is under a phase I clinical trial for treating lung cancer, lymphoma and pancreatic cancer. NPI-0052 has been shown to play a pro-cancerous autophagy role, so delivering the drug and controlling its efficacy in treatment will be critical. NPI-0052 inhibited proteasomes by the phospho-eIF2α-dependent pathway to evoke autophagic flux that was mediated through the expression of Atg5, Atg7 to relieve proteotoxic stress.

**VMP1 inducer**

VMP1 binds with a BH3 domain of Beclin 1 and disrupts Becl2/Beclin 1 interaction leading to increased Yps34 production. Another chemotherapeutic agent, gemcitabine, is reported to mediate pro-death autophagy by VMP1 induction in pancreatic cancer. VMP-1-mediated autophagy was associated with increased Atg16L1 and LC3 participation in autophagosome formation. Besides pancreatic cancer, gemcitabine has been prescribed in ovarian, breast and non-small lung cancer.

Other miscellaneous drugs are listed in Table 1. Albert et al. deduced that inhibiting poly(ADP-ribose) polymerase-1 (PARP-1) with ABT-888 makes irradiated H460 lung cancer cells undergo autophagic cell death. ABT-888 impairs the DNA repair system by acting as an enhancer to radiation therapy. Lonafarnib, a farnesyltransferase inhibitor that prevents post-translational modifications occurring in Ras oncogenic proteins is being investigated in phase I–II clinical studies of highly metastatic cases of pancreatic, oral, breast, ovarian, head and neck cancers. In the complexity of the switch from apoptosis to autophagy, it is not yet resolved whether lonafarnib induces pro-survival or pro-death autophagy. Likewise, CQ-mediated autophagy inhibition reinstates sensitivity of lung cancer cells toward crizotinib (PF02341066, ALK oncoprotein inhibitor). Work on HIV protease inhibitors such as ritonavir and nelfinavir indicated that both the caspase-dependent and caspase-independent pathways of cell death were induced by them. Nelfinavir induced the cytoprotective form of autophagy through Akt activation. Gills et al. deciphered the efficacy of three known HIV protease inhibitors in different cancer cells; presently nelfinavir is in phase I–II clinical trials. These findings highlight the prospective anticancer activity from standard drugs to treat HIV. Xi et al. showed that the mitotic inhibitor, paclitaxel, can impede the chemo-response in A549 cells, after administration of 3-MA or by genetically knocking down Beclin 1, to diminish the protective role of autophagy. The reduction of autophagy proteins like Atg5, Atg7 and Atg9 depleted the chemosensitivity to STF-62247 when it was administered in highly lethal cases of renal cancer. STF-62247 was characterized to selectively induce autophagic cell death in tumor suppressor von Hippel–Lindau (VHL)-deficient cells.
Current investigation revealed the understanding of 2-deoxy-D-glucose-mediated pro-survival autophagy induction in breast cancer cells, which relieved the ER stress induction, that were monitored by a decrease in Grp 78 expression (ER stress marker). Current investigation revealed that autophagy mediated a pro-survival role against 2-deoxy-D-glucose-mediated cell death in breast cancer cells by alleviating the ER stress induction; this finding highlights that combined treatment of autophagy inhibitors and 2-deoxy-D-glucose may be a viable clinical option for breast cancer therapy. Our group recently worked out a crucial switch-over from autophagy to apoptosis in prostate carcinoma cells mediated through the antitumor effects of cytokine melanoma differentiation-associated gene 7/interleukin-24 and highlighted gene therapy-based oncophagy management. 3-MA treatment inhibited autophagy, which induced Ad.mda-7-mediated apoptosis, signifying that the initial autophagy response might be of the protective type. We unraveled a previously unknown autophagic regulation of astrocyte-elevated gene-1 (AEG-1) function, which underscores resistance to drug response and metastasis in astrocytomas. AEG-1 induced protective autophagy, mediated through the AMPK-mTOR pathway, which also involved an increase in Atg5 expression. The outcome further showed that AEG-1 protected the tumor cells from chemotherapy by autophagy induction, and inhibition of this gene increased the chemosensitivity of the cancer cells.

**Oncophagy inhibiting therapy: cutting the hand that feeds tumor**

Suppressing the rise of autophagy flux, which develops as a defense response to any on-going anticancer therapy, is a novel rationale that complements the effectiveness of the treatment. We summarize the role of autophagy inhibitors from the perspective of their clinical relevance to therapy (Table 2). Although there have been many reports regarding the pathways that induce autophagy, we find an incongruous stand when it comes to autophagy inhibition. Upon assessment of most of the clinical trials underway across different countries, we find that the most common therapeutic approach is to suppress the pro-survival form of autophagy that protects the cancer cells. We now review the various targets of autophagy inhibitors that are in clinical trials.

**Beclin 1 inhibitor**

A pre-clinical study by Liu et al. reported an autophagy inhibitor, Spautin-1 (specific and potent autophagy inhibitor-1), that has a specific ability to inhibit ubiquitin-specific proteases, USP10 and USP13, thus targeting the Vps34 subunit of Beclin complex. Spautin-1 impedes autophagy through regulating Beclin 1 deubiquitination and consequently results in the decline in autophagosome production. Characteristically, Beclin 1 was also implicated in modulating tumor suppressor p53 expression by deubiquitination induced by USP10 and USP13. Spautin-1 chemosensitizes imatinib mesylate-mediated apoptosis by PI3K/AKT inactivation that ultimately abrogates antiapoptotic Mc11, Bcl2 expression in CML patients.
Lucanthone was shown to inhibit autophagy by inducing lysosomal membrane permeabilization in breast cancer preclinical models. Lucanthone treatment curbed autophagic flux, as visualized by p62 accumulation, and induced apoptosis through the cathepsin D mediated pathway in vorinostat-insulted breast cancer cells. At present lucanthone is undergoing a phase II clinical trial in brain cancer metastases from non-small cell lung cancer⁶⁰.

**Inhibitor of autophagosome and lysosome fusion**

A wide array of pharmaceutical compounds is available as medicinal options to disrupt the fusion of the autophagosome and lysosome. Among the aminoquinolines, CQ, an antimalarial drug, is one such candidate that possesses safe antitumor properties, especially for short-term use. Use of CQ in combination with other chemotherapeutic drugs causes a decrease in the level of resistance caused by inhibition of protective autophagy in glioma, colorectal cancers as well as many other cancers reported earlier in the text⁶¹,⁶². In addition, Maes et al. showed that CQ affects the tumor endothelial architecture and promotes vessel normalization by abrogating the hypoxic milieu in a tumor by reducing invasive potential and augmenting the drug delivery response⁶³. However, when the pro-death form of autophagy is inducing cell death of tumor cells, then the use of CQ-like autophagy inhibitors should not be used as part of combined therapy.

Oral administration of CQ was shown to be a novel adjuvant therapy to complement conventional treatment in glioma and colorectal cancer patients as well. Compounds like CQ and HCQ are the two presently-available clinical options for inhibiting autophagy during oncophagic therapy. The major limitations of CQ in clinical studies were found to be the inability of the drug to enter malignant cells due to the increased acidity and hypoxic condition in the tumor microenvironment⁶⁵. Like CQ, HCQ is an approved antimalarial drug that is being used for chemotherapy. In comparison to CQ, the higher numbers of clinical trials involving HCQ are aimed to curb the autophagic tolerance to stress in tumor cells⁶⁶.

Clinically, HCQ has been found to be a stronger autophagy inhibitor than CQ. For instance, HCQ suitably sensitizes chronic myeloid stem cells in response to tyrosine kinase inhibitor treatments. Similarly, in gefitinib (a potent inhibitor of EGFR) resistant or sensitive breast cancer cells, combined therapy with HCQ proved to be more efficacious than monotherapy. A more water soluble and potent aminoquinoline is Lys-05, a dimeric form of CQ developed with the lead compound Lys-01 (which was shown to have a 10-fold higher efficacy than HCQ). Unlike CQ, Lys-05 can function at low pH and in hypoxic environments, and readily accretes in tumor cells and deacidifies the lysosomal compartments, thereby diminishing autophagic functioning in HT29-xenografted nude mice. Superior to the CQ, another antimalarial drug, quinacrine, shows high effectiveness in inhibiting autophagy and triggering apoptosis in a p53- and p21-dependent pathway in HCT colon cancer cells.⁷ⁱ.

Aside from the antimalarials involved in autophagy inhibition, other autophagy blockers are yet to be registered for cancer clinical trials.

Another type of autolysosomal formation inhibitor is Bafilomycin-A₁, which blocks vacuolar-type H (+)-ATPases in the membranes of lysosomes and endosomes and which are involved in regulating various cellular activities. Bafilomycin-A₁, in particular, blocks the V-ATPases and triggers inhibition of lysosomal acidification; it was shown to be involved in caspase-mediated apoptotic cell death through the ERK-JNK-p38 pathway in colon cancer cells. The report showed that concomitant autophagy inhibition by monensin escalated apoptosis to sensitize the non-small cell lung cancer cells when combined with rapamycin or erlotinib treatment. In line with the development of autolysosomal blockers, inhibitors like pepstatin A, when combined with E64d, were reported to prevent autolysosomal digestion, unlike inhibitors such as 3-MA that impede autophagy by preventing autophagosome formation. E64d and pepstatin A were found to decrease protective autophagy and inhibit the pro-survival effect to induce apoptosis in nutrient-deprived colon cancer cells. In addition, thymoquine, another pre-clinical molecule, was found to deter the autophagic pathway so as to induce cathepsin-mediated but caspase-independent cell death in glioma cells. As in gliomas, chemoresistance in solid pancreatic tumors is quite common. Proton pump inhibitors such as omeprazole inhibit proliferation and induce cell death by modulating pro-survival autophagy. These findings may add a new angle to the present therapy, as omeprazole is already used in clinical practice against peptic ulcers.

**Class III PI3K inhibitors**

Inhibiting Vps34 (class III PI3K lipid kinase) has been identified to be at the crux of potential treatment strategies to deprive autophagic nutrient fueling in cancer. Likewise, wortmannin-based adjunct therapy in combination with cisplatin in different grades of urothelial cancer shows a better therapeutic outcome than cisplatin treatment alone. Another prominent candidate belonging to this class is 3-MA, which selectively inhibits the class-III PI3K-like wortmannin and breaks the self-protective response that is initiated in tumors by inhibiting the autophagic pathway progression. 3-MA in combination with cisplatin and 5-FU remarkably reduced pro-survival autophagy and initiated apoptosis in esophageal and colon carcinoma cells, respectively.

**Present challenges and future clinical prospects: insights from benchtop phenomenology to bedside therapy**

To the present-day clinician, the main dilemma while prescribing oncophagic drugs is the predicament of autophagic switch-over from the pro-lethal to the pro-survival form. This depends mainly on the cumulative effects of the cancer type, the action of the therapeutic response, and the activity of tumor microenvironment signaling that regulates metabolism; these integrate to develop the capacity to provoke the different types of autophagy change-over along with autophagy-apoptosis crosstalk.
Examining the clinical and pre-clinical aspects of lethal autophagy caused by cancer treatment will reveal the specificity and mechanism of action. Although there are a large number of reports about induction of lethal autophagy in cancer, the essential molecular signal that controls specific progression and that differentiates these responses from a non-specific passive process remains largely underexplored. Reyjal et al. reported that therapeutic trials specifically addressed the induction of cytotoxic autophagy as opposed to passive autophagy. Autophagic cell death appears to be mediated by cell type and genetic constitution, and in a stimulus-specific manner.

A closer inspection of the approved drugs available in the market will show that most of them have certain therapeutic limitations. The main problem among the RTK-targeting drugs such as sorafenib is the short-lived response. Wei and Tan showed the neoplastic recurrences in the case of myelodysplastic and acute myeloid leukemia patients treated with sorafenib. Although this drug shows promising antiangiogenic properties and is presently used to treat liver and thyroid cancer cases, it has side-effects of skin, gastric and metabolic toxicities. Interestingly, a small oral chemotherapeutic drug, lapatinib, is known to decrease HER2 as well as EGFR phosphorylation expression, thereby inactivating ERK and Akt network in breast cancer. It sensitized even the cells conferring resistance to trastuzumab by downregulating the IGF-1/mTORC1 pathways. However, this drug faced impediments in clinical practice because in the phase I clinical trial reported in 2014, Chien et al. showed that lapatinib reached a ceiling limit that counter-acted the treatment regimen.

Interestingly, tamoxifen, the antiestrogen drug, prevents progression and growth arrest of metastatic breast carcinoma cells. The development of antiestrogen resistance is a common clinical impediment in the treatment of estrogen receptor alpha-positive breast cancer patients. Schoenlein et al. showed that Beclin 1 induction leads to a drop in estrogenic growth signaling and to antiestrogen resistance. However, in comparison to the success rate of tamoxifen in women, male breast cancer patients suffer from a 5-fold higher attrition rate, with at least 62.5% of patients complaining about common side-effects of decreased libido, hot flashes and venous thrombosis. Colorectal cancer patients having mutated Ras or where the mutation status was unknown were found to be unsuitable for this drug therapy. Similar to panitumumab, cetuximab is not designated for the cure of K-Ras mutation-positive colorectal cancer. Another member of humanized monoclonal antibody therapy is bevacizumab, which is characterized as evoking pro-survival autophagy; however, a systematic meta-analysis revealed that bevacizumab is associated with high risks of proteinuria, hypertension and renal damage. How these risk factors and the clinical outcomes complicate the patient’s therapy is not entirely understood. Besides this, a double-blinded randomized control trial does not provide strong evidence to validate the effect of the antidiabetic drug, metformin, on cancer cell viability. To circumvent this challenge, long-term follow-up based on randomized trials must be undertaken to answer this problem. A well-known chemotherapeutic drug like cisplatin that has been in clinical practice for over 10 years has been reported to induce renal cell injury that leads to nephrotoxicity-associated death of cancer patients. An additional third-generation, platinum-based drug, oxaliplatin, that is mostly used in FOLFOX (5-FU, leucovorin and oxaliplatin) during the treatment of stage III advanced colorectal cancer cases, is associated with atypical fever and severe hypersensitive reactions as side-effects. In addition to the side-effects, chemoresistance is a severe problem in patients suffering from glioma. Oliva et al. proposed a mechanism of mitochondrial electron transport chain remodeling, to decipher the failure of temozolomide in the second cycle of chemotherapy in 90% of recurrent tumors. On the other hand, to further complicate breast cancer treatment, it was revealed that doxorubicin (adriamycin) administration made the patients vulnerable to cardio-toxicity, for which there was a need for combined therapy with cardioprotectants such as cardoxane (ICRF-187, dexrazoxane). Nevertheless, 5-FU, which is commonly used to treat a broad range of solid tumor cases, has limited biological efficacy owing to rapid metabolism by dihydroxyrimidine dehydrogenase, incomplete oral absorption, and toxicity in gastrointestinal and bone marrow cells due to non-selective attack on healthy cells. Another front-line candidate used in the treatment of mantle cell lymphoma, and newly diagnosed and refractory multiple myeloma is the proteasome inhibitor, bortezomib, that has a major efficacy issue when it comes to tackling treatment-associated neurotoxicity. The clinical response of bortezomib is effective in less than half of patients. In addition, the fact remains that none of the patients has been cured of the disease with this drug. Moreover, there has been very modest clinical efficacy in solid tumor patients who have been treated with only bortezomib. However, interestingly, pre-clinical evidence shows the reduction of bortezomib toxicities in patients treated with vitamin C and green tea-based polyphenol epigallocatechin-3-gallate.

Most of the autophagy inhibitor-based treatments are aimed at suppressing the level of autophagy to remove the chemoresistance support of tumor cell. When autophagy-modulating drugs are prescribed, it must be realized that these drugs can induce autophagy, which has a dual character. During the initiation of treatment, autophagy plays a lethal role by engulfing the mutated proteins; thus drugs that can trigger lethal autophagy during this stage are beneficial. However, during the later stages of malignancy, autophagy plays a protective role that helps the growth and proliferation of tumor cells, so the use of autophagy-inducing drugs can be harmful at this stage. However, combining an autophagy inhibitor with an anticancer agent can prove to be a viable clinical option.

Oncophagy-targeted therapy started in the clinics in 2007, and most trials are awaiting results, so it may be too soon to vouch for the safety of these treatments now. For example, CQ was reported by Kimura et al. to induce kidney damage while increasing cisplatin sensitivity in tumor cells. Other than affecting kidneys, CQ-mediated autophagy inhibition aggrava chemotherapy-associated injuries in the brain, liver, heart and hematopoietic cells. Therefore, chemotherapy combined with CQ may affect different organs in an unexpected manner. For instance, major chemotherapies damage bone marrow, and treatment with CQ may worsen this condition. Determining the exact reason for the
induction of the acute renal injury due to CQ-mediated autophagy inhibition is not yet explained\textsuperscript{202}.

Following pre-clinical trials, several phase I–III studies are underway with HCQ-based autophagy inhibition as an adjuvant to different cytotoxic drugs, as reported in Table 2 and Supplementary Table 1. However, it is also important to mention that sometimes clinical trials are terminated, as in the case of trial identifier number NCT00786682, when HCQ was administered in combination with docetaxel. This combination failed to improve therapeutic efficacy in comparison to historical controls and competing studies. It is notable that HCQ exhibits a long half-life while micromolar level concentrations are required to inhibit autophagy. Recently, the dose-limiting level for HCQ was reported from a clinical trial as 600 mg per day in glioma patients receiving temozolomide and radiation therapy\textsuperscript{203}. Wolpin \textit{et al.} found that HCQ monotherapy failed to achieve autophagy inhibition to augment therapeutic efficacy in metastatic pancreatic cancer patients who had previously undergone treatment\textsuperscript{204}.

Despite the development of a broad range of autophagy-modulating chemotherapeutic pre-clinical trials, few drugs are translated into final clinical studies\textsuperscript{205}. This difference was elucidated by the recent study of Bristol \textit{et al.}\textsuperscript{206} where they showed that CQ treatment failed to inhibit or reverse the tumor growth in the xenografted mice model receiving radiation; these studies deserve special mention as they carried out their studies on syngenic mice that were immune competent, which simulated the actual cancer patient condition in humans, unlike most pre-clinical studies in immunodeficient mice. To add fuel to this debate, a conflicting report by Liang \textit{et al.}\textsuperscript{207} showed that autophagy-based therapy did extend the survival period in immune competent animals. However, there exists a discrepancy in the translation of pre-clinical trial drugs to the clinic. This depends on the different nature of cancer, clonal genotype, interaction with the diverse types of immunogenic patterns and/or other molecular signaling molecules that have the capacity to alter the treatment response as well as autophagic modulation reaching the target tumor cells\textsuperscript{205}.

For an efficient oncophagic line of therapeutics, it is hence imperative to realize that the non-autophagy function of autophagy inducers or inhibitors must be tolerable at the intended doses. Furthermore, the clinical agents must deliver a high benefit-to-risk ratio, with evidence of disease modulation capacity as direct evidence for the effectiveness of the therapeutic approach. In this manuscript, we have comprehensively reviewed ongoing oncophagy-based clinical trials and elaborated on the various shortcomings. As a future treatment strategy, the potential of natural molecules has been comprehensively discussed by Wang and Feng\textsuperscript{208}.

Innovative developments to analyze the autophagy level in cancer patients in a non-invasive, accurate and economical way should revolutionize this field of research which is now limited due to lack of reliability, accuracy and availability of appropriate technology to implement such translational research. Various autophagy markers like LC3, p62, Beclin 1, Atg5 are now being analyzed by RNA \textit{in situ} hybridization and immunohistochemical methods in patient biopsy specimens\textsuperscript{209,210}. Although Beclin 1-mediated inhibition of cytoprotective autophagy is a clinical strategy, in a different report Beclin 1, LC3 expression was increased in 89 clinical cases of multiple myeloma biopsies collected from 2001 to 2004\textsuperscript{211}. Attempts to interpret autophagy biomarkers using peripheral white blood cells in patients’ plasma are in the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Cracking the oncophagic therapy riddle: (A) Highlighting the vicious cycle depicting the therapeutic bottleneck in cancer. (B) Some outstanding questions which the community is presently trying to figure out.}
\end{figure}
Figure 5. Killing the cancer, before the devil knows – oncophagy based diagnosis: We propose a model scoring system to determine the level of autophagy from different normal and tumor specimens of various types, grades, and from patients of different ages, sex and ethnicity. This score will be based on the level of various autophagy markers (discussed in the text) to identify the lethal and protective form of autophagy level and submitted in a repository. Validating a patient’s score prior to and during diagnosis will give physicians ample opportunity to monitor the effectiveness of treatment.
pipeline for modern autophagy-based clinical analysis. Elevation in glycolysis level upon autophagy inhibition using HCQ in pancreatic cancer cases may be successfully determined by positron emission tomography (PET) scanning to monitor fluorodeoxyglucose accrual in the tumor212. More reports on the specific autophagic markers were elucidated by Zhao et al., where depletion of caveolin-1 led to a toxic autophagic response in breast tumor stroma that strongly regressed lymph node metastasis and spontaneous mortality213. Consequently, the clinical evidence showed that high level of stromal monocarboxylate transporter 4 (MCT4) indicates higher possibilities of TNBC and ductal carcinoma in situ. Combining MCT4 and Cav-1 is a novel biomarker strategy using the previously discussed PET imaging in autophagic tumor stroma of patients214. These reports highlight that autophagy induction due to alteration of tumor stromal proteins induces a very specific lethal response. Likewise, the phenomenon of specifically acquired autophagy was further supported by Marino et al.215, where they showed Atg4C to be essential for proper autophagic response under stress, and this enzyme was also reported to modulate tumor progression. Recently, Green and Levine have elegantly revealed that autophagy regulation specifically controls the cell death pattern in an active manner216. Aits et al. reported that the treatment of tumor cells with a potential clinical option called HAMLET (human alpha-lactalbumin made lethal to tumor cells) resulted in lethal autophagy-mediated cell death induction by mTOR inactivation and massive cytoplasmic vacuolization217.

Another potential field of oncophagic therapeutics lies in the evolution of miRNAs-mediated therapeutic strategies218; the authors hypothesized that a low autophagic level abrogates the tumor suppressing miRNAs and promotes oncogenic miRNAs during cancer proliferation. Chen et al. recently constructed the AutomiRDB, an online database connecting the microRNAs involved in autophagic modulation of cancer219. Zhang et al. reported that the autophagy expression induced by miR-216a is involved in binding to the 3’ untranslated region of Beclin 1 to boost the radiosensitivity in pancreatic cancer220. For the first time, the pre-clinical potential of miR-29b was explored by Dai et al. in ovarian cancer patients receiving chemotherapy221. Likewise, ectopic expression of tumor suppressor of miR-101 repressed protective autophagy and targeted enhancer of zeste homolog 2 oncogene to induce apoptosis in liver carcinoma cells treated with doxorubicin or 5-FU222. Moreover, the clinical relevance of miRNA targeted therapies in response to sorafenib treatment in hepatocarcinoma patients was elucidated by autophagic modulation of miR423–5p223. Interestingly, inhibition of miR-183 highlighted the plausible diagnostic importance by triggering autophagy-mediated cell death224 as well as apoptosis225.

Envisaging the use of autophagy-based treatment to target CSC is a futuristic goal for clinicians226. For the first time, Cuff et al. linked the maintenance mechanism of breast CSCs (CD44high/CD24low) to the prevalence of autophagy227. Gong et al. showed that Beclin 1-mediated protective autophagy induction critically maintained breast tumor development228. However, autophagic inhibition by salinomycin treatment in breast CSCs hindered the maintenance of stemness229. Likewise, the caudal type homeobox transcription factor (Cdx1) was showed to exert cytotoxic autophagy through the Cdx1-Bcl-2-LC3 pathway in colon CSCs230. A plausible clinical measure to tackle the problem of therapeutic resistance and cancer recurrence can be achieved by developing targeted oncophagic strategies to challenge the Achilles' heel of CSCs.

The advancement in the research of such oncophagic therapies transcends from basic to translational research, and progresses through rigid regulation of clinical trials for 10–15 years amidst an extremely competitive research funding environment. Realizing the vicious cycle of the therapeutic bottleneck, we initiate a debate by raising the burning questions on unresolved issues to the research community (Figure 4).

In this association, we propose the implementation of a model oncophagy scoring-based clinical investigation as represented in Figure 5. According to our hypothesis, through an initial holistic proteome-wide screening, we need to analyze the autophagy level and designate a score to classify whether it represents a protective or lethal level. All these scores will be collected from normal, benign and various grades of tumor specimens from patients of different ages, sex and ethnicities and curated in an internationally-consorted clinical database. When a patient undergoes diagnosis, his autophagy score would be calculated and matched with the database and the nature of prognosis – oncophagy induction or inhibition – would be decided appropriately. The rationale behind the oncophagy score-based cancer treatment is a serious attempt to simplify the autophagic complexities plaguing the current therapeutic approach. The benefit of this system would be far-reaching as this method would take into account the real molecular proteins defining the progression of cancer. So, the efficacy of this system would be defined accurately by a score that identifies when the nature of the autophagy flips from lethal to protective. The problem of understanding the nature of autophagy in cancer patients is ubiquitous, so the benefit of this oncophagy score would lie in its power to predict the timing as well as the drug dose that would be efficacious to target cancer cells at a particular stage. The bottom line is that, while most applications have been laid out in principle with the aim to target cancer, the therapeutic data differ case-to-case and must be amalgamated in a database to permit use of this scoring system. The advantage of this score would lie in its ability to deliver a simple medical diagnostic test like a signal that would decide the prescription of autophagy inducers or inhibitors.

### Conclusion: the road ahead for oncophagic therapeutics

Investigations in the field of oncophagic-based therapeutics will open a new horizon of opportunities with unbound potential and scope for the betterment of patient survival rates in the coming years. Because autophagy serves a dual role, when this mechanism behaves as a tumor suppressor and when it switches to a tumor promoter function in different stages and types of cancer and with drug treatments creates a
severe dichotomy. Numerous pre-clinical findings are now demonstrating inhibition of the cytoprotective effect of autophagy in conjunction with different combinations of cytotoxic drug and radiation-based therapies. In this review, we have provided an update on clinical findings from across the globe on autophagic involvement in cancer patients and highlighted various ways that researchers are tackling the disease by adopting different trial regimens.

However, the various documented restrictions like time-bound observational biases in survival, selection and referral groups coupled with short follow-up may lead to erroneous conclusions. Nevertheless, with the advancement of oncophagic relevance in the present cancer trials, the future of oncophagy-based remedial measure can make a difference. With the current trials promising better cancer treatment outcome, it is time to recognize the success of oncophagy-based remedial measures that will make a difference in the therapeutic horizon.

Acknowledgements

We thank the National Institute of Technology, Rourkela, for providing a facility for this work. We acknowledge Mr. Deependra Kumar Ban, Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, for fruitful discussion and constructive suggestions. We are highly thankful to Miss. Anuttoma Ray, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, USA, who generously helped us to prepare the final revised form of the manuscript.

Declaration of interest

Research support was partly provided by the Rapid Grant for Young Investigators (RGYI) Award [Grant Number: BT/PR1/5090/GBD/27/309/2011], the Department of Biotechnology [Grant Number: BT/PR7791/BRB/10/1187/2013]; the Science and Engineering Research Board (SERB), the Department of Science and Technology [Grant Number: SR/SO/BB-0101/2012]; the Council of Scientific and Industrial Research (CSIR) [Grant Number: 37(1608)/13/EMR-II] Human Resource Development Group, Government of India. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. We apologize to researchers whose studies on autophagy we were unable to cite due to the length of this review.

References

1. Levine B, Sinha S, Kroemer G. Bcl-2 family members: dual regulators of apoptosis and autophagy. Autophagy 2008;4:600–6.
2. Gundara JS, Zhao J, Robinson BG, Sidhu SB. Oncophagy: harnessing regulation of autophagy in cancer therapy. Endocr Relat Cancer 2012;19:R281–95.
3. Hamáí A, Codogno P, Mehrpour M. Cancer stem cells and autophagy: facts and perspectives. J Cancer Stem Cell Res 2014:2:e1005.
4. Ojha R, Bhattacharyya S, Singh SK. Autophagy in cancer stem cells: a potential link between chemo-resistance, recurrence, and metastasis. Biores Open Access 2015;4:97–108.
5. Lin YH, Huang YC, Chen LH, Chu PM. Autophagy in cancer stem/progenitor cells. Cancer Chemother Pharmacol 2015;75:879–86.
6. Ohsumi Y. Historical landmarks of autophagy research. Cell Res 2012;24:9–23.
7. Jung CH, Ro SH, Cao J, et al. mTOR regulation of autophagy. FEBS Lett 2010;584:1287–95.
8. Jung CH, Seo M, Otto NM, Kim DH. ULK1 inhibits the kinase activity of mTORC1 and cell proliferation. Autophagy 2011;7:1212–21.
9. Jung CH, Jun CB, Ro SH, et al. ULK-Ag13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009;20:1992–2003.
10. Hosokawa N, Hara T, Kaizuka T, et al. Nutrient-dependent mTORC1 association with the ULK1-Ag13-FIP200 complex required for autophagy. Mol Biol Cell 2009;20:1981–91.
11. Bhutia SK, Mukhopadhyay S, Sinha N, et al. Autophagy: cancer’s friend or foe? Adv Cancer Res 2013;118:61–95.
12. Papinski D, Kraft C. Atg1 kinase organizes autophagosome formation by phosphorylating Atg9. Autophagy 2014;10:1338–40.
13. Papinski D, Schuschnig M, Reiter W, et al. Early steps in autophagy depend on direct phosphorylation of Atg9 by the Atg1 kinase. Mol Cell 2014;53:471–83.
14. Webber JL, Young AR, Tooze SA. Atg9 trafficking in Mammalian cells. Autophagy 2007;3:54–6.
15. Petitot A, Ogier-Denis E, Blommaert EF, et al. Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. J Biol Chem 2000;275:992–8.
16. Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T. Beclin-phosphatidylinositol 3′-kinase complex functions at the trans-Golgi network. EMBO Rep 2001;2:330–5.
17. Gozuacik D, Kimchi A. Autophagy and cell death. Curr Top Dev Biol 2007;78:217–45.
18. Fan W, Nassiri A, Zhong Q. Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). Proc Natl Acad Sci USA 2011;108:7769–74.
19. Burman C, Kistakos NT. Regulation of autophagy by phosphatidylinositol 3-phosphate. FEBS Lett 2010;584:1302–12.
20. Funderburk SF, Wang QL, Yue Z. The Beclin 1-VPS34 complex – at the crossroads of autophagy and beyond. Trends Cell Biol 2010;20:355–62.
21. Russell RC, Tian Y, Yuan H, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 2013:15:741–50.
22. Kim YM, Jung CH, Seo M, et al. mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. Mol Cell 2015;57:207–18.
23. Molejon ML, Ropolo A, Re AL, et al. The VMP1–Beclin 1 interaction regulates autophagy induction. Sci Rep 2013;3:1055.
24. Noda T, Fujita N, Yoshimori T. The late stages of autophagy: how does the end begin? Cell Death Differ 2009;16:984–90.
25. Puri C, Renna M, Bento CF, et al. ATG16L1 meets ATG9 in recycling endosomes: additional roles for the plasma membrane and endocytosis in autophagosome biogenesis. Autophagy 2014;10:182–4.
26. Kanki T, Wang K, Cao Y, et al. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. Dev Cell 2009;17:98–109.
27. Moreau K, Puri C, Rubinsztein DC. Methods to analyze SNARE-dependent vesicular fusion events that regulate autophagosome biogenesis. Methods 2015;75:19–24.
28. Itakura E, Mizushima N. Syntaxin 17: the autophagosomal SNARE. Autophagy 2013;9:917–9.
29. Nair U, Jotwani A, Geng J, et al. SNARE proteins are required for macroautophagy. Cell 2011;146:290–302.
30. Moreau K, Renna M, Rubinsztein DC. Connections between SNAREs and autophagy. Trends Biochem Sci 2013;38:57–63.
31. Takáts S, Pires K, Nagy P, et al. Interaction of the HOPS complex with Syntaxin 17 mediates autophagosome clearance in Drosophila. Mol Biol Cell 2014;25:1338–54.
32. Yu L, McPhee CK, Zheng L, et al. Termination of autophagy and reformation of lysosomes regulated by mTOR. Nature 2010;465:942–6.
33. Rong Y, Liu M, Ma L, et al. Clathrin and phosphatidylinositol-4,5-bisphosphate regulate autophagic lysosome reformation. Nat Cell Biol 2012;14:924–34.

34. Liu Y, Shoji-Kawata S, Sumpter Jr RM, et al. Autosis is a Na⁺, K⁺-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. Proc Natl Acad Sci USA 2013;110:20364–71.

35. Ichimura Y, Waguri S, Sou YS, et al. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. Mol Cell 2013;51:618–31.

36. Mathew R, Karp CM, Beaudoin B, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell 2009;137:1062–75.

37. Mathew R, Kongara S, Beaudoin B, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. Genes Dev 2007;21:1367–81.

38. Kimmelman AC. The dynamic nature of autophagy in cancer. Genes Dev 2011;25:1999–2010.

39. Ng TL, Leprivier G, Robertson MD, et al. The AMPK stress response pathway mediates ankois resistance through inhibition of mTOR and suppression of protein synthesis. Cell Death Differ 2012;19:501–11.

40. Tasdemir E, Maiuri MC, Galluzzi L, et al. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol 2008;10:676–87.

41. Guo JY, Karsli-Uzunbas G, Mathew R, et al. Autophagy suppresses progression of K-ras-induced lung tumors to oncocytes and maintains lipid homeostasis. Genes Dev 2013;27:1447–61.

42. Huo Y, Cai H, Teplova I, et al. Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. Cancer Discov 2013;3:894–907.

43. Qu X, Yu J, Bhagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003;112:1890–20.

44. Rosenfeldt MT, O’Prey J, Morton JP, et al. p53 status determines the role of autophagy in pancreatic tumour development. Nature 2013;504:296–300.

45. Yang A, Kimmelman AC. Inhibition of autophagy attenuates pancreatic cancer growth independent of TP53/TRP53 status. Autophagy 2014;10:1683–4.

46. Pavlides S, Tsrigos A, Migonec G, et al. The autophagic tumor-stromal model of cancer: role of oxidative stress and ketone production in fueling tumor cell metabolism. Cell Cycle 2010;9:3485–505.

47. Tang D, Kang R, Livesey KM, et al. High mobility group box 1 (HMGB1) activates an autophagic response to oxidative stress. Antioxid Redox Signal 2011;15:2185–95.

48. Amaravadi RK, Yu D, Lum JJ, et al. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 2007;117:326–36.

49. Golden EB, Cho HY, Jahanian A, et al. Chloroquine enhances temozolomide cytotoxicity in malignant gliomas by blocking autophagy. Neurosurg Focus 2014;37:E12.

50. Ma Y, Galluzzi L, Zitvogel L, Kroemer G. Autophagy and cellular immune responses. Immunity 2013;39:211–27.

51. Kangwan N, Park JM, Kim EH, Hahn KB. Chemoquiescence for ideal cancer treatment and prevention: where are we now? J Cancer Prev 2014;19:89–96.

52. Nishikawa M, Miyake H, Liu B, Fujisawa M. Expression pattern of autophagy-related markers in non-metastatic clear cell renal cell carcinoma: association with disease recurrence following radical nephrectomy. J Cancer Res Clin Oncol 2015;141:1385–91.

53. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

54. Can G, Ekiz HA, Baran Y. Imatinib induces autophagy through BECLIN-1 and ATG5 genes in chronic myeloid leukemia cells. Hematology 2011;16:95–9.

55. Gupta A, Roy S, Lazar AJ, et al. Autophagy inhibition and antimarialarials promote cell death in gastrointestinal stromal tumor (GIST). Proc Natl Acad Sci USA 2010;107:14333–8.

56. Miselli F, Negri T, Gronchi A, et al. Is autophagy rather than apoptosis the regression driver in imatinib-treated gastrointestinal stromal tumors? Transl Oncol 2008;1:177–86.
81. Saleem A, Dvorzhinski D, Santanam U, et al. Effect of dual inhibition of apoptosis and autophagy in prostate cancer. Prostate 2012;72:1374–81.
82. Hann CL, Daniel VC, Sugar EA, et al. Therapeutic efficacy of ABT-737, a selective inhibitor of BCL-2, in small cell lung cancer. Cancer Res 2008;68:2321–8.
83. Zinn RL, Gardner EE, Dobromilskaya I, et al. Combination treatment with ABT-737 and chloroquine in preclinical models of small cell lung cancer. Mol Cancer 2013;12:16.
84. Trisciuoglio D, Desideri M, Ciuffreda L, et al. Bcl-2 overexpression in melanoma cells increases tumor progression-associated properties and in vivo tumor growth. J Cell Physiol 2005;205:414–21.
85. Trisciuoglio D, De Luca T, Desideri M, et al. Removal of the BH4 domain from Bcl-2 protein triggers an autophagic process that impairs tumor growth. Neoplasia 2013;15:315–27.
86. Heidari N, Hicks MA, Harada H. GX15-070 (obatoclax) overcomes glucocorticoid resistance in acute lymphoblastic leukemia through induction of apoptosis and autophagy. Cell Death Dis 2010;1:e76.
87. Gao P, Barvy C, Souquière S, et al. The Bcl-2 homology domain 3 mimetic gossypol induces both Beclin 1-dependent and Beclin 1-independent cytoprotective autophagy in cancer cells. J Biol Chem 2010;285:25570–81.
88. Hamái A, Codogno P. New targets for acetylation in autophagy. Sci Signal 2012;5:pe29.
89. Del Bufalo D, Desideri M, De Luca T, et al. Histone deacetylase inhibition synergistically enhances pemetrexed cytotoxicity through induction of apoptosis and autophagy in non-small cell lung cancer. Mol Cancer 2014;13:230.
90. Dupré-Richer D, Kinal M, Ménasché V, et al. Verinostat-induced autophagy switches from a death-promoting to a cytoprotective signal to drive acquired resistance. Cell Death Dis 2013;4:e486.
91. Rao R, Balusu R, Fiskus W, et al. Combination of pan-histone deacetylase inhibitor LY294002 activates autophagy and induces apoptosis in cancer cells. Br J Pharmacol 2011;164:731–42.
92. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and future directions. Sci Signal 2012;5:pe29.
93. Maira SM, Stauffer F, Brueggen J, et al. Identification and characterization of NVP-BEZ235, a new orally available dual mTOR inhibitor, PI-103, cooperates with stem cell-delivered autophagy in prostate cancer cells. Mol Cancer Ther 2010;9:3673–85.
94. Yothaisong S, Dokduang H, Techasen A, et al. Increased mTORC1/2 inhibition synergistically enhances pemetrexed cytotoxicity through induction of apoptosis and autophagy in non-small cell lung cancer. Mol Cancer 2014;13:230.
95. Yothaisong S, Dokduang H, Techasen A, et al. Increased activation of PI3K/AKT signaling pathway is associated with cholangiocarcinoma metastasis and PI3K/mTOR inhibition presents a possible therapeutic strategy. Tumour Biol 2013;34:1181–90.
96. Li H, Jin X, Zhang Z, et al. Inhibition of autophagy enhances apoptosis induced by the PI3K/AKT/mTOR inhibitor NVP-BEZ235 in renal cell carcinoma cells. Cell Biochem Funct 2013;31:427–33.
97. Youthsong S, Dokduang H, Techasen A, et al. Increased activation of PI3K/AKT signaling pathway is associated with cholangiocarcinoma metastasis and PI3K/mTOR inhibition presents a possible therapeutic strategy. Tumour Biol 2013;34:1181–90.
98. Hong SW, Shin JS, Moon JH, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, induces cell death through alternate routes in prostate cancer cells depending on the PTEN genotype. Apoptosis 2014;19:895–904.
99. Mukherjee B, Tomimatsu N, Amacherla K, et al. The dual PI3K/mTOR inhibitor NVP-BEZ235 is a potent inhibitor of ATM- and DNA-PKcs-mediated DNA damage responses. Neoplasia 2012;14:34–43.
100. Xing C, Zhu B, Liu H, et al. 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;40:194–201.
101. Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. Mol Cancer Ther 2011;10:1533–41.
102. Bagci-Onder T, Wakimoto H, Anderregg M, et al. A dual PI3K/mTOR inhibitor, PI-103, cooperates with stem cell-delivered TRAIL in experimental glioma models. Cancer Res 2011;71:154–63.
103. Tong Y, Liu YY, You LS, Qian WB. Perifosine induces protective autophagy and upregulation of ATG5 in human chronic myelogenous leukemia cells in vitro. Acta Pharmacol Sin 2012;33:542–50.
104. Evangelisti C, Ricci F, Tazzari P, et al. Preclinical testing of the Akt inhibitor triciribine in T-cell acute lymphoblastic leukemia. J Cell Physiol 2011;226:822–31.
105. Tseng CH. Metformin reduces thyroid cancer risk in Taiwanese patients with type 2 diabetes. PLoS One 2014;9:e109852.
106. Tsai MJ, Yang CJ, Kung YT, et al. Metformin decreases lung cancer risk in diabetic patients in a dose-dependent manner. Lung Cancer 2014;86:137–43.
107. Wang N, Pan W, Zhu M, et al. Fangchinoline induces autophagic cell death via p53/ser2/AMPK signalling in human hepatocellular carcinoma cells. Br J Pharmacol 2011;164:731–42.
108. Ge J, Liu Y, Li Q, et al. Resveratrol induces apoptosis and autophagy in T-cell acute lymphoblastic leukemia cells by inhibiting Akt/mTOR and activating p38-AMPK. Biomed Environ Sci 2013;26:902–11.
109. Bishayee A. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. Cancer Prev Res (Phila) 2009;2:409–18.
110. Ling LU, Tan KB, Lin H, Chiu GN. The role of reactive oxygen species and autophagy in safinogin-induced cell death. Cell Death Dis 2011;2:e129.
111. Yu HC, Lin CS, Tai WT, et al. Nitotinib induces autophagy in hepatocellular carcinoma through AMPK activation. J Biol Chem 2013;288:18249–59.
112. Yang PM, Liu YL, Lin YC, et al. Inhibition of autophagy enhances anticancer effects of avatistatin in digestive malignancies. Cancer Res 2010;70:7699–709.
113. Bristol ML, Di X, Beckman MJ, et al. Dual functions of autophagy in the response of breast tumor cells to radiation: cytoprotective autophagy with radiation alone and cytotoxic autophagy in radiosensitization by vitamin D3. Autophagy 2012;8:739–53.
114. DeMasters G, Di X, Newsham I, et al. Potentiation of radiation sensitivity in breast tumor cells by the vitamin D3 analogue, EB 1089, through promotion of autophagy and interference with proliferative recovery. Mol Cancer Ther 2006;5:2786–97.
115. Dando I, Donadelli M, Costanzo C, et al. Cannabinoids inhibit energetic metabolism and induce AMPK-dependent autophagy in pancreatic cancer cells. Cell Death Dis 2013;4:e664.
116. Liu X, Chhipa RR, Nakano I, et al. The AMPK inhibitor Compound C is a potent AMPK-independent anti-glioma agent. Mol Cancer Ther 2014;13:596–605.
117. Beer TM, Armstrong AJ, Rathkopf DEV, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424–33.
118. Nguyen HG, Yang JC, Kung HJ, et al. Targeting autophagy overcomes Enzalutamide resistance in castration-resistant prostate cancer cells and improves therapeutic response in a xenograft model. Oncogene 2014;33:4521–30.
119. Arcella A, Biagoni F, Antonietta Oliva M, et al. Rapamycin inhibits the growth of glioblastoma. Brain Res 2013;1495:37–51.
120. Korets SB, Musa F, Curtin J, et al. Dual mTORC1/2 inhibition in a preclinical xenograft tumor model of endometrial cancer. Gynecol Oncol 2013;132:468–73.
121. Cai Y, Xia Q, Su Q, et al. mTOR inhibitor RAD001 (everolimus) induces apoptotic, not autophagic cell death, in human nasopharyngeal carcinoma cells. Int J Mol Med 2013;31:904–12.
122. Witzig TE, Geyer SM, Ghobrial I, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 2010;28:1596–604.
123. Galimberti S, Petrini M. Temsirolimus in the treatment of relapsed indolent non-Hodgkin lymphoma. Leuk Lymphoma 2009;50:1427–33.
124. Chresta CM, Davies BR, Hickson I, et al. AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Cancer Res 2010;70:288–98.
Zheng B, Mao JH, Qian L, et al. Preclinical evaluation of AZD-2508. *Mol Cancer Ther* 2013;12:1798–810.

Francipane MG, Lagasse E. Selective targeting of human colon cancer stem-like cells by the mTOR inhibitor Torin-1. *OncoTarget* 2013;4:1948–62.

Zeng B, Mao JH, Qian L, et al. Aurora kinase A inhibition triggers drug resistance in breast cancer cells. *Autophagy* 2012;8:1798–810.

Sun Y, Liu JH, Jin L, et al. Inhibition of Beclin 1 expression enhances cisplatin-induced apoptosis through a mitochondrial-dependent pathway in human ovarian cancer SKOV3/DDP cells. *Oncol Res* 2014;21:261–9.

Wang J, Wang X. Role of autophagy in cisplatin resistance in ovarian cancer cells. *J Biol Chem* 2014;289:17163–73.

Shi Y, Tang B, Yu PW, et al. Autophagy protects against oxaplatin-induced cell death via ER stress and ROS in Caco-2 cells. *PLoS One* 2012;7:e51076.

Leng S, Hao Y, Du D, et al. Ursolic acid promotes cancer cell death by inducing Atg5-dependent autophagy. *Int J Cancer* 2013;133:2781–90.

Liu J, Xia H, Kim M, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Acta Pharmacol Sin* 2013;34:669–79.

Akar U, Chaves-Reyez A, Barria M, et al. Silencing of Bcl-2 expression by small interfering RNA induces autophagic cell death by inducing Atg5-dependent autophagy. *Int J Cancer* 2014;134:164–72.

Ding ZB, Hui B, Shi YH, et al. Autophagy activation in hepatocellular carcinoma contributes to the tolerance of oxaplatin via reactive oxygen species modulation. *Clin Cancer Res* 2011;17:6229–38.

Maede Y, Shimizu H, Fukushima T, et al. Differential and common DNA repair pathways for topoisomerase I- and II-targeted drugs in a genetic DT40 repair screen panel. *Mol Cancer Ther* 2014;13:214–20.

Paillas S, Causse A, Marzi L, et al. MAPK14/p38α confers irinotecan resistance to TP53-defective cells by inducing survival autophagy. *Autophagy* 2012;8:1098–112.

Cosan D, Soyocak A, Tekedereli I, et al. Abstract 5109: a novel mTORC1/2 dual inhibitor, against renal cell carcinoma. *Clin Cancer Res* 2014;20:1953c. September 15;20:1831c. September 15.

Lotze MT, Maranchie J, Appleman L. Inhibiting autophagy: a novel approach for the treatment of renal cell carcinoma. *Cancer J* 2013;19:190–206.

Chen B, Su CH, et al. Autophagy induced by farnesyltransferase inhibitors in cancer cells. *Cancer Biol Ther* 2011;10:141–8.

Turcotte S, Chan DA, Sutphin PD, et al. A molecule targeting VHL-deficient renal cell carcinoma that induces autophagy. *Cancer Cell* 2008;14:90–102.

Jia L, Gopinathan G, Sukumar JT, Gribben JG. Blocking IL-21 induces protective autophagy. *Proc Natl Acad Sci USA* 2010;107:22243–8.

Li X, Xu HL, Liu YX, et al. Autophagy modulation as a target for anticancer drug discovery. *Acta Pharmacol Sin* 2013;34:612–24.

Li J, Xia H, Kim M, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell Death Dis* 2011;4:1798–810.

Carew JS, Espitia CM, Esquivel 2nd JA, et al. Lucenthione is a novel inhibitor of autophagy that induces cathepsin D-mediated apoptosis. *J Biol Chem* 2011;286:6602–13.

Sotelo J, Briceno E, Lopez-Gonzalez MA. Adding chloroquine to paclitaxel-induced apoptosis functions as a pro-survival pathway in breast cancer cells. *Front Cell Neurosci* 2015;9:56679.

Schonewolf CA, Mehta M, Schiff D, et al. Autophagy inhibition by chloroquine sensitizes HT-29 colorectal cancer cells to concurrent chemoradiation. *World J Gastrointest Oncol* 2014;6:74–82.

Maes H, Kuchnio A, Peric A, et al. Tumor vessel normalization by chloroquine independent of autophagy. *Cancer Cell* 2014;26:190–206.

Sui X, Chen R, Wang Z, et al. Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. *Cell Death Dis* 2013;4:e838.

Costa C, Strambi A, Zolpil C, et al. Acidic extracellular pH neutralizes the autophagy-inhibiting activity of chloroquine: implications for cancer therapies. *Autophagy* 2014;10:562–71.

Lotze MT, Maranchie J, Appleman L. Inhibiting autophagy: a novel approach for the treatment of renal cell carcinoma. *Cancer J* 2013;19:341–71.

Helgason GV, Mukhopadhyay A, Karvela M, et al. Autophagy in chronic myeloid leukemia: stem cell survival and implication in therapy. *Curr Cancer Drug Targets* 2013;13:724–34.

Dragowska WH, Weppler SA, Wang JC, et al. Induction of autophagy is an early response to gefitinib and a potential therapeutic target in breast cancer. *PLoS One* 2013;8:e76503.

Amaravadi RK, Winkler JD. Lys05: a new lysosomal autophagy inhibitor. *Autophagy* 2012;8:1383–4.
of a genetic autophagy deficiency. Proc Natl Acad Sci USA 2012; 109:8253–8.

171. Mohapatra P, Preet R, Das D, et al. Quinacrine-mediated autophagy and apoptosis in colon cancer cells is through a p53- and p21-dependent mechanism. Oncol Res 2012; 20:801–91.

172. Wu YC, Wu WK, Li Y, et al. Inhibition of macroautophagy by bafilomycin A1 lowers proliferation and induces apoptosis in colon cancer cells. Biochem Biophys Res Commun 2009; 382: 451–6.

173. Choi HS, Jeong EH, Lee TG, et al. Autophagy inhibition with mTOR inhibitors enhances cell cycle arrest and apoptosis induced by mTOR or epidermal growth factor receptor inhibitors in lung cancer cells. Tuberc Respir Dis (Seoul) 2013; 75:19–77.

174. Tanida I, Minematsu-Ikeguchi N, Ueno T, Kominami E. Lyssosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. Autophagy 2005;1:84–91.

175. Sato K, Tsuchihiara K, Fujii S, et al. Autophagy is activated in colorectal cancer cells and contributes to the tolerance of nutrient deprivation. Cancer Res 2007;67:9677–84.

176. Racoma IO, Meisen WH, Wang QE, et al. Thymoquinone inhibits autophagy and induces cathepsin-mediated, caspase-independent cell death in glioblastoma cells. PLoS One 2013;8:e72882.

177. Udelenow A, Kreyes A, Ellinger S, et al. Omeprazole inhibits proliferation and modulates autophagy in pancreatic cancer cells. PLoS One 2011;6:e20143.

178. Ojha R, Singh SK, Bhattacharyya S, et al. Inhibition of grade dependent autophagy in uterine carcinoma increases cell death under nutritional limiting condition and potentiates the cytotoxicity of chemotherapeutic agent. J Urol 2014;191:1889–98.

179. Liu D, Yang Y, Liu Q, Wang J. Inhibition of autophagy by 3-MA potentiates cisplatin-induced apoptosis in esophageal squamous cell carcinoma cells. Med Oncol 2011;28:105–11.

180. Li J, Hou N, Faried A, et al. Inhibition of autophagy by 3-MA enhances the effect of 5-FU-induced apoptosis in colon cancer cells. Ann Surg Oncol 2009;16:761–71.

181. Hamai A, Botti J, Mehrpour M, Codogno P. Autophagy and tumor cell metabolism. In: Mazurek S, Shoshan M., eds. Tumor Cell Metabolism, Switzerland: Springer, 2015:45–63.

182. Rejyal J, Cormier K, Turcotte S. Autophagy and cell death to target cancer cells: exploiting synthetic lethality as cancer therapies. Adv Exp Med Biol 2014;772:167–88.

183. Choi KS. Autophagy and cancer. Exp Mol Med 2012;44:109–20.

184. Wei A, Tan P. Limitations of targeted therapy with sorafenib in elderly high-risk myelodysplastic syndrome and acute myeloid leukemia. Leuk Lymphoma 2013;54:675–6.

185. Chen AJ, Munster PN, Melisko ME, et al. Phase I dose-escalation study of 5-day intermittent oral lapatinib therapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer. J Clin Oncol 2013;32:1427–9.

186. Schoenlein PV, Periyasamy-Thandavan S, Samaddar JS, et al. Autophagy facilitates the progression of ERalpha-positive breast cancer cells. Tumor Cell Death 2009;14:4025–38.

187. Anelli TF, Anelli A, Tran KN, et al. Tamoxifen administration is a marker for autophagy. Exp Mol Med 2014;46:1085–7.

188. Launay-Vacher V, Rey JB, Imsard-Bagnis C, et al. European Society of Clinical Pharmacy Special Interest Group on Cancer Care. Prevention of cisplatin nephrotoxicity: state of the art and recommendations from the European Society of Clinical Pharmacy Special Interest Group on Cancer Care. Cancer Chemother Pharmacol 2008;61:903–9.

189. Rosenfeld MR, Grossman SA, Brem S, et al. Pharmacokinetic analysis and pharmacodynamic evidence of autophagy inhibition in patients with newly diagnosed glioblastoma treated on a phase I trial of hydroxychloroquine in combination with adjuvant temozolomide and radiation (ABTC 0603). J Clin Oncol 2010; 28:15s.

190. Wolpin BM, Rubinson DA, Wang X, et al. Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. Oncologist 2014;19:637–8.

191. Aryan B, Denison AT, Gonzalez Y, Rao VA. Autophagy-based protein biomarkers for in vivo detection of cardiotoxicity in the context of cancer therapy. In: Hayat MA, ed. Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection and Aging. Vol. 3. San Diego, CA: Academic Press, 2013:299–303.

192. Bristol ML, Emery SM, Maycotte P, et al. Autophagy inhibition for chemoensitization and radiosensitization in cancer: do the preclinical data support this therapeutic strategy? J Pharmacol Exp Ther 2013;344:544–52.

193. Liang X, De Vera ME, Buchser WJ, et al. Inhibiting systemic autophagy during interleukin 2 immunotherapy promotes long-term tumor regression. Cancer Res 2012;72:2791–801.

194. Wang N, Feng Y. Elaborating the role of natural products-induced autophagy in cancer treatment: achievements and artifacts in the state of the art. Biomed Res Int 2015;2015:934207.

195. Liu JL, Chen FF, Lung J, et al. Prognostic significance of p62/SQSTM1 subcellular localization and LC3B in oral squamous cell carcinoma. Br J Cancer 2014;111:944–54.

196. Park JM, Huang S, Wu TT, et al. Prognostic impact of Beclin 1, p62/sequestosome 1 and LC3 protein expression in colon carcinomas from patients receiving 5-fluorouracil as adjuvant chemotherapy. Cancer Biol Ther 2013;14:100–7.

197. Jung G, Roh J, Lee H, et al. Autophagy markers BECLIN 1 and SQSTM1 subcellular localization and LC3B in oral squamous cell carcinoma. Br J Cancer 2014;111:944–54.

198. Vander Heiden MG, Cantley LC, Thompson CB. Understanding metabolic ''fertilize'' the tumor microenvironment with hydrogen peroxide, driving the Warburg effect: implications for PET imaging of human tumors. Cell Cycle 2011;10:2504–20.
215. Mariño G, Salvador-Montoliu N, Fueyo A, et al. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *J Biol Chem* 2007;282:18573–83.

216. Green DR, Levine B. To be or not to be? How selective autophagy and cell death govern cell fate. *Cell* 2014;157:65–75.

217. Aits S, Gustafsson L, Hallgren O, et al. HAMLET (human alphalactalbumin made lethal to tumor cells) triggers autophagic tumor cell death. *Int J Cancer* 2009;124:1008–19.

218. Jing Z, Han W, Sui X, et al. Interaction of autophagy with microRNAs and their potential therapeutic implications in human cancers. *Cancer Lett* 2015;356:332–8.

219. Chen Y, Huang J, Liu B. AutomiRDB: a web resource connecting microRNAs and autophagy in cancer. *Apoptosis* 2015;20:1016–17.

220. Zhang X, Shi H, Lin S, et al. MicroRNA-216a enhances the radiosensitivity of pancreatic cancer cells by inhibiting beclin-1-mediated autophagy. *Oncol Rep* 2015;34:1557–64.

221. Dai F, Zhang Y, Chen Y. Involvement of miR-29b signaling in the sensitivity to chemotherapy in patients with ovarian carcinoma. *Hum Pathol* 2014;45:1285–93.

222. Xu L, Beckebaum S, Iacob S, et al. MicroRNA-101 inhibits human hepatocellular carcinoma progression through EZH2 downregulation and increased cytostatic drug sensitivity. *J Hepatol* 2014;60:590–8.

223. Stiuso P, Potenza N, Lombardi A, et al. MicroRNA-423-5p promotes autophagy in cancer cells and is increased in serum from hepatocarcinoma patients treated with sorafenib. *Mol Ther Nucleic Acids* 2015;4:e233.

224. Abraham D, Jackson N, Gundara JS, et al. MicroRNA profiling of sporadic and hereditary medullary thyroid cancer identifies predictors of nodal metastasis, prognosis, and potential therapeutic targets. *Clin Cancer Res* 2011;17:4772–81.

225. Li J, Fu H, Xu C, et al. miR-183 inhibits TGF-beta1-induced apoptosis by downregulation of PDCD4 expression in human hepatocellular carcinoma cells. *BMC Cancer* 2010;10:354.

226. Sinha N, Mukhopadhyay S, Das DN, et al. Relevance of cancer initiating/stem cells in carcinogenesis and therapy resistance in oral cancer. *Oral Oncol* 2013;49:854–62.

227. Cufi S, Vazquez-Martín A, Oliveras-Ferraros C, et al. Autophagy positively regulates the CD44(+) CD24(-/low) breast cancer stem-like phenotype. *Cell Cycle* 2011;10:3871–85.

228. Gong C, Bauvy C, Tonelli G, et al. Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene* 2013;32:2261–72, 2272e.1–11.

229. Yue W, Hamai A, Tonelli G, et al. Inhibition of the autophagic flux by salinomycin in breast cancer stem-like/progenitor cells interferes with their maintenance. *Autophagy* 2013;9:714–29.

230. Wu S, Wang X, Chen J, Chen Y. Autophagy of cancer stem cells is involved with chemoresistance of colon cancer cells. *Biochem Biophys Res Commun* 2013;434:898–903.

Supplementary material available online