Targeting autophagy in cancer

Angelique Onorati[1], **Matheus Dyczynski**[2], **Rani Ojha**[1], and **Ravi K. Amaravadi**[1,#]

[1]Abramson Cancer Center and Department of Medicine; University of Pennsylvania, Philadelphia, PA, 19104; U.S.A

[2]Department of Oncology & Pathology Karolinska Institute, Stockholm, Sweden

Abstract

Background—Autophagy is a conserved, self-degradation system that is critical for maintaining cellular homeostasis during stress conditions. Dysregulated autophagy has implications in health and disease. Specifically, in cancer, autophagy plays a dichotomous role by inhibiting tumor initiation but supporting tumor progression. Early results of clinical trials repurposing hydroxychloroquine for cancer suggest autophagy inhibition could be a promising approach for advanced cancers.

Methods—Literature Review

Results—Here we review fundamental advances in the biology of autophagy, approaches to targeting autophagy, the preclinical rationale and clinical experience with HCQ in cancer clinical trials, the potential role of autophagy in tumor immunity, and recent developments in next generation autophagy inhibitors that have clinical potential.

Conclusions—Autophagy is a promising target for drug development in cancer.

Keywords

Autophagy; HCQ; chemotherapy; immunotherapy

Autophagy in health and disease

Autophagy is a tightly orchestrated process that sequesters misfolded proteins, damaged or aged organelles, and mutated proteins in double membrane vesicles called autophagosomes that ultimately fuse to lysosomes leading to the degradation of the sequestered components[1]. In 2016 the Nobel Prize in Medicine was awarded to Dr. Yoshinori Ohsumi for his groundbreaking work in yeast unraveling the regulation of autophagy[2]. The recycling capacity of autophagy is conserved from yeast to man, and regulates cellular homeostasis in both physiological and pathophysiological contexts. Dysregulated autophagy has been implicated in a number of diseases including neurodegenerative diseases[3], cardiomyopathy[4],

[#]Corresponding author: Ravi K. Amaravadi, MD, 852 BRB 2/3, 421 Curie Blvd, Philadelphia, PA 19104, Ravi.amaravadi@uphs.upenn.edu, (215) 796-5159.

Conflict of Interest Statement: None

infectious disease[5], type II diabetes[6], fatty liver[7], and cancer[8]. Another way autophagy is categorized is into nonselective and selective autophagy[9]. On the one hand, nonselective autophagy occurs when cells degrade their cytoplasm in a bulk manner. On the other hand, the study of how selective autophagy targets specific organelles or proteins has engendered terms such as mitophagy, pexophagy, and xenophagy[10]. Recently autophagy has been identified as a target for therapeutic intervention in a number of diseases including cancer. In order for effective development of therapeutics that target autophagy, a thorough understanding of molecular components of autophagy, with a special focus on druggable targets, will be reviewed here.

A mechanistic understanding of autophagy

The process of autophagy is broken down into four critical steps: initiation, nucleation, maturation, and degradation[10]. During the initiation step, the Unc-51-like kinase 1(ULK1)-Autophagy related gene 13 (ATG13)-family interacting protein 200kD (FIP200) kinase complex gets activated by coordinated inputs from the mechanistic target of rapamycin complex 1 (mTORC1), and AMP-activated protein Kinase (AMPK). In the nucleation step of autophagy, The ULK1 complex phosphorylates and activates the Beclin-1-VPS34 complex. This complex includes Beclin-1, VPS34 (a class III phosphatidylinositol 3-kinase (PI3K)), and other proteins such as VPS15, ATG14L, and autophagy and beclin 1 regulator 1 (AMBRA-1), depending on the subcellular localization of the complex[1]. Both the initiation and nucleation proteins promote the formation of the autophagic vesicle membrane. This membrane can be derived from mitochondria, plasma membrane, or the endoplasmic reticulum[11–13]. During the maturation step, two unique protein conjugation events are necessary for autophagosome formation: 1) ATG7 and ATG10 conjugate ATG5 to ATG12. 2) ATG7 and ATG3 conjugate LC3 (ATG8) to the lipid phosphatidylethanolamine (PE)[14]. The ATG5-ATG12 conjugate forms a complex with ATG16L1, and the ATG5-ATG12-ATG16L1 complex gets anchored onto phosphoinositol 3-phosphate generated by VPS34 on emerging autophagosomal membranes through WIPI-2b[15].. Meanwhile, the cleavage of LC3 by ATG4 leads to the soluble form (LC3-1), which is then conjugated to (PE) on the surface of the emerging autophagosome by ATG3 and ATG7, and guided by the ATG5-ATG12-ATG16L1 complex [16]. The lipidated form of LC3 is referred to as LC3-II. LC3-II migrates faster than LC3-I on gel electrophoresis allowing, the ratio of lipidated to free LC3 to utilized to reflect the number of autophagosomes forming at any given time.

The ultimate effect of this complex system is to place an ubiquitin-like protein (LC3-I) on membrane to label it as an autophagic membrane and to allow for interaction with cargo receptors that bring autophagic cargo to the autophagosome. SQSTM1/P62 and NBR1 are cargo receptors that recruit cargo destined for autophagic degradation to LC3-II on forming autophagasomes[17]. Once the isolation membrane is enclosed, it is called the autophagosome[18]. After autophagosome formation and cargo sequestration, the cargo-bound autophagosomes are transported on microtubules to the perinuclear region where lysosomes are present[19]. The multiprotein HOPS complex along with syntaxin 17 helps tether the autophagosomes to the lysosome[20] Emerging evidence indicates there are adaptor proteins such as EPG5 that recognize autophagy proteins to increase the specificity of autophagosome-lysosome fusion events[21]. Lastly, in the degradation phase, autophagic

cargo are degraded by lysosomal hydrolases[18]. This degraded material is then recycled through nutrient transporters and used to fuel growth of the cell[22].

The dichotomous role of autophagy in cancer

Autophagy plays a dichotomous role in cancer by suppressing benign tumor growth but promoting advanced cancer growth. In the past decade, numerous research groups have established autophagy as a potential therapeutic target in cancer. However, there is some debate about whether to inhibit or induce autophagy. The rationale for inducing autophagy is based on studies which show that mice with loss of one allele of the autophagy gene *Beclin 1* developed spontaneous tumors[23–25]. Initially this was thought to translate into patients with breast, ovarian, and prostate cancers, which are known to harbor monoallelic loss of Beclin 1[23, 24, 26]. However, it was shown that the human homolog of the mouse *Beclin1/Atg6* gene, *BECN1* gene resides adjacent to *BRCA1* on the same chromosome and other tumor suppressor genes. Tumorigenesis in human tumors may therefore be driven by neighboring genes lost rather than *BECN1*.

Many preclinical studies have demonstrated a variety of targeted therapies and DNA damaging agents that can induce autophagy, but in the majority of these studies the autophagy induced by the anticancer agent is cytoprotective, rather than cytotoxic[27]. Even if the goal was to induce autophagy to the point of activating cell death (what some refer to as autophagic cell death), to date there are almost no specific autophagy inducers. Most agents that induce autophagy inhibit other important process in the cell, such as mTOR signaling, or activate other stress responses such as the unfolded protein response[28]. Only Tat-Beclin1, a fusion peptide with an unclear mechanism of action, has been reported as a specific autophagy inducer[29]. These inducers could be useful in preventing the development of benign lesions such as polyps, but further work is needed to identify the best targets and chemical agents that can specifically induce autophagy.

There is mounting evidence that autophagy inhibition could be an effective approach in advanced cancer[18]. Genetically engineered mouse models (GEMMs) of lung cancer, pancreatic cancer and melanoma driven by either mutant *RAS* or *BRAF* in which autophagy genes were deleted have demonstrated that autophagy suppresses the growth of benign tumors, but accelerates the growth of advanced cancers[30–34]. This was also found in a mouse model of breast cancer[35, 36].

Mouse models of tumorigenesis should not be used to understand the utility of inhibiting autophagy in the therapeutic context because autophagy genes are usually deleted in utero at the same time that oncogenes and tumor suppressor genes are altered. In patients, autophagy inhibitors will be deployed after the cancer is already formed in the adult, and likely in combination with other agents. Therefore modulating autophagy in this context may produce different results than modulating autophagy at the origin of tumorigenesis. Accumulating evidence supports that autophagy promotes resistance during cancer therapy in established tumors. This was first demonstrated in a therapeutic mouse model of lymphoma, where autophagy inhibition augmented the efficacy of chemotherapy[37]. Recently a complex GEMM model which allows sudden genetic suppression of autophagy by conditionally

deleting ATG7 throughout the adult animal harboring a growing tumor was reported[38, 39]. This model is the closest model of autophagy inhibition in a cancer therapeutic context to the human clinic. In this model complete loss of autophagy in the mouse was well tolerated for months, during which time dramatic tumor shrinkage was observed. After a few months of complete genetic suppression of autophagy throughout the mouse, mice began to develop fatal neurodegeneration. Despite this fatal toxicity, collectively, these findings strongly support the use of autophagy inhibitors for cancer in clinic, and there may be a therapeutic window for potent extra-central nervous system (CNS) autophagy inhibition. Chronic autophagy inhibition, especially with agents that cross the blood brain barrier must be evaluated cautiously to balance between potency and toxicity, as autophagy plays an important role in normal cell and organismal homeostasis[40].

Autophagy inhibitors for laboratory research

There are a number of tool compounds that can be used to study autophagy in the laboratory. Examples include inhibitors which block the activity Beclin-vps34 complex (3 methyladenine[41–43], LY294002[44, 45], and Wortmannin[46], the Spautin[47, 48]); potent and specific VPS34 inhibitors (SAR405[49–51]; PIK-III[52]); the ULK1 inhibitor (SBI-0206965[53]); ATG4B inhibitors (UAMC-2526[54]; autophagin-1[55], NSC185058[56]); vacuolar-type H+-ATPase inhibitors (bafilomycin[57], salinomycin[58]); lysosomal inhibitors (ROC325[59, 60], VATG-027[61], Mefloquine[61], Verteporfin[62, 63]). 3-methyladenine may impact cancer cell metabolism independent of autophagy by serving as an ROS scavenger at high concentrations typically used. PI3K complex inhibitors (LY294002, Wortmannin) have activity against both class I and class III PI3K so interpretation of effects on autophagy may be difficult especially at the high doses often utilized. Spautin targets deubiquitinases that regulate the degradation of other client proteins besides BECLIN. Vps34 inhibitors target endocytic trafficking in addition to autophagy as vps34 activity is required for many of these autophagy independent trafficking events. SBI-020695 is also a potent FAK1 inhibitor. The potency of ATG4 inhibitors described in the literature thus far have been low, raising the possibility that these inhibitors also inhibit the protease activity of other cysteine proteases. There is very little in vivo evidence of efficacy published for any of the upstream autophagy inhibitors. In contrast, lysosomal inhibitors have had the most convincing in vivo activity. However the lack of a molecular target for these agents makes it even more difficult to determine their autophagy-dependent and autophagy independent effects. In summary while numerous compounds are available, concerns about off-target effects, and suitability for *in vivo* systems underscores the need to develop more potent, specific and translatable inhibitors of autophagy.

Autophagy inhibition in clinical trials

Despite a growing number of tool compounds that can be used to study autophagy in the laboratory, to date, no specific inhibitor that targets an autophagy protein has entered clinical trials. Hydroxychloroquine (HCQ) is the clinically available drug that could function as an autophagy inhibitor. HCQ is thought to inhibit autophagy by acting as a weak base that when trapped inside acidic cellular compartments, such as lysosomes, increases the pH of those compartments[64]. However numerous drugs are weak bases, and they do not function as

autophagy inhibitors. Recent work completed with potent lysosomal inhibitors suggests there may actually be a molecular target for chloroquine (CQ) and HCQ (See below). Preclinical studies in tumor cell lines and animal models have shown that HCQ increases tumor cell death alone or through enhancing tumor killing in combination with targeted agents or cytotoxic chemotherapy. One study showed that with the addition of HCQ with the mTOR inhibitor temsirolimus (TEM), there was an increase in cytotoxicity against renal cell carcinoma cell lines *in vitro*[65]. Additionally, the combination of HCQ and tamoxifen (TMX) was more effective than either monotherapy in estrogen receptor-positive breast cancer cells[66]. HCQ treatment has been effective against pancreatic ductal adenocarcinomas tumors as well[61]. These are examples of preclinical papers where chloroquine was used as an anticancer agent. A pubmed search of chloroquine and cancer results in > 9000 entries, so there are too many examples to discuss here. [67].

As outlined below numerous groups have launched clinical trials with either chloroquine (CQ) or HCQ. The rationale for choosing HCQ in the majority of the studies comes from the predicted ocular toxicity when chloroquine is administered. Dose escalation with chloroquine from the standard 150 mg/day is likely to cause significant toxicity. In contrast dose escalation with HCQ has been accomplished successfully in rheumatoid arthritis[68]. The first phase I trials involving HCQ that included autophagy markers, combined HCQ with vorinostat (VOR), temsirolimus (TEM), temozolomide (TMZ), doxorubicin (DOX), or bortezomib in patients with refractory solid tumors, melanoma, glioblastoma multiforme (GBM) and relapsed/refractory myeloma (Table 1) [46, 61, 63, 69–75]. In these trials across over 200 patients enrolled on these studies there was a < 10% grade 3–4 non hematological adverse event rate, which is surprising since each of the drugs that were combined with HCQ has significant toxicity (e.g. fatigue (VOR), mouth sores and hyperglycemia (TEM), myelosuppression(TMZ), neuropathy (BOR)) that could become dose limiting, and the population was a very sick phase I population or GBM patients. Multiple patients with melanoma, colorectal carcinoma, myeloma, and renal cell carcinoma demonstrated partial response or prolonged stable disease on these HCQ combinations; however, overall response rates were not high (detailed below). None of the combinations tested were pursued in phase II studies except VOR and HCQ (see below). These studies were important however, because they incorporated pharmacokinetic-pharmacodynamic assays that demonstrated for the first time that the highest doses of HCQ allowed by the FDA (600 mg p.o. b.i.d.) were able to produce a modest but reproducible degree of autophagy inhibition in patient tumors or surrogate tissues (peripheral blood mononuclear cells)[46, 61, 63, 69–75]. This allowed the launch of numerous phase II trials with more effective chemotherapy or targeted therapy backbones. Promising preliminary results for some of these trials have been presented in abstract form at meetings, including trials in colon cancer (presented by O'Hara et al ASCO 2015), pancreatic cancer (presented by Miller-Ocuin et al ASCO annual meeting 2017), and melanoma (presented by Gangadhar et al. Society of Melanoma Research 2017). Below we have provided more details about the published HCQ studies (Table 1).

Randomized phase II trial of temozolomide, radiation and chloroquine in malignant glioma

In a trial conducted in Mexico, 30 patients with resected glioma were randomized to standard temozolomide and radiation with placebo or chloroquine 150 mg/day[76]. No

pharmacodynamic markers were assessed and adverse events were not recorded. A follow-up retrospective study in 41 additional patients treated with carmustine and CQ compared to 82 patients treated with carmustine alone reproduced the survival curves of the original randomized study[77].

Randomized phase II trial of whole brain radiation with or without chloroquine

73 patients with non-small cell lung cancer or breast cancer with brain metastases appropriate for whole brain radiation were randomized to receive chloroquine 150 mg daily for 28 days or a matching placebo along with whole brain radiation. Patients treated with chloroquine had a 1 year brain metastases progression free survival rate of 83% versus 55% for the placebo treated arm. This benefit was not associated with a difference in response rate or overall survival.[78],

Phase I trial of erlotinib and HCQ in EGFR inhibitor treated non-small cell lung cancer

A phase I dose escalation of either HCQ alone or HCQ and erlotinib was conducted in EGFR mutant non-small cell lung cancer patients who had previously benefited temporarily from EGFR inhibitor therapy. The HCQ alone arm was closed early due to lack of accrual. The combination of erlotinib and HCQ 1000 mg daily was well tolerated. One out of 19 patients had a partial response and 4 patients had stable disease[79].

HCQ alone in refractory pancreatic cancer

The safety and activity of single agent HCQ in 20 patients with metastatic pancreatic cancer that did not respond to standard chemotherapy was assessed in a phase II study[69]. This trial included ten patients treated twice daily with 400 mg and the other ten patients with 600 mg of HCQ. Of note these patients were mostly Eastern Cooperative Oncology Group (ECOG) performance status 1–2 (restricted - minimal activity), reflecting a sick population of patients. Out of the 20 patients involved, only 2 patients did not experience progressive disease at 2 months[69]. Median progression-free-survival (PFS) was 46.5 days and overall survival was 69 days. One patient experienced grade 3–4 lymphopenia and one had grade 3–4 transaminitis[69]. Overall this study demonstrated no activity for single agent HCQ in ECOG PS 1–2 patients with treatment refractory pancreatic cancer.

Phase I trial of HCQ and temsirolimus in solid tumors with expansion in melanoma patients

This dose-escalation study included 27 patients with advanced solid malignancies followed by a cohort expansion at the top dose level in 12 patients with metastatic melanoma. [72]. Overall the combination of HCQ and TEM was well tolerated with almost no Grade 3–4 toxicities. Common Grade 1 and 2 toxicities included anorexia, fatigue, and nausea. This study demonstrated that an effective and safe dose for this combination was 600 mg of HCQ twice daily in combination with 25 mg of TEM weekly. Fourteen of 21 patients in the dose escalation achieved stable disease. In melanoma patients including the dose escalation and disease specific cohort expansion 14/19 (74%) patients achieved stable disease. The median PFS in 13 melanoma patients treated with HCQ 600 mg twice daily and TEM on the disease specific cohort expansion was 3.5 months[72].

Phase I trial of HCQ and bortezomib in multiple myeloma

The effects of HCQ and bortezomib were studied in 25 patients with relapsed and refractory multiple myeloma[71]. No dose limiting toxicities were observed. Lack of neurotoxicity exacerbation with HCQ was an important finding in this study. A recommended phase II HCQ dose was determined to be 600 mg twice daily in addition to standard doses of bortezomib. Partial response was observed in 3/22 patients, 3/22 had minor responses, and 10/22 had a period of stable disease. However, given the patients with response were bortezomib-naïve, this signal of activity was not deemed enough to warrant phase II study[71].

HCQ and vorinostat in solid tumors with expansion in patients with colon cancer

Another phase I clinical trial studied the combination HCQ with VOR[75]. Twenty four evaluable patients with refractory solid tumors were treated on the dose escalation portion of the study which identified 600 mg daily HCQ and 400 mg daily VOR as the recommended phase II doses for this regimen. The dose limiting toxicities encountered in this study were fatigue and gastrointestinal disturbances[75].. A phase I dose expansion study in 19 colorectal cancer patients found that the combination of VOR and HCQ produced a 2.9-month PFS in patients refractory to standard chemotherapy included regorafenib, and was tolerable[80]. Five of 19 patients experienced prolonged stable disease. This finding justified the launch of an ongoing randomized phase II study of VOR + HCQ versus regorafenib (NCT02316340).

Adjuvant HCQ and temozolomide in glioma

In a phase I/II clinical trial, HCQ in combination with radiation therapy and concurrent and adjuvant TMZ was tested in patients with newly diagnosed gliobastoma multiforme[74]. In this study patients treated with standard low dose continuous TMZ and HCQ 400 mg p.o. b.i.d. experienced Grade 3 and 4 neutropenia and thrombocytopenia[74]. A maximal tolerated dose (MTD) of 600mg/day HCQ was established and a phase II clinical trial was conducted through a national brain tumor consortium. PK-PD studies showed only modest treatment associated accumulation of autophagic vesicles, a surrogate for autophagy inhibition, at HCQ 600 mg/day. No significant improvement in overall survival of patients with malignant glioma was observed[74]. Concern was raised in regards to the dose limiting myelosuppression observed, that prevented adequate autophagy inhibition to produce efficacy.

A phase I study of dose intense temozolomide in patients with solid tumors

In this phase I clinical trial of HCQ and TMZ 40 patients with the vast majority being melanoma patients were enrolled[73]. For 7/14 days, these patients were treated orally with 200–1200 mg of HCQ daily with dose-intense oral TMZ 150 mg/m^2. Unlike the low dose continuous TMZ study in glioma, the dose-intense TMZ and HCQ combination was well-tolerated with no recurrent dose-limiting toxicities. Grade 2 fatigue, anorexia, nausea, constipation, and diarrhea was occasionally observed. Clinical benefit was observed in 9/22 (41%) at the highest dose level of HCQ (600 mg bid) with refractory BRAF-wild type melanoma had a near complete response or prolonged stable disease[73].

Phase I trial of HCQ, rapamycin, cyclophosphamide, and dexamethasone in multiple myeloma

A safety pilot and phase I study was conducted to determine the MTD of HCQ and safety of the 4-drug combinations[81]. Patients with relapsed or refractory multiple myeloma were eligible for this study if they received prior treatment with lenalidomide or bortezomib. All subjects received cyclophosphamide and dexamethasone. In the pilot study, 3 subjects were treated with 12 mg loading of rapamycin followed by 4 mg daily dose of rapamycin for 5 further days[64]. Also in the pilot study, 3 subjects received 800 mg of HCQ daily and had no dose limiting toxicities (DLT). Two DLTs including grade 3 diarrhea and grade 4 thrombocytopenia occurred at 1200 mg HCQ daily. Dose de-escalation to 800 mg HCQ daily resulted in one DLT (grade 4 thrombocytopenia). Therefore, 800 mg of HCQ was determined to be the MTD when combined with rapamycin, cyclophosphamide and dexamethasone[64].

Phase I neoadjuvant study of HCQ and gemcitabine in pancreatic cancer

A phase I/II trial of 35 patients examined the neoadjuvant treatment with HCQ and gemcitabine in patients with borderline resectable pancreatic adenocarcinomas[82]. 1200 mg/day of HCQ was taken for 31 days up until the day of surgery combined with two doses of a fixed-dose 1500 mg/m^2 gemcitabine[69]. DLT or grade 4/5 events did not occur during this trial. 19/35 patients had a decrease in surrogate biomarker response (CA 19-9) and 29/35 patients underwent surgical resection as scheduled[69]. This trial demonstrated that HCQ with gemcitabine is safe and tolerable, and showed encouraging activity in the neoadjuvant setting. A randomized phase II trial with gemcitabine, abraxane, with or without HCQ in resectable pancreatic cancer patients has been launched (NCT01978184), and a randomized phase II study of gemcitabine, abraxane, with or without HCQ in the metastatic setting has been launched (NCT01506973).

Overall, HCQ alone and in combination therapy is being used in clinical trials across a range of cancers (Figure 1). Examples of recently completed and actively recruiting clinical trials which may be reporting results soon are organized in Table 2. Even though HCQ provided the clinical and basic research communities insight into the use of autophagy inhibitors in clinic, the exact mode of action and potency remain to be issues with HCQ. Recent progress into a potential molecular target has been made with more potent autophagy inhibitors in the laboratory (see below). Another issue is that there are no validated biomarkers to identify tumors most likely to respond to HCQ studies. A recent paper identified patterns of expression at the protein level of aldehyde dehydrogenase1A1 (ALDH1A1) and helicase-like transcription factor (HLTF), to predict HCQ sensitivity and resistance[83]. This 2 gene signature can distinguish HCQ sensitive from HCQ resistant tumors *in vitro*. Analysis of The Cancer Genome Atlas (TCGA) demonstrated that the ALDH1A1/HLTF HCQ-S pattern is prevalent in a number of cancer[83].

Autophagy in cancer immunity

The paradigm changing effect of immune checkpoint inhibitors and chimeric antigen receptor T cells has demonstrated the need to consider the immune system when targeting

cancer pathways. Early studies demonstrated that loss of autophagy genes promotes tumor inflammation[84, 85]. In this context conflicting reports on the role of autophagy in tumor immunity continue to cloud the picture of whether or not anti-autophagy therapy will promote or suppress tumor immunity. Inhibiting autophagy may blunt cross-priming of tumor-specific CD8+T cells[86]. Additionally, autophagy has been shown to play an important role in effector and memory differentiation in T cell activation[87]. Autophagy has been shown to dictate the immunogenicity of cell death in tumors[88]. The absence of appropriate response to autophagy induction in tumor cells could potentially predict resistance to immune checkpoint blockers (ICB) therapies in human cancers[89]. Michaud et al demonstrated that autophagy was dispensable for chemotherapy-induced cell death but required for its immunogenicity since only autophagy competent cells attracted dendritic cells and T lymphocytes into the tumor. [90]. These authors proposed that autophagy is essential for immunogenic release of ATP from dying cells, and increased extracellular ATP concentrations improve the efficacy of antineoplastic chemotherapies when autophagy is disabled[90].

To the contrary, emerging data indicates that autophagy limits immune cell-mediated cytotoxicity, and therefore inhibiting autophagy would enhance antitumor immunity. During hypoxia, autophagy inhibits T cell mediated cytotoxicity in lung cancer cells[91]. Additionally, lysosomal exocytosis enhances anti-melanoma therapy[91]. It was found that autophagy inhibition also enhances cell death from natural killer (NK) cells[92]. Within the same study, deletion of Beclin1 caused influx of immune cells into the tumor microenvironment[92]. Emerging data also suggests autophagy deficiency in host cells or tumor cells promotes T cell mediated antitumor immunity[36, 93]. Therapeutic translation of the findings is ongoing. For instance studies designed to determine if combining an anti-PD-L1 or anti-PD-1 with autophagy inhibitor may produce a synergistic effect of cancer immunotherapy.

Novel lysosomal inhibitors

Lys05, a water-soluble dimeric chloroquine[94], was reported as a novel lysosomal autophagy inhibitor that is 10-fold more potent than HCQ. *In vivo* studies of Lys05 alone[95] and in combination with BRAF inhibition treatment[96] demonstrated its single agent and in combination efficacy. The efficacy of Lys05 has also been demonstrated in a range of *in vitro* and *in vivo* tumor models[97–100]. Recently, our group developed an even more potent lysosomal inhibitor, DQ661, a derivative of quinacrine[101]. Leveraging its potency and increased localization to the lysosome, DQ661 was used to pull down its previously unknown molecular target palmitoyl-protein thioesterase 1 (PPT1). DQ661 has *in vivo* activity in models of melanoma, pancreatic and colorectal cancer [101]. Current efforts are ongoing to develop Lys05 and or DQ661 derivatives into clinical drug candidates.

In conclusion, autophagy inhibition is gaining traction as a potentially new therapeutic approach in cancer. The field looks forward to clinical development of novel agents that target upstream components of autophagy (e.g. ULK1, ATG7, ATG4) or lysosomal agents. Efforts are underway to determine if PPT1 is the molecular target of dimeric chloroquines like Lys05, and even HCQ itself. This would change the paradigm of considering these agents as simply weak bases that accumulate and disrupt the lysosome, and instead they

would be considered targeted therapies. More research is needed to understand the role of autophagy inhibition in the context of immunotherapy. Additionally, identifying tumor subsets that are susceptible to autophagy inhibition would enhance translational research outcomes. Substantial progress has been made both in the lab and the clinic to credential autophagy inhibition as a therapeutic strategy, but more work remains to determine how autophagy can be exploited to benefit clinical outcomes.

Acknowledgments

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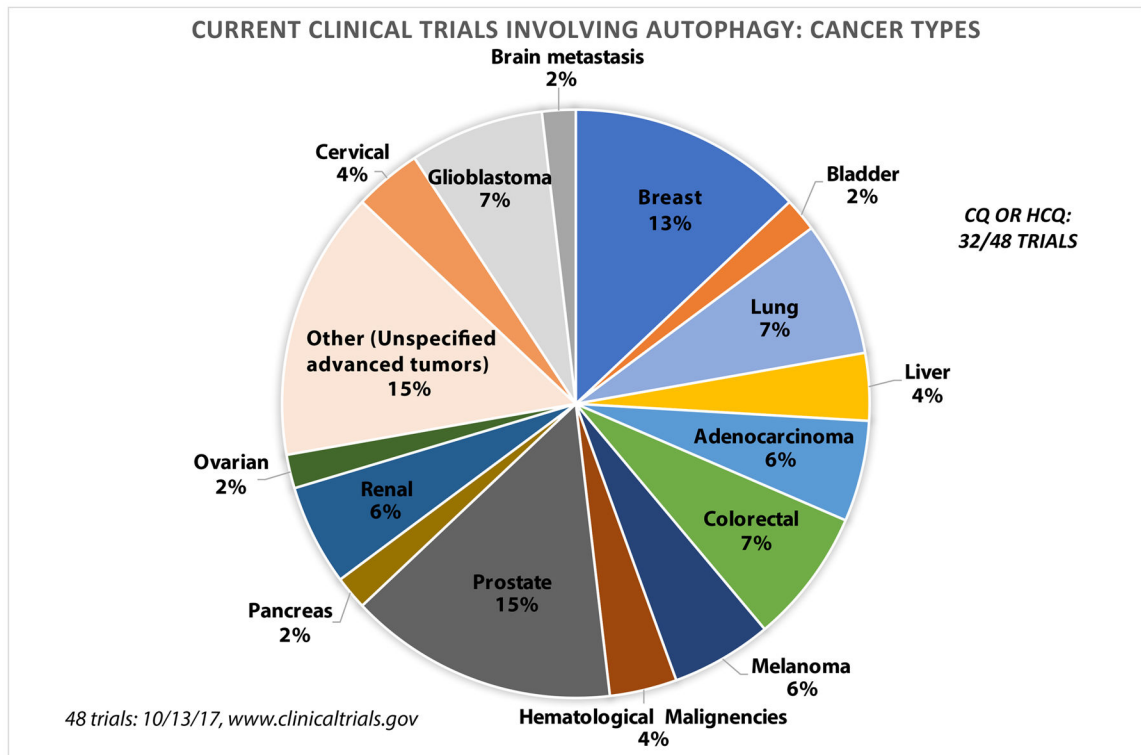
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Summary box

Overview of targeting autophagy in cancer

- New fundamental mechanistic insights into autophagy uncover new druggable targets

- Mouse models of tumorigenesis indicate autophagy suppresses benign tumors but promotes advanced cancer

- More tool compounds are being developed to inhibit autophagy in cancer

- Hydroxychloroquine is the first drug to be repurposed as an autophagy inhibitor in multiple cancer trials.

- Phase I trials have demonstrated safety and target engagement biologically

- Phase II trials may show promise in specific cancers

- More potent and specific autophagy inhibitors and autophagy inducers are being developed.

CURRENT CLINICAL TRIALS INVOLVING AUTOPHAGY: CANCER TYPES

Brain metastasis 2%
Cervical 4%
Glioblastoma 7%
Breast 13%
Bladder 2%
Lung 7%
CQ OR HCQ: 32/48 TRIALS
Liver 4%
Other (Unspecified advanced tumors) 15%
Adenocarcinoma 6%
Colorectal 7%
Ovarian 2%
Renal 6%
Prostate 15%
Melanoma 6%
Pancreas 2%
Hematological Malignencies 4%
48 trials: 10/13/17, www.clinicaltrials.gov

Figure 1.
Current clinical trials targeting autophagy in cancer. The pie chart shows the breakdown of which cancers have clinical trials targeting autophagy registered at www.clinicaltrials.gov.

Table 1

Published Clinical trials involving HCQ

Trial	Disease	DLT/MTD	N	Comments	Reference
Randomized double blind phase II study carmustine, radiation and chloroquine	Glioma	None	30	Median survival 24 v 11 months favoring chloroquine but not significant	78
Randomized Phase II trial of chloroquine and whole brain radiation	No-n-small cell lung cancer; breast cancer	None	73	Chloroquine significantly improved CNS progression-free survival	80
Phase I erlotinib + HCQ	Prior EGFR inhibitor-treated non small cell lung cancer	None	35	Well tolerated, erlotinib + HCQ: 5% response rate	81
Phase II HCQ	Pancreas ca	Grade 3 lymphopenia, transaminitis	20	2/19 evaluable with stable disease	71
Phase I/II temozolomide/radiation + HCQ	Glioma	Myelo-suppression at 800 mg 600 mg MTD	92	MTD was lower than other titrals. No survival benefit in a9 center trial	76
Phase I bortezomib + HCQ	Myeloma	None	30	Responses seen in bortezomib naïve patients	73
Phase I temozolomide + HCQ	Solid tumors, Melanoma expansion	None	40	PK-PD established dose dependent autophagy inhibition	75
Phase I temsirolimus + HCQ	Solid Tumors, melanoma expansion	None	35	Serial PET scans, tumor biopsies; 72% stable disease rate in BRAF WT melanoma	74
Phase I vorinostat + HCQ	Solid tumors,	Grade 3 fatigue at 800 mg 600 mg MTD	24	Tumor biopsies; Clinical activity	77
Phase IB vorinostat	Colon cancer	None	19	5/19 patients stable disease	82
Pilot and phase I cyclophosphamide, dexamethasone, rapamycin+ HCQ	Myeloma	Grade 3 diarrhea, grade 4 thrombocytopenia HCQ 1200 mg daily; MTD HCQ 800 mg daily	18	29% response rate at MTD	83
Phase I/II neoadjuvant gemcitabine + HCQ	Pancreatic cancer	None	35	61% CA19-9 decrease 70% R0 resection; modulation of autophagy correlated with disease free survival	84

Table 2

Examples of ongoing or recently completed HCQ clinical trials

Condition	Treatment	ClinicalTrials.gov Identifier	Phase	Recruiting
Colorectal Cancer	vorinostat + HCQ versus regorafenib	NCT02316340	II	yes
Breast Cancer (Adjuvant)	everolimus + HCQ	NCT03032406	II	yes
Pancreatic Cancer	gemcitabine + nab-paclitaxel +/− HCQ	NCT01978184	II	yes
Solid Tumor	HCQ	NCT03015324	I	yes
Leukemia, Acute Myelogenous	mitoxantrone + etoposide + HCQ	NCT02631252	I	yes
Metastatic Renal Cell Carcinoma	HCQ + IL-2	NCT01550367	I/II	yes
Advanced BRAF Mutant Melanoma	dabrafenib + trametinib + HCQ	NCT02257424	I/II	yes
Hepatocellular Carcinoma	TACE + HCQ	NCT02013778	I/II	yes
Prostate Cancer	HCQ	NCT00726596	II	yes
Pancreatic Cancer	capecitabine + Proton or Photon Radiation Therapy + HCQ	NCT01494155	II	no
Advanced Cancers	sirolimus + HCQ Vorinstat+ HCQ	NCT01266057	I	no
Rectal, Colon Cancer; Metastasis; Adenocarcinoma	HCQ + oxaliplatin + leucovorin + 5-fluorouracil + bevacizumab	NCT01206530	I/II	no
Metastatic Clear Cell Renal Cell Carcinoma	HCQ + RAD001	NCT01510119	I/II	no