Targeting autophagy in cancer

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Abstract | Autophagy is a mechanism by which cellular material is delivered to lysosomes for degradation, leading to the basal turnover of cell components and providing energy and macromolecular precursors. Autophagy has opposing, context-dependent roles in cancer, and interventions to both stimulate and inhibit autophagy have been proposed as cancer therapies. This has led to the therapeutic targeting of autophagy in cancer to be sometimes viewed as controversial. In this Review, we suggest a way forwards for the effective targeting of autophagy by understanding the context-dependent roles of autophagy and by capitalizing on modern approaches to clinical trial design.

Autophagic flux
A measure of the amount of cellular cargo and the rate at which it is degraded by the autophagy pathway.

Advances in the understanding of autophagy and how this pathway can be harnessed to improve clinical outcomes have come a long way since the introduction of the term by Christian de Duve in 1963 (REF. 1) (FIG. 1). The importance of autophagy in health and disease was recently highlighted when Yoshinori Ohsumi was awarded the Nobel Prize for Physiology or Medicine for his work on elucidating the mechanisms of autophagy[2]. Of particular importance is the role of autophagy in cancer. It is thought that autophagy prevents cancer development. Conversely, once cancer is established, increased autophagic flux often enables tumour cell survival and growth[3,4]. Thus, there is an important question in cancer therapy: should we try to enhance autophagy or should we try to inhibit it? In pre-malignant lesions, much evidence suggests that enhancers of autophagy might prevent cancer development[5]. Conversely, in advanced cancers, both enhancing autophagy and inhibiting it have been suggested as therapeutic strategies[3,6,7].

Despite this potential for confusion, clinical interventions to deliberately manipulate autophagy in cancer therapy are already under way[7], with the vast majority focused on inhibiting autophagy. Indeed, a search of the ClinicalTrials.gov website in February 2017 using the search term 'autophagy and cancer' returned 51 studies focused on inhibiting and evaluating autophagy to improve patient outcomes. As with other areas of cancer biology, such as the potential for the immune system to both promote and inhibit tumour formation and progression, the key to successful autophagy-focused therapeutic intervention comes from understanding the biology of how autophagy affects tumour initiation and progression. In this Review, we discuss recent studies that clarify and support this concept. By considering past clinical trial results, current clinical trial design, the development of biomarkers of autophagy dependence and response, and the role of autophagy in chemoresistance we explore how cancer therapy can be maximized by autophagy manipulation. These topics are especially timely, with the continued convergence of a better mechanistic understanding of how autophagy influences therapeutic response both at the tumour cell intrinsic level and within the host, with increasing information from autophagy-focused clinical studies. This convergence will allow us to better target autophagy to improve clinical outcomes in cancer patients.

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Autophagy

Macroautophagy (referred to throughout this Review as autophagy) is an evolutionarily ancient and highly conserved catabolic process that involves the formation of double-membraned vesicles known as autophagosomes that engulf cellular proteins and organelles for delivery to the lysosome[8,9] (FIG. 2). Autophagy is controlled by a highly regulated set of signalling events, occurs at a basal level in all cells, and is induced by diverse signals and cellular stresses[7]. There may be important differences between stimulus-induced autophagy and basal autophagy but our understanding of these differences is poor. The formation and turnover of the autophagosome involves evolutionarily conserved genes, autophagy-related genes (ATGs)[9], and is typically divided into distinct stages: initiation, nucleation of the autophagosome, expansion and elongation of the autophagosome membrane, closure and fusion with the lysosome, and the degradation of intravesicular products (FIG. 2). Initiation begins with the activation of the ULK1 (also known as ATG1) complex (comprising ULK1, ULK2, ATG13, FIP200 (also known as RB1CC1) and ATG101), which activates a class III PI3K complex comprising VPS34 (also known as PIK3C3), ATG14, UV radiation resistance-associated gene protein (UVRAG; also known as p63) and activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1), all of which are scaffolded by a putative

Nutraceuticals
Food with a medicinal benefit.

tumour suppressor, Beclin 1 (REF. 10). The ATG5–ATG12 complex conjugates with ATG16 to expand the autophagosome membrane, and members of the LC3 and GABARAP families of proteins are conjugated to the lipid phosphatidylethanolamine (PE) and recruited to the membrane. ATG4B, in conjunction with ATG7, conjugates LC3I and PE to form LC3II (also known as MAP1LC3B). This lipid-conjugated form of LC3 commonly serves as an autophagosome marker[11]. Ultimately, the autophagosome fuses with the lysosome, the contents are degraded and macromolecular precursors are recycled or used to fuel metabolic pathways. The adaptor protein sequestosome 1 (also known as p62), which targets specific substrates to autophagosomes, and LC3II are degraded along with other cargo proteins and can be used as a measure of autophagic flux[11].

Many of these steps in the autophagy pathway represent potentially druggable targets and provide ways to both positively and negatively influence autophagy (FIG. 2). Although current efforts in the clinic to inhibit autophagy are focused on inhibiting the lysosome using chloroquine (CQ) or the related hydroxychloroquine (HCQ), inhibitors against other autophagy regulators such as VPS34 (REFS 12–14), ULK1 (REFS 15,16) and ATG4B[17] have been reported and shown to inhibit tumour cell growth or to induce tumour cell death *in vitro*[15–17] and in preclinical mouse models[17]. Next-generation lysosomal inhibitors are also in development, including Lys05, which is a bisaminoquinoline that inhibits autophagy and impairs melanoma and colorectal adenocarcinoma growth when used as a single agent in preclinical mouse models[18]. Lys05 is a more potent autophagy inhibitor than HCQ due to its greater deacidification of the lysosome[18]. Other potent lysosomal inhibitors such as quinacrine, VATG-027 and VATG-032 (novel acridine and 1,2,3,4-tetrahydroacridine derivatives of quinacrine) have also been shown to be effective in patient-derived BRAF-mutant melanoma cell lines[19]. Conversely, the induction of autophagy is feasible not only using existing drugs (for example, BH3 mimetics[20] and mTOR inhibitors[21]) but also using nutraceuticals, such as trehalose[22] and caloric restriction mimetics[23], and exercise[24].

Other less well-studied forms of autophagy include microautophagy and chaperone-mediated autophagy (CMA). Non-selective microautophagy is mediated by the direct engulfment of cytoplasm and its components by tubular membrane invaginations into lysosomes. Selective microautophagy involves the direct targeting of specific organelles into lysosomes, such as peroxisomes (micropexophagy), nonessential components of the nucleus (piecemeal microautophagy of the nucleus) and mitochondria (micromitophagy). Although microautophagy has been associated with the development of neurodegenerative disorders, such as Alzheimer disease and Huntington disease, as well as lysosomal glycogen storage diseases, such as Pompe disease, it has not been implicated in cancer[25]. CMA is a form of selective autophagy in which cytosolic proteins with motifs related to the pentapeptide KFERQ are recognized by heat shock cognate 71 kDa protein (HSC70; also known as HSPA8), forming a chaperone complex[26,27] that translocates into the lysosome through lysosomal-associated membrane protein 2A (LAMP2A). CMA has been implicated in cancer[28] and drugs that target the lysosome could affect all types of autophagy.

Substrates that are degraded by autophagy may differ depending on the autophagic stimulus. One example is the role of autophagy in iron homeostasis[29]. The degradation of ferritin by autophagy is initiated when cells sense that they are deficient in iron and is mediated by nuclear receptor co-activator 4 (NCOA4), allowing the release

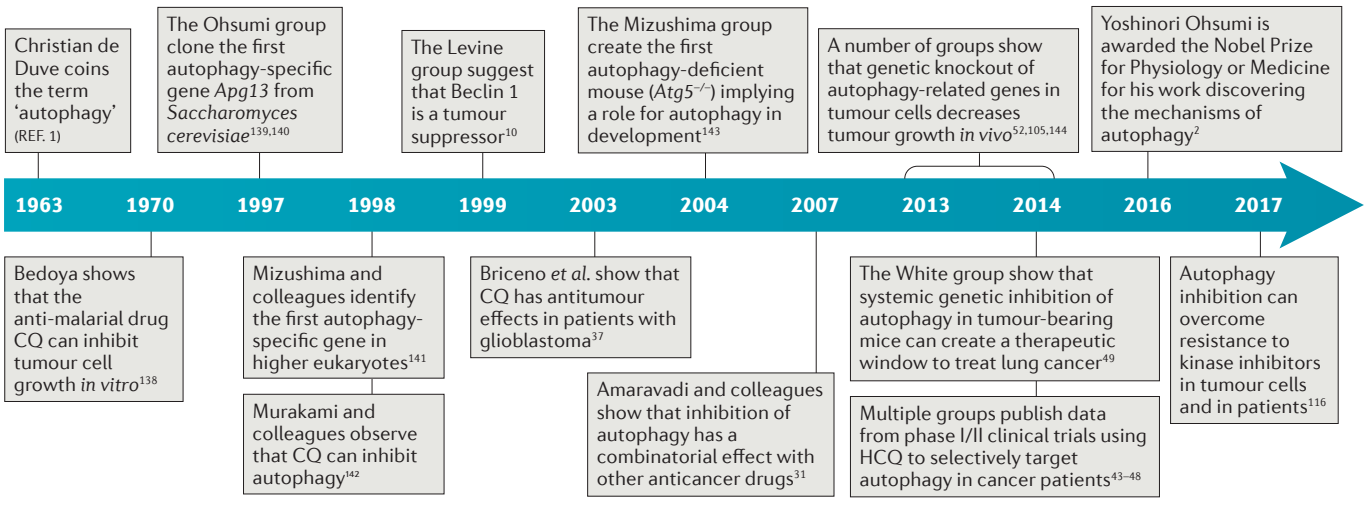

Figure 1 | **Timeline of the major discoveries that led to the successful targeting of autophagy in cancer.** Christian de Duve first coined the term 'autophagy' during a lysosomal conference in 1963. Since then, key discoveries have been made that have elucidated the mechanisms of autophagy from yeast to cultured cell lines, into mice, and culminating in successful clinical trials in cancer patients. The Nobel Prize for Physiology or Medicine was awarded to Yoshinori Ohsumi in 2016, emphasizing the importance of his work, as well as that of many others, on autophagy. ATG, autophagy-related gene; CQ, chloroquine; HCQ, hydroxychloroquine.

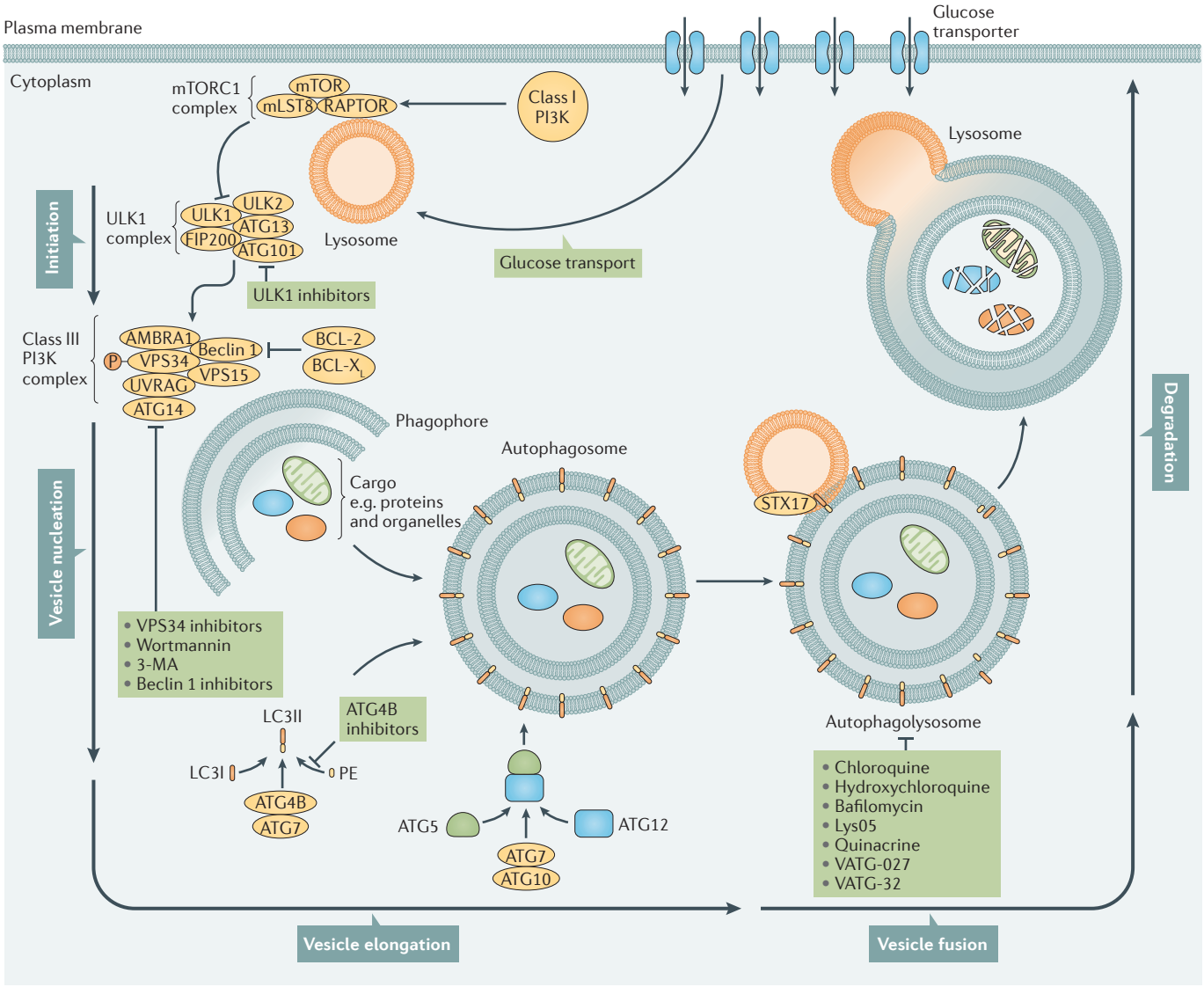

Figure 2 | **Autophagy can be inhibited at multiple stages.** The process of autophagy is divided into five distinct stages: initiation, vesicle nucleation, vesicle elongation, vesicle fusion and cargo degradation. Nonspecific macroautophagy (known as autophagy) is initiated by upstream activation through either nutrient starvation or growth factors. Under starvation conditions, a decrease in glucose transport results in the release of mTOR inhibition of the ULK1 complex, allowing for the progression of autophagy. The ULK1 complex (comprising ULK1, ULK2, FIP200, ATG101 and ATG13) induces vesicle nucleation, which is then mediated by a class III PI3K complex that consists of multiple proteins. Beclin 1, a BH3 domain-only protein, is phosphorylated by ULK1 and functions as an overall scaffold for the PI3K complex, facilitating the localization of autophagic proteins to the phagophore. BCL-2 and BCL-X_L interact with Beclin 1 at the BH3 domain to decrease the pro-autophagic activity of Beclin 1 by interrupting Beclin 1–VPS34 complex formation and by decreasing the interaction of Beclin 1 with UV radiation resistance-associated gene protein (UVRAG). Additional negative regulation of this process occurs with the phosphorylation of VPS34, which decreases its interaction with Beclin 1. By contrast, activating molecule in BECN1 regulated autophagy protein 1 (AMBRA1) binds to Beclin 1 and stabilizes the PI3K complex. ATG14 and UVRAG also bind Beclin 1 to promote interactions between Beclin 1 and VPS34 and to promote phagophore formation. VPS15 is required for optimal VPS34 function by enhancing VPS34 interaction with Beclin 1. The growing double membrane undergoes vesicle elongation to eventually form an autophagosome: a process that is mediated by two ubiquitin-like conjugation systems. The first system involves the conjugation of phosphatidylethanolamine (PE) to cytoplasmic LC3I to generate the lipidated form, LC3II, which is facilitated by the protease ATG4B and the E1-like enzyme ATG7, whereby LC3II is incorporated into the growing membrane. The second conjugation system is also mediated by ATG7, as well as by the E2-like enzyme ATG10, resulting in an ATG5–ATG12 conjugate. Subsequently, the SNARE protein syntaxin 17 (STX17) facilitates autophagosome fusion with the lysosome, resulting in an autophagolysosome. The low pH of the lysosome results in the degradation of the autophagosome contents. This process can be targeted pharmacologically upstream by means of direct ULK1, VPS34 or Beclin 1 inhibition. It can also be targeted by wortmannin and 3-methyladenine (3-MA), which act as PI3K inhibitors. Downstream targets include direct ATG4B inhibitors, as well as chloroquine, hydroxychloroquine and bafilomycin, which prevent autophagosome fusion with the lysosome.

Pharmacokinetic–pharmacodynamic parameters
(PK–PD parameters). The study of the time course of metabolism (PK) and the biochemical and physiological effects (PD) of a drug.

Maximum tolerated dose
(MTD). The highest dose of a treatment that is effective and that does not cause unacceptable side effects.

Myelosuppression
A decrease in bone marrow activity that results in fewer red blood cells, white blood cells and platelets.

of iron into the cell. The selective autophagy of specific substrates can also occur due to oncogenic stress. For example, the degradation of the nuclear lamina occurs in human primary fibroblast cells that have been transformed with oncogenic HRASV12 and genotoxic insults but does not occur during starvation stress[30]. It is often implicitly assumed that a measured increase in autophagy must have the same consequence irrespective of the stimulus. These studies suggest that this assumption is false, and that there may be a high degree of selection with regard to the cargo being degraded, depending on the autophagy stimulus. This could perhaps explain the context-dependent consequences of autophagy on cellular processes, and a better understanding of such mechanisms in cancer could provide a way to more selectively target autophagy for therapeutic purposes.

Cancer clinical trials

Extensive preclinical evidence exists to support the idea of inhibiting autophagy to improve clinical outcomes in cancer patients. Animal tumour models that are driven by specific oncogenes have been shown to cause tumours that regress upon subsequent genetic or pharmacological inhibition of autophagy (see below for further discussion). Similarly, following an initial finding in 2007 by Amaravadi and colleagues[31] (FIG. 1), a large number of *in vitro* studies, genetically engineered mouse models (GEMMs) and patient-derived xenograft (PDX) mouse models have demonstrated improved antitumour effects when various types of anticancer drug are combined with either genetic or pharmacological autophagyinhibition[3,6,32].

CQ and HCQ are currently the only clinically available drugs to inhibit autophagy. These drugs deacidify the lysosome and block the fusion of autophagosomes with lysosomes, thus preventing cargo degradation[33] (FIG. 2). CQ is also able to sensitize cancer cells to chemotherapeutic agents through autophagy-independent mechanisms[34] and has other anticancer effects that are independent of its effect on autophagy[35,36]. Some of the first clinical evidence of improving outcomes through the use of autophagy inhibition was provided by a small trial that involved 18 patients with glioblastoma. The patients who were treated with CQ in conjunction with radiation and the alkylating agent temozolomide had a significantly prolonged median survival compared with controls (33 months compared with 11 months)[37]. Follow-up clinical trials, and retrospective data from Briceno *et al.*[38,39] supported the findings of the initial study (TABLE 1). Additional early studies combining CQ with radiation therapy for brain metastasis also demonstrated improved intracranial tumour control[40,41].

The next major series of clinical trials used HCQ and had the additional benefit of attempting to correlate pharmacokinetic–pharmacodynamic parameters (PK–PD parameters) with autophagy inhibition[42–48]. These early phase clinical trials were carried out in patients with a wide variety of malignancies and involved multiple drug combinations (TABLE 1). Notably, these trials provided important lessons on the implementation of autophagy-targeted therapy. A canine lymphoma study of HCQ combined with the chemotherapy drug doxorubicin, which modelled a dose-escalation phase I human study, provided the initial proof of principle that combining HCQ with chemotherapy is safe[42]. Importantly, it also provided preliminary evidence of the clinical activity of HCQ, with an observed objective response rate of 93%[42]. Additional human studies included a wide range of tumours, such as advanced solid tumours and melanoma[43–45], glioblastoma[46] and refractory myeloma[47]. As predicted, the maximum tolerated dose (MTD) of HCQ varied in relation to the concurrent therapy that was used. A phase I study of histone deacetylase (HDAC) inhibitor vorinostat with HCQ in refractory solid tumours defined the MTD of HCQ as 600 mg daily when combined with vorinostat at a dose of 400 mg daily[43]. Similar safety-related findings were observed when HCQ was combined with concurrent radiation therapy and temozolomide in patients with glioblastoma[46]. By contrast, the combination of HCQ with 25 mg daily of the mTOR inhibitor temsirolimus in another solid tumour patient population was found to be safe when HCQ was used at a dose of 600 mg twice daily[44]. Common dose-limiting toxicities in these trials included gastrointestinal toxicity and fatigue[43–45]. Importantly, HCQ-induced neurotoxicity was not observed, as might have been predicted from *Atg7*-knockout mouse models in which the mice developed substantial neurodegeneration upon complete deficiency of autophagy[49]. The MTD of HCQ as a single agent has not been measured, and 600 mg twice daily of HCQ is the highest dose yet tested when administered in combination with standard chemotherapy agents[44]. Additional studies of potentially higher HCQ doses or of more potent lysosomal autophagy inhibitors, such as Lys05, quinacrine and VATG-032 (REFS 18,19,50,51), might maximize autophagy inhibition and antitumour activity.

Clinical response to autophagy inhibition has varied widely (TABLE 1). Although initial glioblastoma studies that used CQ in combination with chemotherapy and radiation therapy found that median survival more than doubled compared with controls[37–39], a phase I/II trial that used HCQ in combination with chemotherapy and radiation therapy found no significant improvement in the survival of patients with glioblastoma[46]. Notably, in this particular study, there was inconsistent inhibition of autophagy between patients and dose-limiting toxicities, such as myelosuppression, that prevented the intensification of the HCQ therapy, which may explain the different responses in these trials. A phase II trial of HCQ monotherapy in patients with previously treated metastatic pancreatic cancer demonstrated no clinical benefit and provided inconsistent evidence of autophagy inhibition[48]. However, this study was carried out in patients with advanced disease and so there was limited potential for single-agent HCQ to improve end-stage disease outcomes. Preclinical data from PDX studies of pancreatic cancer had demonstrated a response to single-agent HCQ[52]. Furthermore, preoperative treatment with HCQ in combination with gemcitabine resulted in a decrease in the serum tumour marker cancer antigen (CA) 19-9 in 61% of patients with pancreatic adenocarcinoma[53]. Interestingly, in the same cohort, patients with a greater

Table 1 | **Published autophagy trials in cancer**

Tumour type	Autophagy inhibitor	Clinical trial phase	Additional treatment	Clinical response	Grading of side effects*	Biomarker measures	Refs
Non-Hodgkin lymphoma	HCQ	I (in dogs)	Doxorubicin	• PFS: 5 months • ORR: 93.3%	• Grade 1 or 2: mild lethargy and GI upset • Grade 3 or 4: none	• Plasma concentrations of HCQ • LC3-positive cells by flow cytometry • EM of PBMCs for AVs	42
Solid tumours	HCQ	I	Vorinostat	• 1 patient (renal cell carcinoma), durable PR • 2 patients (colorectal cancer), prolonged SD	• Grade 1 or 2: nausea, diarrhoea, fatigue, weight loss, anaemia and elevated creatinine • Grade 3: fatigue and/or myelosuppression in a minority of patients	• EM of PBMCs for AVs • IHC for LC3II	43
Solid tumours and melanoma	HCQ	I	Temsirolimus	• Solid tumours: 67% SD • Melanoma: 74% SD	• Grade 1 or 2: fatigue, anorexia, nausea, stomatitis, rash and weight loss • Grade 3 or 4: anorexia, fatigue and nausea	EM of PBMCs for AVs	44
Solid tumours and melanoma	HCQ	I	TMZ	• Solid tumour patients: 10% PR; 27% SD • Metastatic melanoma patients: 14% PR; 27% SD	Grade 2: fatigue, anorexia, nausea, constipation and diarrhoea	EM of PBMCs for AVs	45
Solid tumours	HCQ	I	Rapamycin with metronomic cyproterone and docetaxel	40% PR; 44% SD	• Grade 1 and 2: fatigue, diarrhoea and mucositis • Grade 3: fatigue, myelosuppression, diarrhoea, nausea, vomiting, cardiotoxicity and hepatic toxicity	Not evaluated	145
Sarcoma	HCQ	Case series (10 patients)	Rapamycin	6 PR; 3 SD; 1 PD	Grade 1: rash, nausea, diarrhoea and constipation	Evaluation of 18FDG-PET as measure of tumour response after 2 weeks	146
Glioblastoma	CQ	III	TMZ and radiation	Median survival: 24 months (controls: 11 months)	Grade 1: myelosuppression	Not evaluated	39
Relapsed glioblastoma	CQ	Case series (5 patients)	Radiation	Two-month response: 2 PR; 1 SD	None	Not evaluated	147
Glioblastoma	HCQ	I/II	TMZ and radiation	Median survival: 15.6 months	• Grade 2 or 3: myelosuppression, nausea, fatigue, constipation and diarrhoea • Grade 4: myelosuppression and constipation	• Plasma concentrations of HCQ • EM of PBMCs for mean AVs • PBMC LC3II:β-actin ratio	46
Glioblastoma	CQ	III	TMZ and radiation	Median survival: 33 months (controls: 11 months)	Increased seizure frequency	Not evaluated	37
Brain metastases: NSCLC, SCLC, breast cancer and ovarian cancer	CQ	Pilot	Radiation	• Median OS: 5.7 months • PFS of brain metastasis at 1 year: 55%	• Grade 1: radiation dermatitis • Grade 2: alopecia	Not evaluated	40
Brain metastases: NSCLC and breast cancer	CQ	II	Radiation	• ORR: 54% (controls: 55%) • PFS of brain metastasis at 1 year: 83.9% (controls: 55.1%)	• Grade 1 or 2: headache, dizziness, nausea, vomiting, anorexia and myelosuppression • Grade 3: nausea, constipation, headache and drowsiness	Not evaluated	41

Table 1 cont. | **Published autophagy trials in cancer**

Tumour type	Autophagy inhibitor	Clinical trial phase	Additional treatment	Clinical response	Grading of side effects*	Biomarker measures	Refs
Refractory myeloma	HCQ	I	Bortezomib	• 14% very good PR • 14% minor response • 45% period of SD	• Grade 1 or 2: myelosuppression fatigue, peripheral neuropathy, nausea, vomiting, diarrhoea and constipation • Grade 3 or 4: nausea, constipation, diarrhoea, anorexia, myelosuppression and fatigue	• Plasma concentrations of HCQ • EM of PBMCs and bone marrow plasma cells for mean AVs • PBMC LC3II: β-actin ratio	47
Metastatic PDAC	HCQ	II	None	• Two-month PFS: 10% • Median PFS: 46.5 days (1.5 months) • OS: 69 days (2.3 months)	Grade 3 or 4: lymphopenia and elevated alanine aminotransferase	PBMC LC3II:β-actin ratio	48
PDAC	HCQ	I/II	Gemcitabine	• 61% with decrease in CA19-9 • If >51% increase in LC3II, improved disease-free survival to 15.03 months versus 6.9 months OS: 34.83 months versus 10.83 months	Grade 3: myelosuppression, hyponatraemia, elevated AST, hypoalbuminaemia, hyperbilirubinaemia rash and hyperglycaemia ileus	• CA19-9 as measure of tumour response • LC3II in PBMCs	53
NSCLC	HCQ	I	Erlotinib	1 PR; 4 SD; ORR 5%	• Grade 1 or 2: nausea, fatigue, vomiting, anaemia and anorexia • Grade 3 or 4: rash, nausea, nail and skin changes and myelosuppression • Grade 5: pneumonitis	Plasma concentrations of HCQ	148

18FDG-PET, [^{18}F]fluorodeoxyglucose-positron emission tomography; AST, aspartate aminotransferase; AVs, autophagic vacuoles; CA19-9, cancer antigen 19-9; CQ, chloroquine; EM, electron microscopy; GI, gastrointestinal; HCQ, hydroxychloroquine; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer; ORR, overall response rate; OS, overall survival; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PDAC, pancreatic adenocarcinoma; PFS, progression-free survival; PR, partial response; SD, stable disease; SCLC, small-cell lung cancer; TMZ, temozolomide. *Side effects are graded according to the Common Terminology Criteria for Adverse Events using a scale of 0–5, with 0 representing no adverse side effects and 5 representing death as a result of an adverse side effect.

than 51% increase in LC3II puncta labelling in peripheral blood mononuclear cells (PBMCs), which is suggestive of effective autophagy inhibition, experienced an increase in both progression-free survival (PFS; 15.03 months compared with 6.9 months) and overall survival (OS; 34.83 months compared with 10.83 months)[53].

Biomarkers

A major limitation in all of these clinical studies has been the identification of appropriate pharmacodynamic biomarkers to evaluate the changes in autophagy. Barnard *et al.*[42] showed that increased intra-tumoural HCQ was associated with an expected increase in LC3II puncta formation (a measure of autophagosome turnover) and with the accumulation of sequestosome 1 as measured by immunohistochemistry compared with treatment-naive tumours. This study[42] provided evidence that the clinical use of HCQ could inhibit autophagic flux within tumours, and supports the use of LC3II and sequestosome 1 as potential biomarkers for future trials. Several human trials have also used transmission electron microscopy (TEM) to evaluate the number of double-membraned vesicles (presumed to be autophagosomes) in PBMCs. However, this was found to be an unreliable method to monitor autophagy inhibition owing to a lack of correlation with the levels of autophagy inhibition in tumour samples, as measured by changes in the immunohistochemical labelling of lysosomal protease cathepsin D (CTSD), as well as sequestosome 1 and LC3II[43].

There can be up to a 100-fold difference in HCQ uptake in tumours compared with plasma, suggesting that plasma analysis is a poor surrogate for tumour specimen analysis[42]. Additionally, CQ uptake into tumour tissue is affected by tumour pH, thus making it difficult to block autophagy in more acidic tumours[50]. Such pH variations could explain some of the differences found between tumours in the accumulation of the drug. Finally, higher doses of HCQ (1,200 mg daily) may be better at causing an accumulation of autophagic vesicles in both PBMCs and tumour biopsy samples[47], although this cannot always be achieved, owing to dose-limiting toxicities. This highlights the potential benefit of newer autophagy inhibitors. For example, Lys05 (and its parent compound Lys01) more potently accumulate within and deacidify the lysosome, allowing for greater autophagy inhibition at lower doses. These effects can be observed when using standard biomarkers, including the accumulation of LC3II by western blot analysis and the accumulation of autophagosomes as measured by TEM[18]. Owing to the limitations of current autophagy inhibitors, and as new inhibitors continue to be evaluated, better biomarkers of autophagy manipulation are needed.

Ongoing clinical trials are aimed at defining additional biomarkers (TABLE 2). Functional imaging techniques are being used to correlate intra-tumour hypoxia with autophagy via positron emission tomography (PET) and computed tomography (CT) scans using hypoxia tracers 2-(2-nitro-1H-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5) labelled with [^{18}F]fluorine isotope (18F-EF5) and [^{18}F]flortanidazole (18F-HX4) (NCT01881451 (REF. 54) and NCT02233387 (REF. 55)). Similarly, the relationship between cancer metabolism and autophagy is being evaluated in a clinical trial combining HCQ with chemotherapy in patients with advanced colorectal cancer (NCT01206530)[56]. Other studies are aimed at correlating the effects of combined proteasome and HDAC inhibition on autophagy and serum metabolic profiles (NCT02042989)[57]. HDAC family members have been shown to regulate autophagy through several mechanisms, including the regulation of the transcription of essential genes[58]. The increased activity of autophagy after treatment with HDAC inhibitors has been shown to significantly blunt HDAC inhibitor anticancer activity[58]. The induction of autophagy has also been shown to occur in response to proteasome inhibitors and is believed to have a role in resistance[59]. This is the basis for early phase and ongoing clinical trials that aim to inhibit autophagy in combination with HDAC[43] and proteasome[47] inhibitors.

Preclinical studies have also identified transcriptional regulators of the microphthalmia/transcription factor E (MiT/TFE) family as potential biomarkers of autophagy regulation. Microphthalmia-associated transcription factor (MITF) or TFE3 overexpression was associated with an increase in autophagy and with MiT/TFE-dependent autophagy and lysosome gene expression in established pancreatic ductal adenocarcinoma (PDAC) cell lines, primary PDAC tumours and primary patient-derived PDAC cell lines[60]. Therefore, evaluating the expression levels of MiT/TFE family members, as well as their associated proteins within tumour samples, has the potential to identify patients with autophagy activation that is under the control of MiT/TFE proteins. Another interesting study by Follo *et al.*[61] found that the quantification of autophagy initiation by ATG13 puncta was correlated between patient tumour-derived *ex vivo* spheroids and formalin-fixed clinical tumour samples, and that differences between ATG13 levels correlated with clinical outcomes in mesothelioma. This is especially important as current measures of autophagic flux require the use of inhibitors of lysosomal proteases to detect the accumulation of LC3II, which is not possible in formalin-fixed samples[11]. By contrast, ATG13 is a static marker, and so it is potentially a much more clinically relevant biomarker of autophagy.

Surrogate markers from peripheral blood could provide another method to assess autophagy inhibition. Autophagy regulates the cellular secretion of cytokines and other signalling molecules[62]. The autophagy-regulated secretome[63], for example, the secretion of the cytokine interleukin-6 (IL-6)[64], has been suggested as a potential biomarker of autophagic activity. Modern clinical procedures such as endoscopic retrograde cholangiopancreatography (ERCP) allow the sampling of such factors from organ-associated ducts[65] or from the peripheral blood, so such an approach is technically feasible. A better understanding of how autophagy regulates secretion, as well as the molecules that are secreted, may enable us to incorporate such methods into a biomarker strategy. Together, these studies suggest a potential multi-dimensional biomarker strategy that would incorporate the direct molecular evaluation of autophagy in biopsy samples, monitoring of autophagy-regulated soluble factors and functional imaging techniques. Although somewhat involved, these assays are clinically feasible and could be incorporated into clinical trial protocols.

Targeting autophagy: a good idea?

The collective results of published clinical trials (TABLE 1) present evidence for the safe use of CQ and HCQ as cancer therapies. The reported positive clinical outcomes

Table 2 | **Autophagy biomarker identification clinical trials**

Autophagy biomarker	Methods of measurement	Tumour type	Clinical trial identification number	Refs
Tumour hypoxia	• 18F-EF5 PET • Tissue LC3II staining • Autophagy gene expression	Clear cell ovarian	NCT01881451	54
Tumour hypoxia	• 18F-HX4 PET • Autophagy gene expression	Cervical	NCT02233387	55
Autophagosomes	Autophagic vesicles in PBMCs	Myeloma	NCT01594242	149
Metabolic alterations	• 18FDG-PET • Autophagic vesicles in PBMCs	Colorectal	NCT01206530	56
Metabolic alterations	Serum metabolic studies	Advanced p53 malignancies	NCT02042989	57
Metabolic alterations	MRI, including magnetic resonance spectroscopy and diffusion weight imaging	Cervical	NCT01874548	150

18F-EF5, [^{18}F]2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5); 18FDG, [^{18}F]fluorodeoxyglucose; 18F-HX4, [^{18}F]flortanidazole; MRI, magnetic resonance imaging; PBMCs, peripheral blood mononuclear cells; PET, positron emission tomography.

Table 3 | **Open clinical trials targeting autophagy-dependent cancers**

Tumour type	Autophagy-dependence marker	Clinical trial phase	Autophagy inhibitor	Additional treatment	Clinical trial identification number	Refs
Colorectal	JNK1	I/II	HCQ	FOLFOX + bevacizumab	NCT01206530	56
Glioblastoma	EGFRvIII	I/II	CQ	Temozolomide + radiation	NCT02378532	114
Pancreatic	Mutant RAS	I/II	HCQ, nab-paclitaxel	Gemcitabine and nab-paclitaxel	NCT01506973	151
Pancreatic	Mutant RAS	II	HCQ, nab-paclitaxel	Gemcitabine and nab-paclitaxel	NCT01978184	152
Pancreatic	Mutant RAS	I/II	HCQ	Gemcitabine	NCT01128296	153
BRAF-mutant melanoma	Mutant BRAF	I	HCQ	Vemurafenib	NCT01897116	154
BRAF-mutant melanoma	Mutant BRAF	I/II	HCQ	Dabrafenib and trametinib	NCT02257424	113

CQ, chloroquine; EGFRvIII, epidermal growth factor receptor variant III; HCQ, hydroxychloroquine, JNK1, JUN N-terminal kinase 1; nab-paclitaxel, nanoparticle albumin-bound paclitaxel.

are encouraging for the role of autophagy inhibition in cancer therapy, but care needs to be taken to understand the underlying contexts in which autophagy inhibition will be beneficial and those in which it could be detrimental.

Autophagy is a known survival mechanism that is conserved from yeast to mammals[66]. It has also been identified as a survival mechanism across several tumour types[67–70]. The association between tumour cell survival and autophagy can be partly explained by the role of autophagy in protecting cells from undergoing programmed cell death[71]. This provides a logical rationale for the inhibition of autophagy improving response to other agents and forms the basis for both completed (TABLE 1) and ongoing (TABLE 3) clinical trials. However, the effect of autophagy on the ability of tumour cells to undergo apoptosis is not always protective. For example, within the same tumour cell population, autophagy can either promote or inhibit apoptosis under different cellular contexts in response to similar death stimuli, such as CD95 ligand (CD95L; also known as FASLG) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10), which both function as death receptor agonists[72]. The mechanisms underlying these opposing effects are related to the degradation of different pro-apoptotic or anti-apoptotic regulators by autophagy[72,73]. An important conclusion from this work is that a much better understanding of how autophagy regulates apoptosis sensitivity (that is, what substrates it degrades) is needed if the aim is to predict whether a tumour cell is more or less likely to be killed in response to a particular death signal when autophagy is blocked. Moreover, increases in cell death can be due to the stage of the autophagy pathway that is inhibited. For example, the prevention of autophagosome maturation can decrease necroptosis and the inhibition of autophagosome turnover can potentiate necroptosis in the same prostate cancer cells[74]. These observations exemplify the underlying problem of autophagy manipulation in cancer therapy: autophagy has context-dependent and even opposing effects on tumour cell behaviour. Such context-dependent effects are poorly understood, emphasizing the importance of a better understanding of the molecular mechanisms that determine how autophagy affects cancer cell behaviour.

Arguments against inhibiting autophagy in cancer therapy. Several studies, especially those by Kroemer and colleagues, have suggested that autophagy inhibition is a bad idea in cancer treatment because it would reduce antitumour T cell responses[75–77]. The rationale behind this assertion is that autophagy in dying tumour cells is required for immunogenic cell death, which leads to efficient recognition by the immune system and the activation of an effective antitumour immune response[78,79]. One caveat to these studies is that they focused on highly immunogenic tumour models, including the CT26 colon cancer mouse model[80]. In opposition to this idea, a recent study[81] using less-immunogenic B16 mouse melanoma and 4T1 human mammary carcinoma cell mouse models, found equivalent T cell responses between autophagy-competent tumour-bearing mice and tumour-bearing mice in which autophagy was either blocked by genetic deletion of autophagy genes or blocked pharmacologically through treatment with CQ[81]. Another study by the Kroemer laboratory[82] took the idea of autophagy as a requirement of immunogenic cell killing one step further by concluding that enhanced autophagy (using caloric restriction mimetics) could boost antitumour immune responses[82]. This led to the suggestion that not only should autophagy not be inhibited but that interventions to increase autophagy during cancer therapy should also be considered.

Autophagy can stimulate tumour antigen cross-presentation[83], which provides another potential mechanism whereby autophagy inhibition could interfere with a robust antitumour immune response. Correlative evidence suggests that these mechanisms may be associated with better outcomes[83]. Higher LC3II puncta combined with the presence of nuclear high mobility

group protein B1 (HMGB1), a non-histone chromatin-binding protein that is known to stimulate anticancer immune responses, in resected breast cancer specimens was associated with improved metastasis-free survival and breast cancer-specific survival[84] and increased immune cell infiltration of the tumour[85]. A caveat to these studies is that the available markers (for example, autophagosome vesicles in PBMCs) are, as noted above, poor measures of the actual level of autophagic flux that is taking place in the tumour tissue. Countering these ideas, other studies report that some antitumour immune responses are enhanced by autophagy inhibition[86,87]. Thus, there are arguments both for and against autophagy inhibition even when considering only the effects on antitumour immune responses.

Another potential use of autophagy to influence an immune response has been demonstrated in an ongoing study led by the Second Affiliated Hospital School of Medicine at Zhejiang University, China, which has proposed the use of a combination of autophagy and proteasome inhibition in *ex vivo* tumour cells for the development of a tumour vaccine (NCT03057340 (REF. 88)). Preclinical data have shown that the inhibition of autophagy in tumour cells treated with a proteasome inhibitor results in the enrichment of short-lived proteins (SLiPs) and misfolded proteins known as defective ribosomal products (DRiPs) in autophagosomes, named DRibble corpuscles[89]. DRiPs and SLiPs are highly expressed in tumours and can support an antitumour immune response, but are inherently unstable and are degraded by proteasomes under normal conditions. The inhibition of proteasome degradation stabilizes these proteins, which are then concentrated in DRibble corpuscles. The inhibition of autophagy at this stage prevents the breakdown of DRiPs and SLiPs that have concentrated in the autophagosome, and enables the fractionation and collection of the DRibble corpuscles to provide the protein required to create effective DRibble vaccines[89]. DRibble tumour vaccines that have been developed from these proteins have been shown to induce cross-reactive T cell responses and tumour antigen cross-protection[90]. A preliminary analysis of a phase II trial that evaluated the use of DRibble vaccines in patients with non-small-cell lung cancer (NSCLC) demonstrated that, at 12 weeks, PBMCs from treated patients had multiple induced and increased antibody responses[91]. Other studies are also attempting to exploit autophagy to improve the efficacy of cancer immunotherapies. For example, a phase I trial evaluating DNX2401, an oncolytic adenovirus, in patients with glioblastoma (NCT01956734 (REF. 92)) hypothesizes that autophagy that is stimulated in response to temozolomide therapy could help viral replication in tumour cells. This is an example in which autophagy inhibition would be counterproductive to the intended purpose of the primary therapy.

In patients, autophagy inhibition is not specifically targeted to tumour cells, thus the potential toxicity from global autophagy inhibition represents another reason to pause when considering the value of targeting autophagy. This is exemplified by a study in which the knockout of an essential autophagy gene (*Atg7*) in all tissues was achieved in adult mice[49]. *Atg7* deletion led to the eventual death of all the mice owing to severe neuronal toxicity, the disruption of glucose homeostasis and increased susceptibility to infection. However, it is important to remember that, although the removal of an essential component of the canonical autophagy pathway in every cell in the body might mimic the effect of a 'perfect' autophagy inhibitor, such a strategy is markedly different from that of the clinical application of an autophagy inhibitor, which is unlikely to be as effective at inhibiting autophagy as the complete genetic deletion of an essential autophagy regulator. In support of this idea, the chronic use of HCQ for the treatment of rheumatological disorders and the treatment of some cancer patients with CQ as an autophagy inhibitor for extended time periods without adverse toxicity[93] demonstrates that long-term treatment with lysosomal autophagy inhibitors is feasible. Most importantly, as long as cancer cells are more dependent than normal tissues on autophagy, even a drug that causes some normal tissue toxicity can have a useful therapeutic window that allows it to be an effective cancer treatment. Indeed, in inducible *Atg7*-knockout mice the growth of KRAS-driven lung tumours was considerably inhibited before any signs of neurotoxicity[49], indicating that such a window for autophagy inhibition exists in some cancers.

Possible mechanisms and markers of autophagy dependence. Although autophagy may be functional in many cancer cells and may be required to respond to stresses, such as amino acid deprivation, some cancer cells may be especially dependent on autophagy even in the absence of added stress[94]. This idea has been named autophagy addiction or autophagy dependence and is important because it has been recognized in some studies that only autophagy-dependent tumours respond to pharmacological autophagy inhibition *in vivo*[94]. Moreover, drug synergy between autophagy inhibitors and other anticancer drugs can occur in autophagy-dependent tumour cells, and the same drug combination was even occasionally antagonistic in autophagy-independent tumour cells[93,94]. This implies that the effects could be counterproductive if autophagy inhibitors were combined with other drugs in autophagy-independent tumours in the clinic. A reliable method of identifying autophagy-dependent cancers is now required to incorporate this concept into clinical decisions. Multiple mechanisms of autophagy addiction are beginning to be uncovered (FIG. 3) that may help to identify the most autophagy-dependent tumours, and many of these mechanisms are amenable to the development of biomarkers that could potentially be used to select patients with tumours that are most likely to respond to autophagy inhibition therapy.

Mutations in the RAS pathway are often associated with the high levels of autophagy that are required to maintain tumour cell metabolism[95–97]. For example, pancreatic cancer has very high rates of *KRAS* mutation and, together with increased activity of transcription factors that promote autophagy[60] and pancreatic stellate cells in the tumour microenvironment that use autophagy to fuel tumour cell metabolism[98], is thought to cause pancreatic tumours to be especially dependent on autophagy[97].

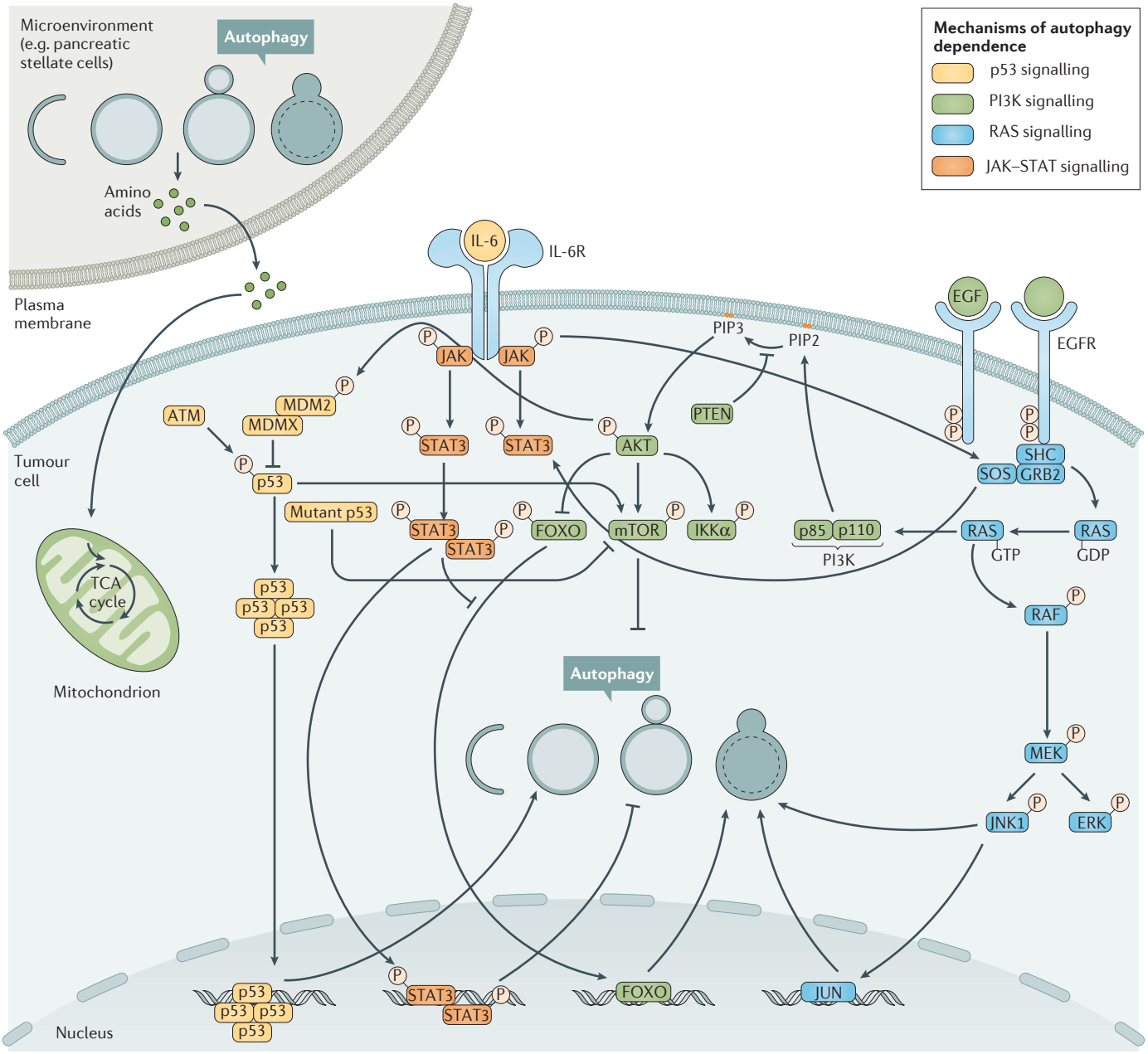

Figure 3 | **Molecular mechanism of autophagy dependence.** Preclinical and clinical models have indicated that the tumour microenvironment, for example, pancreatic stellate cells in the case of pancreatic cancer, p53 status, RAS family status, the activation of Janus kinase (JAK)–signal transducer and activator of transcription (STAT) and PI3K signalling may all have roles in the determination of autophagy dependence within cancer cells, both *in vitro* and in patients. These pathways have all been shown to affect autophagy either positively or negatively and many pathways participate in cross-pathway signalling. Signalling through p53 can both promote and inhibit autophagy, depending on the status of p53, and may interact with other proteins that are activated by mutations to enhance autophagy dependence, especially in pancreatic cancer. The activation of epidermal growth factor receptor (EGFR) through amplification or mutation leads to the downstream upregulation of the PI3K–AKT–mTOR pathway, as well as the activation of STAT3 and the RAS pathway. Although autophagy inhibition can occur through mTOR activation, these downstream effects collectively result in the stimulation of autophagy and an increase in autophagy dependence. Mutations or alterations in the RAS family (specifically, KRAS) have been shown to promote autophagy, enhancing tumour growth and therapy resistance. Specific mutations in *RAF*, such as *BRAF*^{V600E}, promote autophagy dependence in multiple tumours, including central nervous system (CNS) tumours and melanoma. Finally, autophagy regulation of JAK–STAT signalling through interleukin-6 (IL-6) has been identified as a mechanism of autophagy dependence in breast cancer. All of these pathways are complex and interact on multiple levels. The identification of tumours with these pathways activated, and as yet unidentified pathways will provide methods of detection of autophagy-dependent tumours. FOXO, Forkhead box O; GRB2, growth factor receptor-bound protein 2; IKKα, inhibitor of nuclear factor-κB kinase subunit-α; JNK, JUN N-terminal kinase; TCA, tricarboxylic acid.

Similarly, tumours in mouse models of lung cancer and melanoma that are driven by the *Braf*^{V600E} mutation are highly sensitive to *Atg7* gene deletion[99,100], and autophagy inhibition is sufficient to kill BRAF-V600E-expressing, but not wild-type BRAF-expressing, brain tumour cell lines[93].

These data might lead us to conclude that RAS- and BRAF-mutant tumours define autophagy dependency and would be good markers to select patients in whom autophagy should be inhibited therapeutically[101,102]. However, even in this case there are context-dependent effects that should be kept in mind. Nuclear p53 has been shown to facilitate autophagy and cytoplasmic p53 is associated with inhibition of autophagy[103,104], indicating that, overall, the role of p53 in autophagy is complex. Although p53 has both autophagy-promoting and autophagy-inhibiting activities, it is not known whether these activities determine whether tumour cell growth is increased or decreased through cell death upon autophagy inhibition.

In one KRAS-mutant mouse pancreatic cancer model, homozygous deletion of *Trp53* in the pancreas switched the loss of autophagy from being an inhibitor of tumour growth to being a promoter of tumour growth[105]. On the basis of this study, it was suggested that patients with tumours that had both KRAS and p53 mutations might experience tumour growth following autophagy inhibition[106]. However, this concern may be unfounded because human pancreatic tumours do not present with homozygous deletion of *TP53* occurring simultaneously with the activation of KRAS; instead, these tumours usually present as p53 loss of heterozygosity (LOH)[52]. Subsequent studies in mouse models that used conditional pancreatic *Trp53* LOH that more closely resembled the human disease indicated that p53 status does not affect response to autophagy inhibition in pancreatic cancer[52]. Huo *et al.*[107] were able to show that impaired autophagy following the monoallelic loss of *Becn1* in mice resulted in a reduction in partner and localizer of BRCA2 (PALB2)-associated mammary tumorigenesis (a model of hereditary breast cancer) in the presence of wild-type p53 but not on a p53-null background[107]. A similar conclusion was reached using immortalized, HRAS mutant-expressing primary human ovarian surface epithelial cells, skeletal muscle myoblasts and embryonic kidney cells; some cell types displayed growth inhibition when autophagy was blocked but others showed growth promotion[108]. Moreover, an analysis of a large number of human cancer cell lines with *KRAS* mutations did not find them to be more sensitive to knockdown of ATG genes than tumour cell lines without *KRAS* mutations[35]. Taken together, these data suggest that although studying RAS and p53 may provide further important insights into the biological mechanism by which autophagy can both promote and inhibit tumour growth, the status of these two genes alone may not identify tumours for which autophagy inhibition would be most valuable.

In a panel of breast cancer cell lines, selection for or against a library of shRNAs that targeted more than 100 autophagy regulators was used to identify tumour cell lines that could survive and/or proliferate following global genetic interference of the autophagy pathway[94]. This study revealed that some breast cancer cells grow well when autophagy is globally inhibited and that others are dependent on autophagy for survival. These effects were associated with autophagy regulation of signal transducer and activator of transcription 3 (STAT3) activity and autophagy-dependent secretion of interleukins, particularly IL-6 (REF. 64). In colon cancer, functional JUN N-terminal kinase 1 (JNK1) was required for hypoxia-induced autophagy[109], and ongoing clinical studies are evaluating the use of JNK1 as a marker of autophagy dependence (NCT01206530 (REF. 56)). Epidermal growth factor receptor (EGFR)-mutated or EGFR-amplified tumours are another potential target for autophagy inhibitors. The activation of EGFR leads to the downstream regulation of several pathways that influence autophagy, including PI3K–AKT–mTOR, STAT3 and RAS family signalling and Beclin 1-associated signalling pathways[110]. Specifically, tumours expressing EGFR variant III (EGFRvIII), a common mutation in the extracellular domain of EGFR, have been shown to require the upregulation of metabolism[111] and are autophagy dependent[112].

Importantly, clinical trials are already using these markers of dependence or are gathering further data for biomarker validation (TABLE 3). For example, the BRAF, autophagy and MEK inhibition in metastatic melanoma (BAMM) trial (NCT02257424 (REF. 113)) is specifically assessing HCQ autophagy inhibition for BRAF-V600E or BRAF-V600K-expressing metastatic melanoma. An additional trial in glioblastoma will evaluate the use of EGFRvIII to identify patients who will respond to CQ autophagy inhibition in combination with chemotherapy and radiation therapy (NCT02378532 (REF. 114)).

Autophagy in cancer escape mechanisms. There is mounting evidence of a potential role for autophagy in the ability of cancers to develop resistance to chemotherapy. Patients with melanoma who have tumours that become resistant to the BRAF inhibitor vemurafenib through an ER stress response have higher levels of autophagy[115]. Moreover, the inhibition of autophagy could reverse acquired resistance to vemurafenib that was the result of the continued culture of melanoma cell lines in the presence of the drug[115]. Similarly, in the clinical setting, a patient with BRAF-mutant brain cancer, who had initially responded to vemurafenib treatment, but who then acquired resistance to the drug, was successfully treated with a combination of CQ and vemurafenib[93]. Thus, in this patient, the tumour could be resensitized by treatment with an autophagy inhibitor. Importantly, only the combination therapy of kinase inhibitor and autophagy inhibitor, and not autophagy inhibition as a single agent, was effective for the long-term control of tumour growth, indicating that the clinical benefit is derived from overcoming resistance rather than from the acquisition of new sensitivity to autophagy inhibition alone[93].

Further laboratory and clinical studies have found that genetic and pharmacological autophagy inhibition could overcome multiple molecularly distinct

mechanisms of resistance to BRAF inhibition and is effective in both low-grade and high-grade BRAF-mutant brain tumours[116]. Although only a few patients with clinically acquired resistance to BRAF inhibitors have been treated with combinations of CQ and vemurafenib, it is encouraging that each patient obtained clinical benefit, suggesting that the autophagy inhibitor was consistently able to overcome resistance to the kinase inhibitor in patients[93,116]. Additional preclinical studies have shown the ability of autophagy inhibition to overcome resistance to tyrosine kinase inhibition in bladder cancer[117], thyroid cancer[118], NSCLC[119,120] and ALK-positive lung cancer[121]. As current attempts to circumvent resistance to kinase inhibitors tend to focus either on targeting the same pathway (often the same kinase) in a different way, or on targeting a parallel signalling pathway, this strategy of inhibiting an entirely independent process (that is, autophagy) may represent a fundamentally different way of tackling acquired drug resistance.

Autophagy has also been implicated in resistance to multiple standard chemotherapeutic agents, often in the tumours that are the most difficult to treat. Recent studies have found autophagy induction to cause resistance to the cytotoxic drug paclitaxel in ovarian cancer[122]. Resistance to the chemotherapy cisplatin has been shown to be due to autophagy induction in ovarian and oesophageal cancer[123,124], and to occur through hypoxia-induced autophagy in lung cancer[125]. As in melanoma[115], autophagy induction due to an ER stress response results in resistance to cyclin-dependent kinase (CDK) inhibitors in primary patient chronic lymphocytic leukaemia (CLL) cells[126] and in resistance to HDAC inhibitors such as Tubastatin A in glioblastoma cell lines[127]. As the link between autophagy and resistance to chemotherapy is strengthened, autophagy will undoubtedly continue to develop as a promising target in cancer therapy[128–132].

Autophagy has also been implicated in the survival of dormant tumour cells and, more importantly, may be crucial for such tumour cells to begin growing again. In pancreatic cancer mouse models in which tumour regression was induced by silencing oncogenic KRAS, rare surviving tumour cells that persisted after complete inhibition of the oncogenic driver partly relied on autophagy[133]. A recent study using a *Drosophila melanogaster* tumour model found that dormant tumours from autophagy-deficient animals reactivated tumour growth when transplanted into autophagy-proficient animals. This suggests that non-tumour-cell autonomous autophagy in the surrounding cells of the microenvironment is crucial for the re-growth of dormant tumours[134]. If similar effects occur in mammals, then this study[134] would suggest that efforts to enhance autophagy after the apparently successful treatment of cancer might have the unintended side effect of promoting recurrence from residual dormant tumour cells.

Conclusions

In the world of oncology, autophagy has competing and context-dependent effects; thus, a 'one size fits all' approach with interventions that are designed to inhibit or to enhance autophagy in cancer therapy will not be successful. Given this situation, it might be presumed that the best strategy would be to simply avoid trying to manipulate autophagy at all in cancer therapy. However, altered autophagy is unavoidable. Many current treatments (for example, those that affect the mTOR pathway) affect autophagy. In addition, physiological stimuli, especially those that have different effects on tumours compared with normal tissues, such as nutrient deprivation or hypoxia, will also alter autophagy in the tumour. This means that the effects of these changes need to be understood to tailor interventions to the particular situation. Initially at least, such interventions are most likely to revolve around inhibiting autophagy. This means that deciding which patients would benefit from autophagy inhibition therapy is key.

Clinical trials of CQ or HCQ as autophagy inhibitors have demonstrated the safety of targeting autophagy for cancer therapy. No devastating neurological toxicities have been observed in patients who have received these agents, suggesting that the neurodegeneration that is seen in mouse models after complete and irreversible inhibition of autophagy is not necessarily informative of the extent of toxicity that will occur after pharmacological treatment with autophagy inhibitors. The survival benefit associated with combining CQ with vemurafenib in patients with brain tumours[93,116] provides clinical evidence that autophagy-targeted therapy is a feasible clinical strategy in appropriately selected patient populations. The focus of clinical trials has so far been on the use of lysosomal inhibition with CQ and its derivatives. More potent and autophagy-specific inhibitors are in development, including better lysosomal inhibitors such as Lys05 (REF. 18) and drugs that target earlier steps in the autophagy pathway, such as the steps involving ULK1 (REFS 15,16), VPS34 (REFS 12–14) and ATG4B[17]. Although the preliminary data are encouraging, these compounds are still in early preclinical studies. Issues with selectivity, as well as the need for the use of higher drug concentrations, may limit clinical utility, and optimization of the current lead drugs through chemical modifications of the structures will be needed before moving to clinical trials[135].

An important unanswered question raised by the use of inhibitors that target early steps in the autophagy pathway remains: is it better to stop the formation of autophagosomes or to block the degradation of autophagosomes with lysosomal inhibitors? Autophagosomal structures can serve as scaffolds to induce apoptosis[136] and necroptosis[74,136]. Thus, the accumulation of autophagosomes might promote such signalling under certain circumstances. If this idea is correct, then it might be better to block autophagosome degradation with a lysosomal inhibitor rather than to inhibit autophagosome formation, which might prevent tumour cell killing. Finally, there also remains the question of the use of autophagy inducers to prevent oncogenesis. Arguments have been made that increasing autophagy suppresses the development of cancer by limiting genomic mutations, promoting oncogene-induced senescence and reducing tumour-initiating inflammation[137]. This remains a complex question owing to the interaction of autophagy with different genetic backgrounds, such as p53 mutations in

pancreatic cancer[105] and breast cancer[107], in which p53 status may influence response to autophagy stimulation, making it either protumorigenic or antitumorigenic.

We have begun to combine anticancer drugs of many different classes with autophagy inhibitors and inducers, but with little rationale for deciding which combinations to test or serious attempts to select patients who are most likely to benefit from these therapies. Fortunately, modern clinical trial design often allows the collection of samples from tumours and blood both before and after treatment. This may aid the development of better biomarkers to serve as pharmacodynamic markers of the efficacy of autophagy inhibitors and may help to better identify which patients should or should not be treated. If we combine improved clinical studies with detailed molecular and cellular analysis to understand the mechanisms underlying the context-dependent effects of autophagy on cancer it should be possible to develop a more rational basis for deciding when and in which direction we should try to manipulate autophagy during cancer therapy. As we cannot avoid the alteration of autophagy in tumours and as we know that such alterations will change tumour behaviour, ignoring the problem is not a good option; a better answer is to understand the underlying biology and then to apply this knowledge in well-designed clinical trials.

1. Klionsky, D. J. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat. Rev. Mol. Cell Biol.* **8**, 931–937 (2007).
2. The Nobel Assembly. *The Nobel Assembly at Karolinska Institutet has today decided to award the 2016 Nobel Prize in Physiology or Medicine to Yoshinori Ohsumi* https://www.nobelprize.org/nobel_prizes/medicine/laureates/2016/press.html (2016).
3. Amaravadi, R., Kimmelman, A. C. & White, E. Recent insights into the function of autophagy in cancer. *Genes Dev.* **30**, 1913–1930 (2016).
4. White, E. Deconvoluting the context-dependent role for autophagy in cancer. *Nat. Rev. Cancer* **12**, 401–410 (2012).
5. Galluzzi, L. *et al.* Autophagy in malignant transformation and cancer progression. *EMBO J.* http://dx.doi.org/10.15252/embj.201490784 (2015).
6. Levy, J. M. & Thorburn, A. Targeting autophagy during cancer therapy to improve clinical outcomes. *Pharmacol. Ther.* **131**, 130–141 (2011).
7. Towers, C. G. & Thorburn, A. Therapeutic targeting of autophagy. *EBioMedicine* **14**, 15–23 (2016).
8. Mizushima, N. Autophagy: process and function. *Genes Dev.* **21**, 2861–2873 (2007).
9. Mizushima, N., Yoshimori, T. & Ohsumi, Y. The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell Dev. Biol.* **27**, 107–132 (2011).
A detailed discussion of the protein and membrane interactions required for autophagosome formation.
10. Liang, X. H. *et al.* Induction of autophagy and inhibition of tumorigenesis by *beclin 1*. *Nature* **402**, 672–676 (1999).
Beclin 1 is identified as a putative tumour suppressor.
11. Klionsky, D. J. *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* **12**, 1–222 (2016).
The definitive consensus of experimental methods that are appropriate for the study of autophagy.
12. Bago, R. *et al.* Characterization of VPS34-IN1, a selective inhibitor of Vps34, reveals that the phosphatidylinositol 3-phosphate-binding SGK3 protein kinase is a downstream target of class III phosphoinositide 3-kinase. *Biochem. J.* **463**, 413–427 (2014).
13. Dowdle, W. E. *et al.* Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis *in vivo*. *Nat. Cell Biol.* **16**, 1069–1079 (2014).
14. Ronan, B. *et al.* A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nat. Chem. Biol.* **10**, 1013–1019 (2014).
15. Egan, D. F. *et al.* Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. *Mol. Cell* **59**, 285–297 (2015).
16. Petherick, K. J. *et al.* Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. *J. Biol. Chem.* **290**, 11376–11383 (2015).
17. Akin, D. *et al.* A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Autophagy* **10**, 2021–2035 (2014).
18. McAfee, Q. *et al.* Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc. Natl Acad. Sci. USA* **109**, 8253–8258 (2012).
19. Goodall, M. L. *et al.* Development of potent autophagy inhibitors that sensitize oncogenic BRAF V600E mutant melanoma tumor cells to vemurafenib. *Autophagy* **10**, 1120–1136 (2014).
20. Malik, S. A. *et al.* BH3 mimetics activate multiple pro-autophagic pathways. *Oncogene* **30**, 3918–3929 (2011).
21. Nazio, F. *et al.* mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat. Cell Biol.* **15**, 406–416 (2013).
22. DeBosch, B. J. *et al.* Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. *Sci. Signal.* **9**, ra21 (2016).
23. Marino, G., Pietrocola, F., Madeo, F. & Kroemer, G. Caloric restriction mimetics: natural/physiological pharmacological autophagy inducers. *Autophagy* **10**, 1879–1882 (2014).
24. He, C. *et al.* Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature* **481**, 511–515 (2012).
25. Li, W. W., Li, J. & Bao, J. K. Microautophagy: lesser-known self-eating. *Cell. Mol. Life Sci.* **69**, 1125–1136 (2012).
26. Arias, E. & Cuervo, A. M. Chaperone-mediated autophagy in protein quality control. *Curr. Opin. Cell Biol.* **23**, 184–189 (2011).
27. Kaushik, S. *et al.* Chaperone-mediated autophagy at a glance. *J. Cell Sci.* **124**, 495–499 (2011).
28. Kon, M. *et al.* Chaperone-mediated autophagy is required for tumor growth. *Sci. Transl Med.* **3**, 109ra117 (2011).
29. Mancias, J. D., Wang, X., Gygi, S. P., Harper, J. W. & Kimmelman, A. C. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* **509**, 105–109 (2014).
30. Dou, Z. *et al.* Autophagy mediates degradation of nuclear lamina. *Nature* **527**, 105–109 (2015).
31. Amaravadi, R. K. *et al.* Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J. Clin. Invest.* **117**, 326–336 (2007).
Therapy with autophagy inhibition is identified as having combinatory effects with other anticancer agents.
32. Thorburn, A., Thamm, D. H. & Gustafson, D. L. Autophagy and cancer therapy. *Mol. Pharmacol.* **85**, 830–838 (2014).
33. Yang, Y. P. *et al.* Application and interpretation of current autophagy inhibitors and activators. *Acta Pharmacol. Sin.* **34**, 625–635 (2013).
34. Maycotte, P. *et al.* Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. *Autophagy* **8**, 200–212 (2012).
35. Eng, C. H. *et al.* Macroautophagy is dispensable for growth of KRAS mutant tumors and chloroquine efficacy. *Proc. Natl Acad. Sci. USA* **113**, 182–187 (2016).
36. Maes, H. *et al.* Tumor vessel normalization by chloroquine independent of autophagy. *Cancer Cell* **26**, 190–206 (2014).
37. Briceno, E., Reyes, S. & Sotelo, J. Therapy of glioblastoma multiforme improved by the antimutagenic chloroquine. *Neurosurg. Focus* **14**, e3 (2003).
The results of the first clinical trial to evaluate the antitumour effects of CQ, which showed improved clinical outcomes with autophagy inhibition in glioblastoma.
38. Briceno, E., Calderon, A. & Sotelo, J. Institutional experience with chloroquine as an adjuvant to the therapy for glioblastoma multiforme. *Surg. Neurol.* **67**, 388–391 (2007).
39. Sotelo, J., Briceno, E. & Lopez-Gonzalez, M. A. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* **144**, 337–343 (2006).
40. Eldredge, H. B. *et al.* Concurrent whole brain radiotherapy and short-course chloroquine in patients with brain metastases: a pilot trial. *J. Radiat. Oncol.* **2,** 315–321 (2013).
41. Rojas-Puentes, L. L. *et al.* Phase II randomized, double-blind, placebo-controlled study of whole-brain irradiation with concomitant chloroquine for brain metastases. *Radiat. Oncol.* **8**, 209 (2013).
42. Barnard, R. A. *et al.* Phase I clinical trial and pharmacodynamic evaluation of combination hydroxychloroquine and doxorubicin treatment in pet dogs treated for spontaneously occurring lymphoma. *Autophagy* **10**, 1415–1425 (2014).
43. Mahalingam, D. *et al.* Combined autophagy and HDAC inhibition: a phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. *Autophagy* **10**, 1403–1414 (2014).
44. Rangwala, R. *et al.* Combined MTOR and autophagy inhibition: Phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. *Autophagy* **10**, 1391–1402 (2014).
45. Rangwala, R. *et al.* Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. *Autophagy* **10**, 1369–1379 (2014).
46. Rosenfeld, M. R. *et al.* A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy* **10**, 1359–1368 (2014).
47. Vogl, D. T. *et al.* Combined autophagy and proteasome inhibition: A phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy* **10**, 1380–1390 (2014).
48. Wolpin, B. M. *et al.* Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma. *Oncologist* **19**, 637–638 (2014).
49. Karsli-Uzunbas, G. *et al.* Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discov.* **4**, 914–927 (2014).
An evaluation of the genetic knockout of autophagy-related genes and the growth of tumour cells *in vivo*, and the identification of a therapeutic window to inhibit autophagy in lung cancer growth and development.
50. Pellegrini, P. *et al.* Acidic extracellular pH neutralizes the autophagy-inhibiting activity of chloroquine: implications for cancer therapies. *Autophagy* **10**, 562–571 (2014).
51. Wang, T. *et al.* Synthesis of improved lysomotropic autophagy inhibitors. *J. Med. Chem.* **58**, 3025–3035 (2015).
52. Yang, A. *et al.* Autophagy is critical for pancreatic tumor growth and progression in tumors with p53 alterations. *Cancer Discov.* **4**, 905–913 (2014).

53. Boone, B. A. *et al.* Safety and biologic response of pre-operative autophagy inhibition in combination with gemcitabine in patients with pancreatic adenocarcinoma. *Ann. Surg. Oncol.* **22**, 4402–4410 (2015).
54. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01881451?term=NCT01881451&rank=1 (2016).
55. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02233387?term=NCT02233387&rank=1 (2016).
56. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01206530?term=NCT01206530&rank=1 (2017).
57. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02042989?term=NCT02042989&rank=1 (2017).
58. Fullgrabe, J., Heldring, N., Hermanson, O. & Joseph, B. Cracking the survival code: autophagy-related histone modifications. *Autophagy* **10**, 556–561 (2014).
59. Wang, H. *et al.* Next-generation proteasome inhibitor MLN9708 sensitizes breast cancer cells to doxorubicin-induced apoptosis. *Sci. Rep.* **6**, 26456 (2016).
60. Perera, R. M. *et al.* Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature* **524**, 361–365 (2015).
61. Follo, C., Barbone, D., Richards, W. G., Bueno, R. & Broaddus, V. C. Autophagy initiation correlates with the autophagic flux in 3D models of mesothelioma and with patient outcome. *Autophagy* **12**, 1180–1194 (2016).
62. Lock, R., Kenific, C. M., Leidal, A. M., Salas, E. & Debnath, J. Autophagy-dependent production of secreted factors facilitates oncogenic RAS-driven invasion. *Cancer Discov.* **4**, 466–479 (2014).
63. Kraya, A. A. *et al.* Identification of secreted proteins that reflect autophagy dynamics within tumor cells. *Autophagy* **11**, 60–74 (2015).
64. Maycotte, P., Jones, K. L., Goodall, M. L., Thorburn, J. & Thorburn, A. Autophagy supports breast cancer stem cell maintenance by regulating IL6 secretion. *Mol. Cancer Res.* **4**, 651–658 (2015).
65. Varadarajulu, S. & Bang, J. Y. Role of endoscopic ultrasonography and endoscopic retrograde cholangiopancreatography in the clinical assessment of pancreatic neoplasms. *Surg. Oncol. Clin. N. Am.* **25**, 255–272 (2016).
66. Feng, Y., He, D., Yao, Z. & Klionsky, D. J. The machinery of macroautophagy. *Cell Res.* **24**, 24–41 (2014). **Basic review of the history and core machinery involved in the process of autophagy.**
67. Altman, J. K. *et al.* Autophagy is a survival mechanism of acute myelogenous leukemia precursors during dual mTORC2/mTORC1 targeting. *Clin. Cancer Res.* **20**, 2400–2409 (2014).
68. Kun, Z. *et al.* Gastrin enhances autophagy and promotes gastric carcinoma proliferation via inducing AMPKα. *Oncol. Res.* http://dx.doi.org/10.3727/096504016X14823648620870 (2017).
69. Masui, A. *et al.* Autophagy as a survival mechanism for squamous cell carcinoma cells in endonuclease G-mediated apoptosis. *PLoS ONE* **11**, e0162786 (2016).
70. Tan, Q. *et al.* Role of autophagy as a survival mechanism for hypoxic cells in tumors. *Neoplasia* **18**, 347–355 (2016).
71. Fitzwalter, B. E. & Thorburn, A. Recent insights into cell death and autophagy. *FEBS J.* **282**, 4279–4288 (2015).
72. Gump, J. M. *et al.* Autophagy variation within a cell population determines cell fate through selective degradation of Fap-1. *Nat. Cell Biol.* **16**, 47–54 (2014).
73. Thorburn, J. *et al.* Autophagy controls the kinetics and extent of mitochondrial apoptosis by regulating PUMA levels. *Cell Rep.* **7**, 45–52 (2014).
74. Goodall, M. L. *et al.* The autophagy machinery controls cell death switching between apoptosis and necroptosis. *Dev. Cell* **37**, 337–349 (2016).
75. Rao, S., Yang, H., Penninger, J. M. & Kroemer, G. Autophagy in non-small cell lung carcinogenesis: a positive regulator of antitumor immunosurveillance. *Autophagy* **10**, 529–531 (2014).
76. Ma, Y., Galluzzi, L., Zitvogel, L. & Kroemer, G. Autophagy and cellular immune responses. *Immunity* **39**, 211–227 (2013).
77. Townsend, K. N. *et al.* Autophagy inhibition in cancer therapy: metabolic considerations for antitumor immunity. *Immunol. Rev.* **249**, 176–194 (2012).
78. Ko, A. *et al.* Autophagy inhibition radiosensitizes *in vitro*, yet reduces radioresponses *in vivo* due to deficient immunogenic signalling. *Cell Death Differ.* **21**, 92–99 (2014).
79. Michaud, M. *et al.* Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* **334**, 1573–1577 (2011).
80. Lechner, M. G. *et al.* Immunogenicity of murine solid tumor models as a defining feature of *in vivo* behavior and response to immunotherapy. *J. Immunother.* **36**, 477–489 (2013).
81. Starobinets, H. *et al.* Antitumor adaptive immunity remains intact following inhibition of autophagy and antimalarial treatment. *J. Clin. Invest.* **126**, 4417–4429 (2016).
82. Pietrocola, F. *et al.* Caloric restriction mimetics enhance anticancer immunosurveillance. *Cancer Cell* **30**, 147–160 (2016).
83. Li, Y. *et al.* The vitamin E analogue α-TEA stimulates tumor autophagy and enhances antigen cross-presentation. *Cancer Res.* **72**, 3535–3545 (2012).
84. Ladoire, S. *et al.* Combined evaluation of LC3B puncta and HMGB1 expression predicts residual risk of relapse after adjuvant chemotherapy in breast cancer. *Autophagy* **11**, 1878–1890 (2015).
85. Ladoire, S. *et al.* The presence of LC3B puncta and HMGB1 expression in malignant cells correlate with the immune infiltrate in breast cancer. *Autophagy* **12**, 864–875 (2016).
86. Baginska, J. *et al.* Granzyme B degradation by autophagy decreases tumor cell susceptibility to natural killer-mediated lysis under hypoxia. *Proc. Natl Acad. Sci. USA* **110**, 17450–17455 (2013).
87. Liang, X. *et al.* Inhibiting systemic autophagy during interleukin 2 immunotherapy promotes long-term tumor regression. *Cancer Res.* **72**, 2791–2801 (2012).
88. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03057340?term=NCT03057340&rank=1 (2017).
89. Page, D. B. *et al.* Glimpse into the future: harnessing autophagy to promote anti-tumor immunity with the DRibbles vaccine. *J. Immunother. Cancer* **4**, 25 (2016).
90. Yu, G. L. *et al.* Combinational immunotherapy with allo-DRibble vacciens and anti-OX40 co-stimulation leads to generation of cross-reactive effector T cells and tumor regression. *Sci. Rep.* **6**, http://dx.doi.org/10.1038/srep37558 (2016).
91. Hilton, T. S. *et al.* Preliminary analysis of immune responses in patients enrolled in a Phase II trial of cyclophosphamide wiht allogenic DRibble vaccine alone (DPV-001) or with GM-CSF or imiquimod for adjuvant treatment of stage IIIA or IIIB NSCLC. *J. Immunother. Cancer* **2**, 249 (2014).
92. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01956734?term=NCT01956734&rank=1 (2015).
93. Levy, J. M. M. *et al.* Autophagy inhibition improves chemosensitivity in BRAFV600E brain tumors. *Cancer Discov.* **4**, 773–780 (2014).
94. Maycotte, P. *et al.* STAT3-mediated autophagy dependence identifies subtypes of breast cancer where autophagy inhibition can be efficacious. *Cancer Res.* **74**, 2579–2590 (2014).
95. Guo, J. Y. *et al.* Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev.* **25**, 460–470 (2011).
96. Lock, R. *et al.* Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Mol. Biol. Cell* **22**, 165–178 (2011).
97. Yang, S. *et al.* Pancreatic cancers require autophagy for tumor growth. *Genes Dev.* **25**, 717–729 (2011).
98. Sousa, C. M. *et al.* Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion. *Nature* **536**, 479–483 (2016).
99. Strohecker, A. M. *et al.* Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. *Cancer Discov.* **3**, 1272–1285 (2013).
100. Xie, X., Koh, J. Y., Price, S., White, E. & Mehnert, J. M. Atg7 overcomes senescence and promotes growth of BrafV600E-driven melanoma. *Cancer Discov.* **5**, 410–423 (2015).
101. Mancias, J. D. & Kimmelman, A. C. Targeting autophagy addiction in cancer. *Oncotarget* **2**, 1302–1306 (2011).
102. Thorburn, A. & Morgan, M. J. Targeting autophagy in BRAF-mutant tumors. *Cancer Discov.* **5**, 353–354 (2015).
103. Levine, B. & Abrams, J. p53: the Janus of autophagy? *Nat. Cell Biol.* **10**, 637–639 (2008).
104. Tang, J. Di, J., Cao, H., Bai, J. & Zheng, J. p53-mediated autophagic regulation: a prospective strategy for cancer therapy. *Cancer Lett.* **363**, 101–107 (2015).
105. Rosenfeldt, M. T. *et al.* p53 status determines the role of autophagy in pancreatic tumour development. *Nature* **504**, 296–300 (2013).
106. Iacobuzio-Donahue, C. A. & Herman, J. M. Autophagy, p53, and pancreatic cancer. *N. Engl. J. Med.* **370**, 1352–1353 (2014).
107. Huo, Y. *et al.* Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. *Cancer Discov.* **3**, 894–907 (2013).
108. Morgan, M. J. *et al.* Regulation of autophagy and chloroquine sensitivity by oncogenic RAS *in vitro* is context-dependent. *Autophagy* **10**, 1814–1826 (2014).
109. Vasilevskaya, I. A., Selvakumaran, M., Roberts, D. & O'Dwyer, P. J. JNK1 inhibition attenuates hypoxia-induced autophagy and sensitizes to chemotherapy. *Mol. Cancer Res.* **14**, 753–763 (2016).
110. Jutten, B. & Rouschop, K. M. EGFR signaling and autophagy dependence for growth, survival, and therapy resistance. *Cell Cycle* **13**, 42–51 (2014).
111. Guo, D. *et al.* The AMPK agonist AICAR inhibits the growth of EGFRvIII-expressing glioblastomas by inhibiting lipogenesis. *Proc. Natl Acad. Sci. USA* **106**, 12932–12937 (2009).
112. Jutten, B. *et al.* EGFR overexpressing cells and tumors are dependent on autophagy for growth and survival. *Radiother. Oncol.* **108**, 479–483 (2013).
113. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02257424?term=NCT02257424&rank=1 (2016).
114. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02378532?term=NCT02378532&rank=1 (2016).
115. Ma, X.-H. *et al.* Targeting ER stress-induced autophagy overcomes BRAF inhibitor resistance in melanoma. *J. Clin. Invest.* **124**, 1406–1417 (2014).
116. Mulcahy Levy, J. M. *et al.* Autophagy inhibition overcomes multiple mechanisms of resistance to BRAF inhibition in brain tumors. *eLife* **6**, e19671 (2017). **The first demonstration of the use of autophagy inhibition to overcome kinase inhibitor resistance in patients.**
117. Kang, M. *et al.* Concurrent autophagy inhibition overcomes the resistance of epidermal growth factor receptor tyrosine kinase inhibitors in human bladder cancer cells. *Int. J. Mol. Sci.* **18**, 321 (2017).
118. Wang, W. *et al.* Targeting autophagy sensitizes BRAF-mutant thyroid cancer to vemurafenib. *J. Clin. Endocrinol. Metab.* **102**, 634–643 (2016).
119. Liu, J. T. *et al.* Autophagy inhibition overcomes the antagonistic effect between gefitinib and cisplatin in epidermal growth factor receptor mutant non–small-cell lung cancer cells. *Clin. Lung Cancer* **16**, e55–e66 (2015).
120. Zou, Y. *et al.* The autophagy inhibitor chloroquine overcomes the innate resistance of wild-type EGFR non-small-cell lung cancer cells to erlotinib. *J. Thorac Oncol.* **8**, 693–702 (2013).
121. Ji, C. *et al.* Induction of autophagy contributes to crizotinib resistance in ALK-positive lung cancer. *Cancer Biol. Ther.* **15**, 570–577 (2014).
122. Zhang, S. F. *et al.* TXNDC17 promotes paclitaxel resistance via inducing autophagy in ovarian cancer. *Autophagy* **11**, 225–238 (2015).
123. Wang, J. & Wu, G. S. Role of autophagy in cisplatin resistance in ovarian cancer cells. *J. Biol. Chem.* **289**, 17163–17173 (2014).
124. Yu, L. *et al.* Induction of autophagy counteracts the anticancer effect of cisplatin in human esophageal cancer cells with acquired drug resistance. *Cancer Lett.* **355**, 34–45 (2014).
125. Wu, H. M., Jiang, Z. F., Ding, P. S., Shao, L. J. & Liu, R. Y. Hypoxia-induced autophagy mediates cisplatin resistance in lung cancer cells. *Sci. Rep.* **5**, 12291 (2015).
126. Mahoney, E. *et al.* ER stress and autophagy: new discoveries in the mechanism of action and drug resistance of the cyclin-dependent kinase inhibitor flavopiridol. *Blood* **120**, 1262–1273 (2012).

127. Li, Z. Y. *et al.* A novel HDAC6 inhibitor Tubastatin A: controls HDAC6-p97/VCP-mediated ubiquitination-autophagy turnover and reverses Temozolomide-induced ER stress-tolerance in GBM cells. *Cancer Lett.* **391**, 89–99 (2017).
128. Aveic, S. & Tonini, G. P. Resistance to receptor tyrosine kinase inhibitors in solid tumors: can we improve the cancer fighting strategy by blocking autophagy? *Cancer Cell. Int.* **16**, 62 (2016).
129. Chen, S. *et al.* Autophagy is a therapeutic target in anticancer drug resistance. *Biochim. Biophys. Acta* **1806**, 220–229 (2010).
130. Kumar, A., Singh, U. K. & Chaudhary, A. Targeting autophagy to overcome drug resistance in cancer therapy. *Future Med. Chem.* **7**, 1535–1542 (2015).
131. Sannigrahi, M. K., Singh, V., Sharma, R., Panda, N. K. & Khullar, M. Role of autophagy in head and neck cancer and therapeutic resistance. *Oral Dis.* **21**, 283–291 (2015).
132. Sui, X. *et al.* Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. *Cell Death Dis.* **4**, e838 (2013).
133. Viale, A. *et al.* Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* **514**, 628–632 (2014).
134. Katheder, N. S. *et al.* Microenvironmental autophagy promotes tumour growth. *Nature* **541**, 417–420 (2017).
135. Pasquier, B. Autophagy inhibitors. *Cell. Mol. Life Sci.* **73**, 985–1001 (2016).
136. Basit, F., Cristofanon, S. & Fulda, S. Obatoclax (GX15-070) triggers necroptosis by promoting the assembly of the necrosome on autophagosomal membranes. *Cell Death Differ.* **20**, 1161–1173 (2013).
137. Chen, H. Y. & White, E. Role of autophagy in cancer prevention. *Cancer Prev. Res. (Phila.)* **4**, 973–983 (2011).
138. Bedoya, V. Effect of chloroquine on malignant lymphoreticular and pigmented cells *in vitro*. *Cancer Res.* **30**, 1262–1275 (1970).
The anti-malarial drug CQ is first shown to inhibit tumour cell growth *in vitro*, as indicated by the accumulation of autophagic vacuoles.
139. Funakoshi, T., Matsuura, A., Noda, T. & Ohsumi, Y. Analyses of APG13 gene involved in autophagy in yeast, *Saccharomyces cerevisiae*. *Gene* **192**, 207–213 (1997).
The Ohsumi group cloned the first autophagy-specific gene, *Apg13* in yeast (*ATG1* in humans).
140. Matsuura, A., Tsukada, M., Wada, Y. & Ohsumi, Y. Apg1p, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae*. *Gene* **192**, 245–250 (1997).
The Ohsumi group demonstrated the direct involvement of protein phosphorylation in the regulation of autophagy.
141. Mizushima, N., Sugita, H., Yoshimori, T. & Ohsumi, Y. A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. *J. Biol. Chem.* **273**, 33889–33892 (1998).
The first autophagy-specific genes in higher eukaryotes were identified.
142. Murakami, N. *et al.* Accumulation of tau in autophagic vacuoles in chloroquine myopathy. *J. Neuropathol. Exp. Neurol.* **57**, 664–673 (1998).
The first study to observe that CQ can inhibit autophagy and the connection between the accumulation of cellular proteins and the inhibition of lysosomal degradation.
143. Kuma, A. *et al.* The role of autophagy during the early neonatal starvation period. *Nature* **432**, 1032–1036 (2004).
The first autophagy-deficient mouse (*Atg5*$^{-/-}$) was created, and indicated that autophagy is important during development.
144. Rao, S. *et al.* A dual role for autophagy in a murine model of lung cancer. *Nat. Commun.* **5**, 3056 (2014).
***In vivo* model that demonstrated the ability of autophagy to repress early oncogenesis but to support late-stage cancer growth.**
145. Chi, K. H. *et al.* Addition of rapamycin and hydroxychloroquine to metronomic chemotherapy as a second line treatment results in high salvage rates for refractory metastatic solid tumors: a pilot safety and effectiveness analysis in a small patient cohort. *Oncotarget* **6**, 16735–16745 (2015).
146. Chi, M. S. *et al.* Double autophagy modulators reduce 2-deoxyglucose uptake in sarcoma patients. *Oncotarget* **6**, 29808–29817 (2015).
147. Bilger, A. *et al.* FET-PET-based reirradiation and chloroquine in patients with recurrent glioblastoma: first tolerability and feasibility results. *Strahlenther. Onkol.* **190**, 957–961 (2014).
148. Goldberg, S. B. *et al.* A phase I study of erlotinib and hydroxychloroquine in advanced non-small-cell lung cancer. *J. Thorac Oncol.* **7**, 1602–1608 (2012).
149. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01594242?term = NCT01594242&rank = 1 (2016).
150. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01874548?term = NCT01874548&rank = 1 (2016).
151. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01506973?term = NCT01506973&rank = 1 (2017).
152. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01978184?term = NCT01978184&rank = 1 (2015).
153. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01128296?term = NCT01128296&rank = 1 (2015).
154. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT01897116?term = NCT01897116&rank = 1 (2016).

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Competing Interests
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