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Autophagy in tumour immunity and therapy

Houjun Xia^{1,2}, Douglas R. Green^{3,✉}, Weiping Zou^{1,2,4,5,6,✉}

¹Department of Surgery, University of Michigan School of Medicine, Ann Arbor, MI, USA.

²Center of Excellence for Cancer Immunology and Immunotherapy, University of Michigan Rogel Cancer Center, University of Michigan School of Medicine, Ann Arbor, MI, USA.

³Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN, USA.

⁴Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI, USA.

⁵Graduate Program in Immunology, University of Michigan School of Medicine, Ann Arbor, MI, USA.

⁶Graduate Program in Cancer Biology, University of Michigan School of Medicine, Ann Arbor, MI, USA.

Abstract

Autophagy is a regulated mechanism that removes unnecessary or dysfunctional cellular components and recycles metabolic substrates. In response to stress signals in the tumour microenvironment, the autophagy pathway is altered in tumour cells and immune cells — thereby differentially affecting tumour progression, immunity and therapy. In this Review, we summarize our current understanding of the immunologically associated roles and modes of action of the autophagy pathway in cancer progression and therapy, and discuss potential approaches targeting autophagy to enhance antitumour immunity and improve the efficacy of current cancer therapy.

Autophagy is a tightly regulated and stress-induced catabolic pathway in all eukaryotes, during which double-membraned vesicles (called autophagosomes) are formed that engulf cellular targets — including damaged organelles, unfolded proteins and pathogens — and deliver them to the lysosome to be digested¹. Selective forms of autophagy represent quality control and homeostatic mechanisms (BOX 1). The basic biology of autophagy has been extensively reviewed^{2–4}. The canonical form of autophagy under discussion herein (more formally, macro-autophagy) is best understood under conditions of nutrient starvation⁵ (FIG. 1). In the non-canonical autophagy pathway, proteins also function to lipidate microtubule-associated protein 1A/1B-light chain 3 (LC3; also known as ATG8) family proteins on a single lipid bilayer. The most studied examples of this are LC3-associated phagocytosis (LAP)⁶ and the more recently described LC3-associated endocytosis (LANDO)⁷ (FIG. 1).

✉ douglas.green@stjude.org; wzou@med.umich.edu.

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Competing interests

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Here, we focus on the autophagy pathway in the tumour microenvironment (TME) where, in response to various stresses, the autophagy pathway in various cell types can be modulated, resulting in suppression or promotion of tumour progression. The effects of changes in autophagy in the tumour may be context-dependent, including variable tumour genetics and stages as well as host factors. The immune system plays a critical role in prevention of tumour occurrence, progress and metastasis, and shapes the tumour response to therapy. Immune surveillance provides a way to recognize, control and kill tumour cells. However, tumour cells evade tumour immunity via reducing their immunogenicity, forming immunosuppressive networks and inhibiting immune response⁸. Immunotherapies harness the immune system to fight against the tumour through evoking successful antitumour immune responses. For example, immune checkpoint blockade (ICB) unleashes the ability of T cells to restrain tumour growth^{9,10}. Other immunotherapy approaches can also enhance antitumour immunity, including chimeric antigen receptor T cells (CAR T cells), dendritic cell vaccines and cytokine therapies.

Recent studies have demonstrated the involvement of the autophagy pathway in survival and apoptosis, differentiation, activation, effector function and tumour trafficking of immune cell subsets¹¹. Meanwhile, tumour autonomous autophagy can alter tumour growth through modulating the immune response^{12,13}. Furthermore, the pro-tumour role of autophagy may be abolished by combining immune checkpoint therapy with autophagy inhibitors¹⁴. Given our recent advances in understanding the multiple layers of the relationship between the autophagy pathway and the tumour immune responses, and the potential of targeting the autophagy pathway in cancer therapy, it is timely to summarize and discuss recent findings on the autophagy pathway in the context of tumour immunity and immunotherapy.

Autophagy induction in tumour cells

The TME, including the immunosuppressive networks⁸, plays a key role in cancer progression, metastasis and resistance to therapies. In the TME, autophagy in tumour cells can be induced by integrated intracellular and extracellular stress signals including metabolic stress, hypoxia, redox stress and immune signals.

Metabolic stress

Insufficient nutrient uptake from the TME affects the metabolic machinery, leading to intracellular metabolic stress. In response to metabolic stress, tumour cells rewire their own metabolic pathways via upregulating nutrient transporters¹⁵ and activating autophagy¹⁶. Mechanistically, 5'-AMP-activated protein kinase (AMPK) and mTOR complex 1 (mTORC1) are two opposing regulatory kinases that alter autophagy induction under nutrient deprivation. As an AMP sensor, AMPK is activated via the increased ratio of AMP and ATP, whereas the activity of mTORC1 is reduced due to limited amino acid availability. This leads to phosphorylation of the targets in the autophagy pre-initiation complex (AMPK at an activating site, mTORC1 at an inhibitory site). To initiate autophagy, AMPK phosphorylates unc-51-like kinase 1 (ULK1) at six different sites (S317, S467, S555, T574, S637 and S777)^{17,18} and at S91/S94 in Beclin 1 (REF.¹⁹). Moreover, AMPK targets Raptor, a subunit of mTORC1, and activates tuberous sclerosis complex 2 (TSC2), an upstream

negative regulator of mTOR, to inhibit mTORC1 activity^{20,21}. Unlike AMPK, mTORC1-induced phosphorylation of ULK1 on S757 inhibits its downstream activation of the vacuolar protein sorting 34 (VPS34) complex and autophagy¹⁷ (FIG. 1). In addition, AMPK activates the transcriptional factors forkhead box O3 (FoxO3) and transcription factor EB (TFEB) to amplify autophagy-related gene expression²². Activated autophagy promotes recycling of key metabolites — including nucleotides, amino acids and lipids — thereby supporting tumour cell survival and proliferation in the TME.

Hypoxic stress

Hypoxia is a hallmark of solid tumours. Hypoxia results in suppression of oxidative phosphorylation in the mitochondria, an increase in the ratio of AMP to ATP and activation of AMPK. As discussed above, AMPK then targets ULK1, Beclin 1 and mTORC1 to induce autophagy²³. Furthermore, hypoxia restrains mTOR signalling and unleashes protein phosphatase 2A (PP2A) activity via regulated in development and DNA damage response 1 (REDD1; also known as DDIT4). PP2A directly dephosphorylates prolyl hydroxylase domain-containing protein 2 (PHD2) on S125, which restricts the ability of PHD2 to degrade hypoxia-induced factor 1 α (HIF1 α), thereby increasing HIF1 α stabilization²⁴. HIF1 α is then translocated into the nucleus and induces the expression of downstream target genes, including B cell lymphoma 2 (BCL2) interacting protein 3 (BNIP3) and BNIP3L, which dissociates Beclin 1 from BCL2 and, consequently, activates autophagy²⁵. In addition, hypoxia causes activation of activating transcription factor 4 (ATF4), which results in upregulation of LC3B and autophagy-related protein 5 (ATG5) expression, and maintenance of high levels of autophagic flux²⁶. These events promote autophagy-mediated tumour cell survival under hypoxic conditions²⁴.

Oxidative stress

Oxidative stress reflects an imbalance between free radicals and antioxidants. reactive oxygen species (ROS) are generated within cells through oxygen metabolism and other processes. Excessive ROS may increase the risk of DNA damage and promote tumorigenesis²⁷. In response to elevated ROS, ataxia telangiectasia mutated (ATM) activates the TSC2 tumour suppressor via the liver kinase B1 (LKB1) and AMPK metabolic pathway in the cytoplasm to repress mTORC1 and induce autophagy²⁸. Oxidative stress can also promote autophagy through nuclear factor- κ B (NF- κ B)-mediated upregulation of p62/SQSTM1 (REF.²⁹). In return, activated autophagy can alleviate oxidative stress through p62-induced degradation of kelch-like ECH-associated protein 1 (KEAP1), resulting in the stabilization and accumulation of nuclear factor erythroid 2-related factor 2 (NRF2) and activation of antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase and thioredoxin³⁰. Furthermore, autophagy can mediate a selective degradation of damaged and dysfunctional mitochondria (mitophagy). By controlling oxidative stress, autophagy may inhibit tumorigenesis but may also promote tumour cell survival.

Immune signals

Immune signals can modulate the autophagy pathway in the TME. Damage-associated molecular patterns (DAMPs) and cytokines are major soluble mediators that modulate autophagic responses. Extracellular DAMPs — such as DNA complexes, ATP and high-

mobility group box 1 protein (HMGB1)³¹ — activate innate immune cells and promote inflammation. These signals are perceived by extracellular or intracellular pattern recognition receptors including Toll-like receptors (TLRs), advanced glycosylation end product-specific receptor (AGER), purinergic P2RX7 receptors and absent in melanoma 2 (AIM2)³¹. Autophagy is stimulated by multiple cell death-associated DAMPs³². Various TLRs are proposed as autophagy inducers when they recognize DAMPs and activate downstream signalling. The E3 ligase TNF receptor-associated factor 6 (TRAF6)-mediated ubiquitination of Beclin 1 is critical for TLR4-triggered autophagy in macrophages, which releases Beclin 1 from its inhibitor BCL2 (REF.³³). In non-small cell lung cancer (NSCLC) cells, activation of TLR3 and TLR4 with polyinosinic:polycytidylic acid (poly(I:C)) and lipopolysaccharide (LPS) induces autophagy and increases the production of certain cytokines related to tumour metastasis³⁴. Moreover, TLR engagement activates NF- κ B-dependent transcription and promotes autophagy activation through transcriptional upregulation of autophagy receptor p62, which mediates mitophagy and restricts NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in LPS-treated macrophages³⁵. In pancreatic and colon cancer cells, AGER is important for HMGB1-induced autophagy in a Beclin 1-dependent manner in vitro³⁶. AGER also down-regulates the phosphorylation of mTOR and increases Beclin 1/VPS34-dependent autophagosome formation in pancreatic cancer cells³⁷.

Cytokines regulate the autophagy pathway in a context-dependent fashion. In *Drosophila*, TNF and interleukin-6 (IL-6)-like signalling activates autophagy, thereby promoting early-stage tumour growth and invasion³⁸. Transforming growth factor- β (TGF β) increases the transcript levels of *BECN1*, *ATG5* and *ATG7* via both SMAD-dependent and SMAD-independent pathways, and enhances the degradation rate of long-lived proteins in human HuH7 hepatocellular carcinoma cells³⁹. TGF β activates autophagy to enhance its growth-inhibitory effect and postpones apoptosis in human hepatocellular and mammary carcinoma cells in vitro³⁹. TGF β 2-induced autophagy also supports glioma invasion in mice via alteration of epithelial–mesenchymal transition and metabolism⁴⁰. Additionally, type I and II interferons are autophagy inducers in multiple human cancer cell lines⁴¹. It seems that IFN γ -induced autophagy correlates with an inhibition of carcinogenesis in an inflammation-related gastric cancer model⁴².

Tumour cell autonomous autophagy

Function in tumorigenesis

Most human cancers develop over a long period of time as mutations accumulate in various proto-oncogenes, such as *KRAS* and *BRAF*⁴³. The relationship between oncogenes and autophagy genes in tumorigenesis has been explored. There are more than 40 genes encoding ATGs, which are involved in the autophagy pathway⁴⁴. Mutation of genes encoding ATGs may contribute to tumour initiation and immune system recognition⁴⁵. However, the core autophagy machinery in human tumour tissues remains largely intact at the DNA level and is expressed at similar transcript levels to matched normal tissues, based on data available from The Cancer genome Atlas (TCGA)⁴⁶. This observation is in line with studies showing that intact systemic autophagy is necessary for tumour growth, even if few

mutated ATGs are observed in certain human tumour types^{47,48}. Mosaic deletion of *Atg5* in mice or *Atg7* in mouse liver led to only benign hepatomas, suggesting that complete and specific autophagy deficiency promotes liver tumour initiation but restricts progression to malignant disease⁴⁹. A similar phenomenon was observed in *Kras*^{G12D}-driven pancreatic cancer models; loss of *Atg5* or *Atg7* in the mouse pancreas promoted benign pancreatic intraepithelial neoplasia formation, but also prevented progression of this pancreatic intraepithelial neoplasia to malignant disease^{50,51}. Moreover, tissue-specific inactivation of *Atg5* in mice markedly accelerated the initiation of but impaired the progression of *Kras*^{G12D}-driven lung cancer⁵². Complete loss of ATG7 was also found to reduce the tumour burden in *Braf*^{V600E}-driven melanoma⁵³ and lung cancer⁵⁴. The general observations in ATG-deficient cells include accumulated p62, damaged mitochondria, ROS and genome damage. The accumulation of ROS or p62 can promote genome instability and attenuate NF- κ B signalling, which was shown to initiate tumour formation in autophagy-deficient tumour models^{55,56}. However, the accumulation of damaged cellular components and ROS can induce cell senescence, which inhibits tumour growth by restraining the tumour cell proliferation⁵⁷. In *Braf*^{V600E}-driven melanoma, *Atg7*-deleted tumours showed increased oxidative stress and senescence. Thus, autophagy promotes *Braf*^{V600E}-driven melanoma by limiting oxidative stress and overcoming senescence⁵³. In line with this, reduced cell proliferation but increased cell senescence was observed in mice bearing *Atg7*^{-/-} compared with wild-type *Braf*^{V600E}-driven lung tumours⁵⁴. Hence, autophagy deficiency-induced senescence prevents further tumour progression. Notably, a complete loss of autophagic function in cancer cells is rare in patients. Autophagy was first linked to human cancer based on the detection of monoallelic deletions of *BECN1* in human breast cancer and ovarian cancer^{58,59}. Similarly, heterozygous loss of *ATG5* at chromosome band 6q21 was found to be a distinctive feature of human advanced melanomas⁶⁰. Monoallelic loss of *Atg5* enhanced melanoma metastasis and compromised the response to targeted therapy in mouse models⁶⁰. In addition, although homozygous disruption of *Atg5* did not lead to the development of any advanced tumour, heterozygous disruption of *Atg5* in *Kras*^{G12D}-driven pancreatic cancers with insufficient ATG5 levels led to an increased burden of tumours and metastases compared with wild-type mice⁶¹. In addition to its direct effect on tumour cells, inflammation may provide an indirect mechanism by which autophagy defects promote tumorigenesis⁶². It has been reported that functional loss of ATG16L1 based on the Thr300Ala (T300A) mutation induces chronic inflammation in Crohn's disease, which predisposes patients to colorectal cancer development. ATG16L1 Thr300Ala mutation or *Atg16l1* depletion in macrophages enhances endotoxin-induced inflammasome activation, which makes the ATG16L1-deficient mice highly susceptible to dextran sulfate sodium-induced acute colitis^{63,64}. Interestingly, *Atg16l1*^{T300A} knock-in mice have been shown to manifest an impaired antibacterial host defence, which in turn led to chronic inflammation, tissue damage and an elevated cancer risk⁶⁵. Thus, it appears that a mild or intermediate defect in cancer cell autophagy could promote tumorigenesis and drive cancer progression, whereas complete loss of an autophagy component can trigger tumour cell senescence and slow down cancer progression.

Function in the immune response

Elevated autophagy in tumour cells plays a pro-tumour role in the TME^{12,38}. The antitumour effect of autophagy deficiency on pancreatic tumour growth was observed in immunocompetent mice¹⁴, but not in immune-deficient mice⁶⁶. This suggests a potential impact of tumour autonomous autophagy on antitumour immune response.

MHC class I-mediated tumour antigen presentation.

Major histocompatibility complex (MHC) molecules present antigens to T cells, which usually leads to initiation of an immune response. There are two types of MHC: MHC class I and MHC class II. MHC class I is expressed in all nucleated cell types, whereas MHC class II expression is limited to professional antigen-presenting cells (APCs), such as dendritic cells. MHC class I displays the peptides to prime and activate CD8⁺ cytotoxic T lymphocytes (CTLs). CTLs kill the targeted tumour cells via matched antigen–MHC class I complex recognition⁶⁷. MHC class I-restricted antigens are generally derived from endogenous neosynthesized proteins, which are processed by the ubiquitin–proteasome system and transferred to the endoplasmic reticulum. Here, they are loaded onto newly translated MHC class I complexes. It has been reported that autophagy promotes MHC class I internalization and degradation in dendritic cells, and autophagy-deficient dendritic cells elevate presentation of different viral antigens to CD8⁺ T cells^{68,69}.

Alterations in the genes encoding proteins of MHC class I, such as *B2M* and *HLA* genes, occur in certain types of cancer^{70,71}. Tumour cells express low levels of MHC class I, which enables escape from CD8⁺ T cell-mediated killing. It has been reported that autophagy-related genes are enriched in MHC class I-negative pancreatic carcinoma cells that reside in liver metastasis⁷². Interestingly, the autophagy and lysosome system support pancreatic carcinoma metabolism and growth⁵⁰. It is possible that autophagy mediates MHC class I lysosomal degradation in cancer cells. In support of this, in pancreatic cancer cells, selective autophagy has been shown to reroute MHC class I to lysosomes via the ubiquitin-binding receptor, neighbour of BRCA1 gene 1 protein (NBR1), leading to degradation, thereby precluding T cell recognition¹⁴. This MHC class I degradation depends on canonical autophagy, but not on LAP or LANDO¹⁴. Hence, tumour cells can evade immune surveillance via autophagy-mediated MHC class I degradation (FIG. 2a). Furthermore, it appears that basal autophagy flux varies in different pancreatic tumours, and this is associated with immunogenicity. Autophagy-low tumours exhibit higher levels of MHC class I expression and tumour-infiltrating CD8⁺ T cells compared with autophagy-high tumours¹⁴. In support of this observation, host autophagy — particularly hepatocyte autophagy — suppresses an antitumour T cell response in carcinogen-induced MB49 urothelial carcinoma tumour-bearing mice⁷³. Thus, heterogeneous expression of autophagy genes can shape tumour immunogenicity^{14,74}.

In addition to cytosolic antigens, dendritic cell subsets, such as CD8α⁺ and CD103⁺ dendritic cells, can present exogenous antigens to CD8⁺ T cells via MHC class I — namely, antigen cross-presentation⁷⁵. To this end, dendritic cells take up dead tumour cells, which are transported into the cytosol and processed by the proteasome. The processed antigens can then be loaded on MHC class I molecules in the endoplasmic reticulum or reimported

into the phagosome to be loaded on MHC class I molecules for presentation to CD8⁺ T cells. Induction of autophagy in tumour cells can enhance their phagocytosis and MHC class I-mediated cross-presentation to CD8⁺ T cells. Indeed, the uptake of intact autophagosomes that comprise tumour antigens, released from dead tumour cells, is necessary for dendritic cell-mediated cross-presentation^{76,77} (FIG. 2a). Consistently, in autochthonous mouse models of sporadic intestinal tumour with signal transducer and activator of transcription 3 (STAT3) deficiency in intestinal epithelial cells (IECs), IEC mitophagy can enhance MHC class I-mediated tumour antigen presentation and augment CD8⁺ T cell-mediated antitumour immunity via dendritic cells⁷⁸. Hence, the role of tumour autonomous autophagy in tumour antigen presentation is context-dependent.

Immune cell trafficking in the tumour.—Distinct chemokines mediate the trafficking of different immune cell subsets into the TME⁷⁹. Autophagy can regulate the expression of chemokines in tumour cells and migration of immune cells to the tumour, in turn altering tumour immune responses. *Atg5*-inactivated *Kras*^{G12D}-driven lung cancer cells expressed high protein levels of CXCL5, a pro-tumour chemokine for myeloid-derived suppressor cell (MDSC) migration, compared with control *Kras*^{G12D} lung cancer cells^{52,79}. Monoallelic *Atg5*-deleted *Kras*^{G12D}-driven pancreatic cancer cells produced high levels of chemokines (including CXCL1, CXCL2 and CXCL12) compared with autophagy-competent *Kras*^{G12D} pancreatic cancer cells, attracting more immune-suppressive macrophages into original pancreatic tumour tissues in these transgenic mice⁶¹. Interestingly, in the polyoma middle T antigen (PyMT)-driven spontaneous mouse model of breast cancer, FAK family interacting protein of 200 kDa (FIP200; also known as RB1CC1) ablation suppressed mammary tumour initiation and progression via elevated chemokine expression of CXCL9 and CXCL10, which mediates CD8⁺ T cell tumour trafficking⁸⁰. Similarly, conditional inactivation of *Atg7* in IECs inhibited the formation of precancerous lesions in *Apc*^{+/-} mice. IECs with conditional *Atg7* inactivation, but not those expressing wild-type *Atg7*, produced CXCL9 and CXCL10, and supported CD8⁺ T cell trafficking¹³. Accordingly, targeting autophagy genes or pharmacologically inhibiting autophagy induced the expression of CCL5 in melanoma cells, resulting in an increase of natural killer (NK) cell tumour infiltration⁸¹. These data suggest that autophagy may play an inhibitory role in the expression of pro-tumour chemokines as well as antitumour chemokines, thereby differentially affecting tumour progression (FIG. 2b).

Natural killer cell and CD8⁺ T cell effector function.—NK cells and CD8⁺ T cells are effector immune cells and mediate tumour killing. Tumour autonomous autophagy, induced by hypoxia, has been shown to degrade NK cell-derived granzyme B and weaken NK cell-mediated tumour lysis in B16-F10 and 4T1 mouse tumour models. Inhibition of autophagy restored the levels of granzyme B, recovered NK cell cytotoxicity and prevented tumour progression⁸². Similarly, hypoxia-induced tumour autophagy in IGR-Heu lung carcinoma cells has been shown to regulate tumour cell STAT3 phosphorylation and disable tumour cell sensitivity to CTL-mediated killing⁸³. Therefore, hypoxia-induced autophagy can attenuate the antitumour effector function of NK cells and CTLs (FIG. 2c). In line with this, a genome-wide in vitro clustered regularly interspaced short palindromic repeats (CRISPR) screen across a panel of genetically diverse mouse cancer cell lines has shown

that the autophagy pathway is a conserved mediator of tumour resistance to cytotoxicity induced by CTLs and the effector cytokines IFN γ and TNF. Thus, activated tumour autophagy may be a cancer cell-intrinsic immune evasion mechanism in the TME⁸⁴.

CTL-mediated tumour ferroptosis.—Ferroptosis is a form of necrotic cell death caused by uncontrolled peroxidation of phospholipid membranes via an iron-dependent mechanism⁸⁵. Nuclear receptor coactivator 4 (NCOA4) plays a role in degradation of ferritin (cellular iron storage protein) and supports cell ferroptosis. Blockade of autophagy or knockdown of NCOA4 abrogates the accumulation of ferroptosis-associated cellular labile iron and ROS, and prevents eventual ferroptotic cell death^{86,87}. Tumour ferroptosis is a critical mechanism by which CD8⁺ T cells mediate tumour killing in tumour immunotherapy⁸⁸ and radiotherapy⁸⁹. Thus, autophagy activation may potentiate tumour ferroptosis and sensitize tumour immunotherapy.

Altogether, it seems that tumour autonomous autophagy not only supports tumour cell survival and proliferation but also alters tumour antigen presentation, immune cell trafficking and effector immune cell function in the TME.

Autophagy in tumour-infiltrating immune cells

T cells

Human tumour-infiltrating immune cells — including effector CD8⁺ T cells, regulatory T cells (T_{reg} cells) and myeloid APCs^{90–92} — affect tumour progression and therapeutic response^{8,10}. Apart from tumour cells, multiple stress signals affect the autophagy pathway in immune cell subsets, thereby shaping their survival, activation, differentiation and function in the TME¹¹.

Effector T cell subsets.—CD8⁺ T cells recognize tumour-associated antigens (TAAs) and mediate tumour killing. CD4⁺ T helper cells (T_H cells) help dendritic cells prime and activate CD8⁺ T cells. However, T cells (particularly naive T cells) in the tumour draining lymph nodes and TME undergo apoptosis in tumour-bearing mice and patients with cancer. It has been shown that tumour-infiltrating T cells in tumour-bearing mice manifest impaired autophagy along with a selectively reduced expression of FIP200 (which is essential for autophagosome formation)⁹³. Mechanistically, tumour-derived lactate inhibited FIP200 expression and caused T cell apoptosis by disabling the balance between pro-apoptotic and anti-apoptotic BCL2 family members, thereby supporting tumour immune evasion⁹³.

In addition to T cell survival and apoptosis, autophagy is also involved in the regulation of T cell activation, differentiation, stemness and effector function. After TCR engagement in vitro, autophagy in CD8⁺ T cells is induced, leading to degradation of BCL10 and protecting the cells from adverse consequences of unrestrained NF- κ B activation⁹⁴.

Phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3; also known as VPS34) is an early key player in autophagy. *Pik3c3*-deficient T cells exhibit reduced levels of active mitochondria compared with wild-type controls and fail to differentiate into effector T cells upon T cell activation⁹⁵. Autophagy-deficient effector CD4⁺ T cells with *Atg7* depletion express minimal levels of IL-2 and IFN γ ⁹⁶. Human liver-resident memory CD8⁺ T cells

manifest increased basal autophagy compared with CD8⁺ T cells in blood, which is critical for their proliferation and cytokine production. Blocking autophagy in IL-15-induced resident memory CD8⁺ T cells by MRT68921 dihydrochloride and 3-methyladenine (3MA) results in the accumulation of depolarized mitochondria, a feature of exhausted T cells⁹⁷.

Furthermore, autophagy can regulate T cell metabolism to control T cell phenotypes. In the steady state, naive T cells largely rely on oxidative phosphorylation. Upon antigen stimulation, T cells undergo a metabolic transition from oxidative phosphorylation to glycolysis to support cellular proliferation and effector function. During memory formation, effector T cells revert to oxidative phosphorylation⁹⁸. Although speculative, autophagy might be involved in promoting fatty acid β -oxidation during memory formation. This is because autophagy can contribute to the degradation of lipid droplets⁹⁹, which can in turn support CD8⁺ T cell memory generation¹⁰⁰. Autophagy-deficient effector CD8⁺ T cells are unable to establish effective memory to provide long lasting antiviral immunity^{101,102}. Also, a persistent loss of mitochondrial function and mass has been observed in dysfunctional tumour-infiltrating CD8⁺ T cells¹⁰³. In addition, decreased mitophagy leads to accumulated depolarized mitochondria in both human and mouse melanoma-infiltrating CD8⁺ T cells. These T cells display functional, transcriptomic and epigenetic characteristics of terminally exhausted T cells¹⁰⁴. Hence, it is likely that a suitable level of autophagy is important for CD8⁺ T cell survival, differentiation and function.

Interestingly, it has been reported that potassium in the TME reduces the uptake and consumption of local nutrients in tumour-infiltrating T cells, which induces autophagy, enhances mitochondrially dominant metabolism and increases the depletion of intracellular acetyl-coenzyme A (AcCoA). Consumption of intracellular AcCoA in mitochondrial metabolism results in reduced histone acetylation on the promoters and enhancers of genes encoding effector molecules. Consequently, this causes impaired CD8⁺ T cell effector function, but sustains T cell stemness¹⁰⁵. Blocking autophagy by disrupting *Atg7* in potassium-treated T cells reduces T cell stemness, which is required for CD8⁺ T cell persistence in vivo, multipotency and tumour clearance¹⁰⁵. However, it has also been shown that loss of ATG5 in T cells increases the number of tumour-infiltrating IL-9-expressing CD4⁺ T cells (T_H9 cells)¹⁰⁶, and CD8⁺ T cells isolated from *Atg5*-deficient mice show a profound shift to an effector memory phenotype compared with *Atg5*-proficient CD8⁺ T cells in tumour-bearing mice¹⁰⁷. Thus, a balanced autophagy pathway shapes T cell immunity and alters tumour immune responses (FIG. 3a).

Of note, TAA-specific T cell activation is dependent on antigen processing and presentation. Autophagy is involved in the regulation of antigen processing and presentation in dendritic cells¹⁰⁸. Efficient dendritic cell-mediated TAA presentation requires lysosomal proteolysis through autophagy¹⁰⁹. Genetic knockout of *Pik3c3* in dendritic cells results in a decrease of CD8 α ⁺ dendritic cells, impaired B16 melanoma-specific CTL activity and an increased incidence of lung metastasis in mice compared with controls⁶⁹. In contrast to dendritic cells, MDSCs directly suppress T cell activation. MDSCs accumulate in the human cancer microenvironment, suppress antitumour immune responses, promote tumour progression and hinder cancer therapeutic efficacy^{110,111}. MDSCs in patients with cancer exhibit increased levels of autophagy¹¹², which may be induced by hypoxia¹¹³ and HMGB1 (REF.¹¹²).

Ablation of autophagy in mono-cytic MDSCs results in elevated MHC class II expression, reduced suppressor activity and weakened tumour growth in a melanoma mouse model compared with controls¹¹⁴.

Regulatory T cell subsets.—CD4⁺ T_{reg} cell-mediated suppression of antitumour immunity is a major mechanism of tumour immune evasion and immunotherapy resistance^{92,115,116}. The autophagy pathway has been shown to be involved in T_{reg} cell lineage commitment, thereby affecting antitumour immunity¹¹⁷. Indeed, specific deletion of genes encoding autophagy components, including ATG5, ATG7 and AMBRA1 (REFS^{118,119}) in T_{reg} cells, leads to T cell apoptosis and dysfunction in mice^{120–122}. On the molecular level, autophagy-deficient T_{reg} cells have been shown to exhibit high levels of the mTORC1–MYC pathway and glycolytic activity¹²¹. In line with this, human melanoma-infiltrating T_{reg} cells expressed high levels of arginase 2 (ARG2), which resulted in degradation of intracellular arginine and attenuation of arginine-mediated mTOR activation, thereby potentially leading to autophagy activation¹²³. As a result of this interplay of metabolic and autophagy processes, T_{reg} cell survival and function were enhanced in the TME^{122,123} (FIG. 3a).

Macrophages

Tumour-associated macrophages (TAMs) include peripheral migratory macrophages and long-lived, embryonic-derived and self-maintained residential macrophages¹²⁴. Macrophages take up and process dead tumour cells, and present TAAs to T cells. When a cell dies, it is rapidly cleared by phagocytosis, which induces LAP in macrophages¹²⁵. Clearance of apoptotic cells by macrophages through LAP induces anti-inflammatory cytokine production. However, when LAP is defective, such clearance induces the production of inflammatory cytokines and type I interferons^{125–127}.

In the TME, death of cells and their clearance by TAMs are common events, and LAP can readily be detected in TAMs¹²⁷. When LAP (but not canonical autophagy) was disrupted in TAMs by genetic depletion of various key components, antitumour T cell activity restricted Lewis lung carcinoma and B16-F10 tumour growth; this immunity was dependent on stimulator of interferon genes (STING; also known as TMEM173)-dependent type I interferon production¹²⁷. As discussed above, disruption of autophagy in the TME has been shown to engage antitumour immunity; however, as components of autophagy and LAP are shared, it remains possible that it is LAP rather than autophagy that mediates these effects. This distinction may be particularly important in settings where lysosomal function is inhibited with chloroquine (CQ) or bafilomycin A1 as an adjunct to cancer therapy. Although such treatments block autophagic flux, they do not interfere with LC3 lipidation. Indeed, they are associated with accumulation of autophagosomes. By contrast, inhibition of the lysosomal V-ATPase blocks the lipidation of LC3 proteins at a single membrane, including the phagosome in LAP¹²⁸. The ability of CQ treatment to improve anticancer immunity in preclinical and clinical settings might therefore be an effect of inhibiting LAP. LAP takes part in the MHC class II-mediated presentation of phagocytosed fungal antigens in human macrophages¹²⁹. LAP in macrophages is required not only for efficient clearance of dead cells but also for determining their anti-inflammatory phenotype^{125,127}. In line with

this, defects in LAP induce pro-inflammatory gene expression, trigger STING-mediated type I interferon responses in TAMs and promote T cell-mediated antitumour immunity¹²⁷ (FIG. 3b). Thus, non-canonical autophagy in TAMs may contribute to immune suppression in the TME.

Several human cancers, including ovarian cancer, often metastasize to the peritoneal cavity. The peritoneal cavity spaces contain a distinct population of cavity-resident macrophages. Deficiency of LAP-related components, such as Rubicon and ATG7, does not induce interferon responses in peritoneal residential macrophages¹³⁰. Interestingly, loss of FIP200 in macrophages inhibits peritoneal ovarian cancer progression and results in a potent antitumour T cell response in an ID8 ovarian cancer-bearing mouse model. It appears that genetic knockout of *Fip200* in macrophages causes high apoptosis of T cell immunoglobulin and mucin domain-containing molecule 4 (TIM4)-positive peritoneal residential macrophages in tumour-bearing mice due to the accumulation of mitochondria-related ROS. Relative to TIM4⁻ TAMs, TIM4⁺ TAMs manifest high oxidative phosphorylation and adapt mitophagy to alleviate oxidative stress. Due to low arginine resultant from ARG1-mediated metabolism, high levels of ARG1 in TIM4⁺ TAMs contribute to potent mitophagy activities via weakened mTORC1 activation¹³¹. TIM4⁺ residential TAMs inhibit T cell function and promote tumour cavity metastasis¹³¹ (FIG. 3c). Thus, autophagy activation in tumour-associated residential macrophages supports TAM survival and enforces immunosuppression in the TME.

More recently, it has been recognized that LC3 proteins can also be lipidated at endosomal membranes when cell surface receptors are internalized — a process called LANDO⁷. LANDO functions in the recycling of endosomes from the cytosol to the plasma membrane and, similarly to LAP, also represses inflammatory cytokine production. To date, the molecules involved in LAP and LANDO are the same; therefore, it remains possible that the disruption of LANDO, rather than of LAP, contributes to antitumour immunity.

In summary, both canonical and non-canonical autophagy pathways participate in regulation of the tumour immune response in the TME. Further studies are essential to understand the distinct roles of these two autophagy pathways and their potential crosstalk in the context of tumour immunity and immunotherapy.

Autophagy in cancer therapy

Indirect modulation of tumour immunity

Treatment of cancer in a patient often includes cancer therapies such as chemotherapy, radiotherapy and/or immunotherapy. These therapies may take advantage of the immune system to eliminate tumour cells. In certain cancer therapies, autophagy is indispensable for the modulation of tumour immunity — which suggests that targeting autophagy in combination potentially improves these cancer therapies.

Re-establishing latent immune surveillance is important for inducing an antitumour response. The basis for tumour immune evasion is loss of immunogenicity. Immunogenic cell death (ICD), induced by some cancer therapies, renders tumours immunogenic and

triggers antitumour immune responses. In response to ICD inducers, such as mitoxantrone and oxaliplatin, tumour autophagy was activated in mice bearing subcutaneous CT26 colon tumours, accompanied with increased tumour-infiltrating dendritic cells and T cells¹³². However, it remains unclear whether autophagy is directly induced by ICD inducers or is a consequence of ROS-based endoplasmic reticulum stress in the context of ICD in vivo¹³³. Upon ICD, intact autophagosomes containing multiple tumour antigens and DAMPs from dying cells are released to the extracellular space and are taken up by APCs to initiate T cell immunity³². In addition, autophagy in CT26 cells was required for the preservation of lysosomal ATP stores and was related to the secretion of ATP during mitoxantrone treatment¹³². Extracellular ATP can function as a 'find me' signal to recruit APCs to dying cells and stimulate IL-1 β production in APCs via NLRP3-mediated inflammasome activation, inducing antitumour adaptive immunity¹³⁴. In addition, depletion of *Atg5* or *Atg7* in CT26 cells reduced chemotherapy-elicited ATP release from dying tumour cells and inhibited antitumour immunity¹³². In a similar vein, cisplatin failed to trigger ICD in prostate cancer cells due to weak autophagy induction by cisplatin compared with oxaliplatin¹³⁵. Notably, dying B16-F10, MC38 or 3LL tumour cells released DAMPs in response to cisplatin, camptothecin or gemcitabine, thereby inducing TIM4 expression on tumour-associated myeloid cells. TIM4 mediated the phagocytosis of dying cells and activation of autophagy led to degradation of the ingested tumour cells, resulting in reduced antigen presentation and impaired CTL responses¹³⁶. Because TIM4⁺ resident macrophages can sequester effector T cells and prevent CTL-mediated tumour killing¹³⁷ (unpublished data), blocking the TIM4 and autophagy pathway with anti-TIM4 monoclonal antibody enhanced tumour antigen-specific CD8⁺ T cell infiltration and augmented the antitumour effect of chemotherapeutics¹³⁶. This effect of TIM4 blockade on ICD induction was dispensable and may be useful to treat certain tumours without response to the ICD inducer¹³⁶. Thus, autophagy can confer immunogenic properties to tumours in the context of some chemotherapies.

In addition to chemotherapy, radiotherapy can also induce ICD under specific doses and precise fractionation schedules. For instance, CT26 cells exposed to gamma-radiation or ultraviolet C light showed an increase in membrane calreticulin. Calreticulin serves as an 'eat me' signal for dying tumour cells¹³⁸ and is a sign of early ICD, which can promote phagocytosis of tumour cells by dendritic cells¹³⁹. Interestingly, multiple mouse and human tumour cells express high levels of stannocalcin 1, which blocks calreticulin membrane exposure and abrogates membrane calreticulin-directed phagocytosis by APCs, thereby impairing tumour immunity¹⁴⁰. Moreover, it has been reported that loss of autophagy in tumour cells reduces the efficacy of radiotherapy in immunocompetent mice, where autophagy-induced ATP released from dying tumour cells elicited an antitumour immune response during radiotherapy¹⁴¹. Hence, autophagy may be involved in the induction of tumour immunogenicity in radiotherapy.

Also in response to cancer targeted therapies, autophagy can modulate antitumour immune responses. CD47 on the tumour surface acts as a 'don't eat me' signal and prevents macrophage-mediated phagocytosis through its direct interaction with signal regulatory protein- α (SIRP α)¹⁴². SIRP α D1-Fc, a fusion protein comprising the first extracellular domain of human SIRP α and the Fc fragment of human IgG1, blocked the CD47-SIRP α

signalling pathway and induced autophagy in NSCLC cells. This led to a weakened macrophage-mediated phagocytosis of NSCLC cells and reduced SIRP α D1–Fc-mediated cytotoxicity against NSCLC cells. In line with the possibility that autophagy played a role in reducing cell clearing effectivity, simultaneous blockade of the CD47–SIRP α signalling and autophagy elicited potent antitumour effects in NSCLC xenograft models¹⁴³. In addition, sunitinib, a receptor protein-tyrosine kinase inhibitor, downregulated tumour PDL1 expression by inducing p62-mediated selective autophagy, and increased antitumour immunity in a phase III trial in patients with metastatic breast cancer¹⁴⁴. Thus, autophagy differentially modulates tumour immune responses in cancer therapy.

Targeting autophagy

Autophagy inhibitors are classified as early-stage inhibitors (SBI-0206965, 3MA and wortmannin), targeting ULK1/ULK2 or VPS34, and late-stage inhibitors (CQ, hydroxychloroquine (HCQ), bafilomycin A1 and monensin), targeting the lysosome. More specifically, CQ and HCQ inhibit autophagosome degradation via interfering with lysosome acidification. However, HCQ monotherapy failed to control tumour growth in patients with advanced pancreatic cancer in a clinical trial¹⁴⁵. Given the effects of autophagy inhibition on the immune response as outlined above, autophagy inhibition in combination with other cancer therapies has been considered in order to improve therapy efficacy. For example, patients with resectable pancreatic adenocarcinoma treated with HCQ in combination with the chemotherapeutic agents nab-paclitaxel and gemcitabine exhibited an increase in immune cell tumour infiltration¹⁴⁶. In addition, the combination of CQ and the mutated BRAF inhibitor vemurafenib improved tumour control in a patient with BRAF-mutant brain cancer who acquired resistance to vemurafenib¹⁴⁷. Furthermore, multiple clinical trials have explored the antitumour efficacy of autophagy inhibitors in combination with different treatments (TABLE 1). As hinted above, HCQ mediates a modest lysosomal inhibition in patients with cancer. To enhance the potential of lysosomal inhibition and the antitumour effect, new lysosomotropic agents, such as Lys05 (REF.¹⁴⁸) and DC661 (REFS^{149,150}), are being developed using the CQ structure as a base. These drugs target palmitoyl-protein thioesterase 1 (PPT1) and manifest high tumour inhibition in preclinical studies.

Chemotherapy.—High autophagic flux in cancer correlates with reduced response to chemotherapy and is associated with poor survival in patients with cancer¹⁵¹. Preclinical studies have shown that autophagy inhibition overcomes chemotherapy resistance in NSCLC^{143,152}, bladder cancer¹⁵³, thyroid cancer¹⁵⁴ and pancreatic cancer^{155,156}. The results of several studies suggest that autophagy inhibition might synergize with inhibition of MEK–ERK signalling. In pancreatic ductal adenocarcinoma, inhibition of MEK1 (also known as MAPKK1) and MEK2 by trametinib leads to activation of autophagy. Interestingly, inhibition of MEK1 and MEK2 combined with autophagy inhibition by either CQ or the dominant-negative form of ATG4B synergistically reduced pancreatic ductal adenocarcinoma cell growth in vitro and in vivo¹⁵⁵. In addition, inhibition of ERK in *KRAS*-driven human pancreatic ductal adenocarcinoma cells results in suppression of both glycolysis and mitochondrial function, and boosts autophagic activity¹⁵⁶. In melanoma with *BRAF* mutation, BRAF and MEK inhibitors induced ERK reactivation and autophagy (via ERK-mediated phosphorylation of ATF4), which promoted tumour resistance to these

inhibitors^{157,158}. Expression of a dominant-negative ATF4 mutant in melanoma disrupted autophagy and sensitized tumours to BRAF and MEK inhibitors *in vivo*¹⁵⁸. Therefore, autophagy inhibitors enhanced the ability of MEK, ERK and BRAF inhibitors to mediate antitumour activity^{155–158}. However, there are other mechanisms of autophagy-mediated resistance to chemotherapy that are being uncovered. For example, autophagy induced by *Fusobacterium nucleatum* can protect human colon cancer cells from chemotherapy-induced apoptosis. Autophagy activation and abundant *F. nucleatum* were detected in colorectal cancer tissues in patients with recurrence post chemotherapy¹⁵⁹. Therefore, novel clinical trials for chemotherapy will benefit from a comprehensive understanding of the nature of chemotherapy-induced autophagy.

Radiation therapy.—Autophagy plays a critical role in protecting tumour cells from cell death in response to radiation therapy. In breast cancer cells, radiation induced expression of autophagy-related genes, accompanied by accumulation of autophagosomes. Short-term inhibition of autophagy alongside radiotherapy led to enhanced cytotoxicity of radiotherapy in resistant cancer cells¹⁶⁰. Similarly, hypoxia enhances radioresistance in A549 lung cancer cells by inducing autophagy, which regulates ROS production *in vitro*¹⁶¹. In glioblastoma, radiation therapy induced autophagy by increasing mammalian STE20-like protein kinase 4 (MST4) expression, which stimulates autophagy through phosphorylation of ATG4B at site S383 in a panel of patient-derived glioma stem-like cells *in vitro*. The small-molecule inhibitor NSC185058 (targeting ATG4B), in combination with radiotherapy, impairs intracranial xenograft growth of glioblastoma and extends the survival of treated mice¹⁶². Therefore, targeting tumour autophagy potentially enhances the efficacy of radiation therapy. Indeed, the effect of autophagy inhibitors in combination with radiation therapy has been exploited in clinical trials in patients with cancer (TABLE 1).

Immunotherapy.—Harnessing the immune system provides an important approach to combatting cancer. Inhibition of autophagy may impair systemic immunity as autophagy is involved in immune system development¹¹ and effector T cell survival and function⁹³. However, systemic inhibition of autophagy by CQ in a short time did not impair T cell function in preclinical models of melanoma and breast cancer. The data suggest that the immune system may be tolerant to a certain intensity of autophagy inhibition¹⁶³. Yet, given that autophagy can regulate tumour immune response, targeting autophagy may promote the efficacy of immunotherapy and overcome immunotherapy resistance. For example, suppression of VPS34 kinase activity using its inhibitors SB02024 or SAR405 resulted in an increase in the levels of CCL5, CXCL10 and IFN γ in the TME, leading to high levels of NK cell and T cell tumour infiltration in models of melanoma and colorectal carcinoma. VPS34 inhibition also reversed either anti-PD1 or anti-PDL1 therapy resistance in these models¹⁶⁴. In addition, treatment with CQ blocked autophagy-mediated MHC class I degradation, synergized with dual ICB therapy (anti-PD1 and anti-CTLA4 antibodies) and resulted in an enhanced antitumour immune response in a mouse model of pancreatic cancer¹⁴. Moreover, photodynamic therapy-induced tumour immunogenicity is counteracted by ROS-induced autophagy, and can be enhanced by treatment with the autophagy inhibitor 3MA, also leading to reduced PDL1 expression, in a model of osteosarcoma¹⁶⁵. As an early immunotherapy regimen, high-dose IL-2 has been used to treat patients with renal cell

carcinoma and melanoma¹⁶⁶. High-dose IL-2 therapy shows an antitumour effect with broad stimulation of the immune system. However, high-dose IL-2 treatment can cause cytokine-induced systemic autophagic syndrome with increased serum levels of IFN γ , IL-6 and IL-18. Thus, pairing IL-2 with CQ limited toxicity and enhanced the immunotherapeutic efficacy of IL-2 in an advanced mouse model of metastatic liver cancer¹⁶⁷. Hence, targeting autophagy could potentially enhance immunotherapy (FIG. 4). Accordingly, clinical trials using HCQ in combination with immunotherapy are ongoing to treat patients with different types of cancer (TABLE 2).

In addition, CAR T cells are a type of immunotherapy that involves T cells extracted from patients and genetically modified to express receptors that recognize cancer-specific antigens, followed by transfusion¹⁶⁸. CAR T cell therapy has achieved clinical success in treating haematologic cancers, but failed in treating solid cancer¹⁶⁹. Autophagy modulation may offer several benefits for patients with cancer treated with CAR T cells. It is well known that the TME presents a barrier for CAR T cell infiltration and function in solid tumours. Given that autophagy inhibition could remodel the TME and promote T_H1-type chemokine production^{13,80}, autophagy inhibition may facilitate CAR T cell tumour trafficking. Metabolic stress induces effector T cell apoptosis and impairs their function in the TME¹⁵. Elevated autophagy in CAR T cells may support T cell fitness and survival in the TME. Thus, prior to transfusion to patients with cancer, enforcing expression of autophagy components in vitro in CAR T cells may be a potential approach to improve their therapeutic efficacy. Furthermore, CAR T cells directly recognize antigens on the tumour cell surface, which can be degraded via the autophagy pathway⁷⁷. Therefore, tumour autophagy inhibition may lead to increased expression of antigens, thereby enhancing CAR T cell-mediated tumour killing. Finally, CAR T cell therapy could cause cytokine release syndrome¹⁷⁰. Because autophagic cell death may be involved in cytokine release syndrome, autophagy inhibition may ameliorate cytokine release syndrome and generate clinical benefit for patients. Overall, the potential for autophagy inhibition to increase the efficacy of immunotherapy is a promising area of continuous exploration, which will hopefully see patients benefitting in the future.

Conclusion

Autophagy is an important mechanism studied in multiple cancer fields, including cancer biology, immunology and therapy. Given that autophagy can be induced by different factors in different cells in the TME, its induction and activation can both promote and inhibit tumour progression. Autophagy in T cell subsets may play a positive role in the antitumour immune response¹¹, whereas functional autophagy in tumour cells could support tumour antigen presentation and recognition, particularly in the setting of chemotherapy-induced ICD models¹³². In this context, inhibition of autophagy may be detrimental to antitumour immunity. By contrast, the autophagy pathway may be related to tumour cell survival, tumour antigen degradation, reduced T_H1-type chemokines and enhanced T_{reg} cells and MDSCs. Thus, systemically targeting autophagy as a cancer therapeutic approach is challenging.

This challenge may stem from multiple sources, such as our insufficient understanding of the different roles of the autophagy pathway in different tissues, organs and specific cells — including the immune system. For example, autophagy deficiency in the whole body, but not in specific immune cell subsets, leads to several systemic diseases including neurodegenerative disease, metabolic disease and cancer¹⁷¹. Liver-specific deletion of autophagy pathway components resulted in elevated circulating ARG1, causing a reduction of both serum and tumour arginine, slowing down arginine auxotroph tumour growth in a mouse model¹⁷² and inducing antitumour immunity in tumours with high mutational burden⁷³. In order to target the autophagy pathway in the context of cancer immunotherapy, it is paramount to explore the biological activities of autophagy in the major immune cell subsets. Recent studies have started to assess the autophagy pathway in T cells, macrophages and dendritic cells. Future work may be extended to B cells, NK cells and NKT cells¹⁷³. Additionally, following cancer progression, the autophagy pathway is dynamically altered in response to different stimuli in the TME. The role of autophagy has been largely studied in mouse models with specific genetic deletion of specific autophagy components. Therefore, developmental and functional compensation in murine models with specific genetic deficiency in autophagy components must be taken into account. Finally, it is well defined that autophagy degrades and recycles intracellular components to sustain metabolism and survival in response to stress signals, such as starvation. It is essential to understand how autophagy is simultaneously involved in fine-tuning the function and survival of immune cells, tumour cells and stromal cells in the TME, and whether systemic manipulation of the autophagy pathway eventually leads to increased clinical benefit in patients with cancer.

In fact, current clinical studies have found that systemically targeting tumour autophagy as a monotherapy has failed to yield reliable, objective responses in patients with cancer (TABLES 1 and 2). However, manipulation of autophagy remains an option in combinatorial therapy with conventional therapy and ICB (TABLES 1 and 2). Information on the tumour genetic status and immune infiltration profile may inform the optimal therapeutic combination. For example, if tumour genetic mutations result in autophagic adaptation, autophagy inhibition may potentially improve targeted therapy against specific mutated genes. As tumours with high genetic mutation burden may express relatively high levels of neoantigens alongside increased immune cell infiltration, compared with tumours with a low mutation burden, autophagy inhibition may maintain and boost the immune response via decreasing autophagy-mediated degradation of tumour antigen and MHC class I. Hence, immunotherapy in combination with autophagy inhibition may be a meaningful approach for tumours with a high mutation burden. Furthermore, autophagy manipulation in vitro and ex vivo through a dendritic cell-based cancer vaccine may be feasible. For example, inhibition of dendritic cell autophagy may enhance MHC class I-mediated tumour antigen presentation and T cell activation^{68,69}. Autophagosomes derived from dead tumour cells contain tumour antigens and may be utilized for dendritic cell vaccines to induce cross-presentation^{76,77}. Altogether, in order to pursue the next generation of autophagy modulators for cancer therapy, the immunological parameters should be considered. We expect that autophagy modulators can enhance tumour immunogenicity and antigen presentation, improve tumour-infiltrating T cell survival and function, and potentially disable the immunosuppressive

networks in the TME. It is hopeful that targeting the autophagy pathway will enhance and/or synergize cancer therapy, including immunotherapy^{83,136}.

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Glossary

Immunogenicity

The ability to provoke an immune response in humans or other animals

Chimeric antigen receptor T cells

(CAR T cells). Cells used in a type of cancer treatment in which the patient's T cells are modified in the laboratory to carry CAR and reinfused back into the patient to attack cancer cells recognized by CAR

Oxidative phosphorylation

The electron transfer chain driven by substrate oxidation that is coupled to the synthesis of ATP through an electrochemical transmembrane gradient

Autophagic flux

The measure of autophagic degradation activity

Reactive oxygen species

(roS). The highly reactive form of molecular oxygen formed as a by-product during the metabolism of oxygen

Damage-associated molecular patterns

(DAMPs). Host-derived molecules that can initiate a non-infectious inflammatory response

Epithelial–mesenchymal transition

A reversible cellular programme in which epithelial cells lose their cell polarity and cell–cell adhesion, gain migratory and invasive properties, and, finally, are transformed into mesenchymal cells

The Cancer Genome Atlas

(TCGA). A project to catalogue genetic mutations responsible for cancer that takes advantage of genome sequencing and bioinformatics

Senescence

A process by which cells irreversibly stop dividing and enter a state of permanent growth arrest without undergoing cell death

Clustered regularly interspaced short palindromic repeats

(CrisPr). A family of repetitive DNA sequence used to detect and destroy DNA

Glycolysis

The metabolic pathway that converts glucose into pyruvate independent of oxygen

Fatty acid β -oxidation

The catabolic process by which fatty acid molecules are broken down to generate acetyl-coenzyme A (AcCoA) and utilized in the tricarboxylic acid (TCA) cycle

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Box 1 | Overview of selective forms of autophagy in mammalian cells

Mitophagy is the selective degradation of damaged and aged mitochondria via autophagy. Mitophagy is triggered by a loss of electrical potential across the inner mitochondrial membrane (Ψ_m). although several mechanisms exist, the best understood involves the proteins phosphatase and tensin homologue (PteN)-induced kinase 1 (PiNK1) and the ϵ 3 ubiquitin ligase Parkin¹⁸⁹. when Ψ_m is disrupted, PiNK1 is stabilized at the mitochondrial outer membrane, where it phosphorylates ubiquitin. in turn, phospho-ubiquitin activates the function of Parkin to ubiquitinate outer mitochondrial membrane proteins, including Mitofusin ii. ubiquitinated Mitofusin ii is then targeted by adapters to bring the damaged mitochondria into a forming autophagosome.

Lipophagy targets lipid droplets and catabolizes their components into free fatty acids and glycerol by lysosomal acid lipase (LaL)⁹⁹. Lipophagy is induced by nutrient deprivation and signalling via mtOr complex 1 (mtOrC1) and 5'-aMP-activated protein kinase (aMPK) during lengthy fasting¹⁹⁰. soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (sNares), lipidate microtubule-associated protein 1a/1B light chain 3 (LC3), LaMP1, LaMP2B and LaMP2C as well as small GTPases (such as ras-related protein rab7a (REF.¹⁹¹)) are involved in the fusion of lipophagosome with the lysosome.

Endoplasmic reticulum-phagy is the selective clearance and degradation of endoplasmic reticulum components and membranes by the autophagic machinery. Six endoplasmic reticulum surface proteins have been identified as specific receptors through LC3/GaBaraP-interacting regions to recruit autophagy machinery in mammalian cells, including atlastin GTPase 3 (atL3)¹⁹², cell-cycle progression gene 1 (CCPG1)¹⁹³, family with sequence similarity 134 member B (FaM134B)¹⁹⁴, reticulon 3 long isoform (rtN3L)¹⁹⁵, translocation protein seC62 (seC62)¹⁹⁶ and testis-expressed protein 264 precursor (teX264)^{197,198}. These receptors function to fragment the endoplasmic reticulum into suitable pieces that can be readily incorporated into autophagosomes. the COPII subunit, seC24-related protein C (seC24C), acts with autophagy receptors to deliver endoplasmic reticulum sheets and tubules to lysosomes for degradation¹⁹⁹. Atlastin, an endoplasmic reticulum-resident GTPase, acts downstream of the endoplasmic reticulum-phagy receptors to remodel the endoplasmic reticulum membrane to separate pieces for efficient autophagosomal engulfment²⁰⁰.

Other forms of selective autophagy also exist in mammalian cells. Ferritinophagy is an alternative form of autophagy that selectively degrades ferritin to regulate iron homeostasis via nuclear receptor coactivator 4 (NCOA4)^{201,202}. Nuclear fragile X mental retardation-interacting protein 1 (NuFiP1) is a receptor for starvation-induced ribophagy, which interacts with ribosomes and delivers them to autophagosomes by directly binding to LC3B (REF.²⁰³). in the nucleus, nucleophagy mediates degradation of nuclear lamin B1 which interacts with LC3 in mammals. LC3 assists lamin B1 in transferring from nuclear to cytoplasmic and degrading in the lysosome following ras signalling²⁰⁴. Peroxisomes are targeted to the autophagosome by p62/sQstM1, which is defined as

pexophagy²⁰⁵. in addition, autophagy can regulate the rNa silencing machinery by selectively degrading DiCer and argonaute risC catalytic component 2 (aGO2)²⁰⁶.

Fig. 1 |. The machinery of canonical and non-canonical autophagy.

Canonical autophagy is normally induced in response to the cell undergoing an energy crisis. Autophagy-inducing signals, such as nutrient deprivation, trigger the activation of 5'-AMP-activated protein kinase (AMPK), whose kinase activity simultaneously inhibits mTOR and activates the pre-initiation complex (unc-51-like kinase 1 (ULK1), autophagy-related protein 13 (ATG13), ATG101 and FAK family interacting protein of 200 kDa (FIP200)). This complex, in turn, phosphorylates components in the autophagy initiation complex composed of vacuolar protein sorting 34 (VPS34), VPS15 and Beclin 1, along with ATG14 and AMBRA1. The initiation complex converts phosphoinositides in the endoplasmic reticulum to phosphatidylinositol-3-phosphate (PI3P), which recruits ligation machinery composed of the E1 ligase ATG7, the E2 ligase ATG3 and a complex of ATG16L1 and an ATG5–ATG12 conjugate that together act as an E3 ligase. The ligase machinery functions to ligate lipidate microtubule-associated protein 1A/1B light chain 3 (LC3) family proteins to phosphatidylethanolamine (PE) to form LC3–PE. The activity and coordination of the ATG5–ATG12 system and the LC3–PE system facilitate the curvature and sealing of the autophagosome, as well as the lipidation and embedding of LC3–PE into the autophagosomal membrane. The resulting autophagosomes then fuse to lysosomes (forming autolysosomes), resulting in digestion of the autophagosome contents loaded by the LC3 binding proteins. The non-canonical autophagy pathway also functions to lipidate LC3 family proteins on a single membrane. LC3-associated phagocytosis (LAP) and LC3-associated endocytosis (LANDO) process independently of the autophagy pre-initiation (ULK1) complex, and utilize a different initiation complex composed of VPS34, VPS15, Beclin 1, UVRAG and Rubicon without requirements for ATG14 or AMBRA1. mTORC1,

mTOR complex 1; ROS, reactive oxygen species; TLR, Toll-like receptor; WIPI2, WD repeat domain phosphoinositide-interacting protein 2.

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Fig. 2 | Impact of tumour autonomous autophagy on immunity.

a | Autophagy in dead tumour cells promotes dendritic cell (DC)-mediated cross-presentation via increasing production of autophagosome with tumour antigen. In addition, neighbour of BRCA1 gene 1 protein (NBR1) mediates major histocompatibility complex (MHC) class I degradation via the autophagy pathway, resulting in reduced MHC class I surface expression in pancreatic cancer cells and abolished cytotoxic T lymphocyte (CTL) recognition. **b** | Autophagy blockade or deficiency results in accumulated reactive oxygen species (ROS) in tumour cells and subsequent mitochondria and genome damage, thereby releasing DNA into cytoplasm. Absent in melanoma 2 (AIM2) senses cytosolic DNA, activates inflammasome signalling and promotes tumorigenesis via recruiting immunosuppressive immune cells. c-GAS–stimulator of interferon genes (STING) can also sense cytosolic DNA, stimulate type I interferon responses and enhance antitumour immunity via recruiting CD8⁺ T and natural killer (NK) cells. **c** | Hypoxia induces

autophagy in tumour cells through hypoxia-induced factor 1 α (HIF1 α). Elevated autophagy mediates degradation of granzyme B in tumour cells, which is secreted by activated CD8⁺ T cells and NK cells, and blocks CTL-mediated and NK cell-mediated tumour killing. Meanwhile, autophagy inhibits chemokine CCL5 expression, which recruits the NK cell migration to the tumour microenvironment (TME). MDSC, myeloid-derived suppressor cell; TAA, tumour-associated antigen; T_{reg} cell, regulatory T cell.

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Fig. 3 |. autophagy in immune cells in the tumour microenvironment.

a | In the tumour microenvironment (TME), T cells rely on autophagy to support their survival and differentiation. Basal levels of autophagy in naive T cells maintain their quiescence and protect them from mitochondria-derived reactive oxygen species (ROS)-induced apoptosis. High levels of lactate in tumours disrupt autophagy of naive T cells and impair the antitumour response in mouse models. High levels of potassium or low levels of nutrients in the TME constrain the nutrient uptake in effector T cells and cause functional caloric restriction, which induces autophagy through mTOR complex 1 (mTORC1) inhibition. Autophagy is critical for mitochondrial integrity, which determines effector T cell differentiation via metabolic reprogramming. High levels of arginase 2 (ARG2) expression in tumour-infiltrating regulatory T cells (T_{reg} cells) may maintain their high level of autophagy. The loss of autophagy in T_{reg} cells switches their metabolism from oxidative phosphorylation (OXPHOS) to glycolysis with active mTORC1 and MYC, which leads to

FOXP3 instability. Increased apoptosis and defective function in autophagy-deficient T_{reg} cells contribute to greater tumour resistance. **b** | Tumour-associated macrophages (TAMs) take advantage of lipidated microtubule-associated protein 1A/1B light chain 3 (LC3)-associated phagocytosis (LAP) to degrade apoptotic cancer cells. LAP deficiency in TAMs leads to the release of mitochondrial DNA from apoptotic cancer cells, which induces type I interferon response through the cGAS–stimulator of interferon genes (STING) pathway and antitumour activity. **c** | In ovarian cancer metastasis, $TIM4^{+}$ TAMs are embryonically originated and locally sustained, whereas $TIM4^{-}$ TAMs are replenished from circulating monocytes. Relative to $TIM4^{-}$ TAMs, $TIM4^{+}$ TAMs manifest high levels of oxidative phosphorylation and adapt mitophagy to alleviate oxidative stress. High levels of ARG1 in $TIM4^{+}$ TAMs contribute to potent mitophagy activities via weakened mTORC1 activation due to low arginine levels resulting from ARG1-mediated metabolism. Furthermore, genetic deficiency of autophagy element FAK family interacting protein of 200 kDa (FIP200) results in $TIM4^{+}$ TAM loss via ROS-mediated apoptosis, and elevated T cell immunity and tumour inhibition in vivo. ATG13, autophagy-related protein 13; BCL2, B cell lymphoma 2; MCT1, monocarboxylate transporter 1; TIM4, T cell immunoglobulin and mucin domain-containing molecule 4; ULK1, unc-51-like kinase 1.

Fig. 4 |. Targeting autophagy for tumour immunotherapy.

a | Targeting autophagy remodels the tumour microenvironment (TME). Tumour autophagy inhibition can upregulate production of the T helper 1 (T_H1)-type chemokines, promoting effector immune cell tumour infiltration. Targeting tumour autophagy may be combined with adoptive transfer of engineered chimeric antigen receptor T cells (CAR T cells) and immune checkpoint blockade (ICB). **b** | Autophagy can mediate degradation of potential tumour antigens and major histocompatibility complex (MHC) components in antigen-presenting cells (APCs). Autophagy inhibition can enhance the antitumour T cell response through elevated antigen processing and presentation. **c** | Autophagy can mediate degradation of MHC class I and granzyme B to avoid cytotoxic T lymphocyte (CTL)-mediated and natural killer (NK) cell-mediated killing. Autophagy inhibition can result in increased levels of MHC class I on the cell surface and prevents the autophagic degradation of granzyme B, thereby allowing cell lysis. Autophagy inhibition can enhance ICB therapeutic efficacy. **d** | High-dose interleukin-2 (IL-2) treatment causes systemic autophagic syndrome. Autophagy inhibition protects patients with cancer from systemic damage and improves IL-2 therapy. DAMP, damage-associated molecular pattern; DC, dendritic cell; TAM, tumour-associated macrophage; T_{reg} cell, regulatory T cell.

Table 1 |

Clinical trials targeting autophagy in cancer therapy

Tumour type	Autophagy inhibitor	Clinical trial phase	Combination	Clinical response	Side effects	Ref.
Brain metastases	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	174
GBM	CQ	III	Radiation therapy and temozolomide	Median survival: 33 months (controls: 11 months)	Increased seizure frequency	175
GBM	HQC	I/II	Radiation therapy and temozolomide	Median survival: 15.6 months	Neutropenia and thrombocytopenia	176
mCRPC	Pantoprazole	II	Docetaxel	Median OS: 15.7 months Median PFS: 5.3 months	Grade 3 or 4: fatigue, anaemia, febrile neutropenia, grade 4 neutropenia and anorexia	177
NSCLC	HQC	I/II	Carboplatin and paclitaxel	PFS: 3.3 months ORR: 33%	Neutropenia, neuropathy and anaemia	178
NSCLC	HQC	I	Erlotinib	1 PR; 4 SD ORR: 5%	Rash, nausea, diarrhoea and fatigue	179
PDAC	HQC	I/II	Gemcitabine	Decreased CA 19-9: 61% Median OS: 34.8 months (control: 10.83 months)	Grade 3: myelosuppression, hyponatraemia, elevated AST, hypoalbuminaemia, hyperbilirubinaemia rash and hyperglycaemia ileus	180
Pancreatic cancer	HQC	II	Gemcitabine, nab-paclitaxel	Improved Evans grade histopathologic responses	None	146
Pancreatic cancer	HQC	II	Gemcitabine, nab-paclitaxel	ORR: 38.2% (control: 21.1%)	Neutropenia, anaemia, fatigue, nausea, peripheral neuropathy, visual changes and neuropsychiatric symptoms	145
RCC	HQC	I/II	Everolimus	Disease control: 67% PR: 6% PFS 6 months: 45%	None	181
Refractory myeloma	HQC	I	Bortezomib	Very good PR: 14% Minor response: 14% SD: 45%	Grade 1 or 2: myelosuppression fatigue, peripheral neuropathy, nausea, vomiting, diarrhoea and constipation Grade 3 or 4: nausea, constipation, diarrhoea, anorexia, myelosuppression and fatigue	182
Refractory myeloma	Ricoinostat	I/II	Bortezomib and dexamethasone	ORR: 37% CBR: 39%	Thrombocytopenia, diarrhoea, anaemia, fatigue, nausea, hypokalaemia, vomiting, peripheral neuropathy, hyperglycaemia and renal insufficiency	183
Solid tumours	Pantoprazole	I	Doxorubicin	2 patients with confirmed PR	Fatigue, neutropenia and leukopenia	184
Solid tumours	HQC	I	MK-2206	Overall response: 15%	Fatigue and maculo-papular rashes	185
Solid tumours including melanoma	HQC	I	Temsirolimus	Solid tumours: SD (67%) Melanoma: SD (74%)	Grade 1 or 2: fatigue, anorexia, nausea, stomatitis, rash and weight loss Grade 3 or 4: anorexia, fatigue and nausea	186

Tumour type	Autophagy inhibitor	Clinical trial phase	Combination	Clinical response	Side effects	Ref.
Solid tumours including melanoma	HCQ	I	Temozolomide	Solid tumour patients: PR (10%) and SD (27%) Melanoma: PR (14%) and SD (27%)	Grade 2: fatigue, anorexia, nausea, constipation and diarrhoea	187
Solid tumours	HCQ	I	Vorinostat	1 patient with renal cell carcinoma: durable PR; 2 patients with colorectal cancer: prolonged SD	Grade 1–2: nausea, diarrhoea, fatigue, weight loss, anaemia and elevated creatinine Grade 3: fatigue and/or myelosuppression	188

CBR, clinical benefit rate; CQ, chloroquine; GBM, glioblastoma multiforme; HCQ, hydroxychloroquine; mCRPC, metastatic castration-resistant prostate cancer; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PDAC, pancreatic adenocarcinoma; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; SD, stable disease.

Table 2 |

Ongoing clinical trials of combinations with immunotherapy

Tumour type	Clinical trial phase	Treatment	Clinical trial identification number
Metastatic RCC	I/II	HCCQ/aldesleukin (IL-2)	NCT01550367
Metastatic melanoma	I/II	HCCQ/nivolumab HCCQ/nivolumab/ipilimumab	NCT04464759
Gastrointestinal cancer	I/II	HCCQ/cobimetinib/atezolizumab	NCT04214418
Pancreatic cancer	II	HCCQ/gemcitabine/nab-paclitaxel/avelumab	NCT03344172

Data are collected from US National Library of Medicine [ClinicalTrials.gov](https://clinicaltrials.gov). HCCQ, hydroxychloroquine; RCC, renal cell carcinoma.