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TITLE:
Lysosomes as a therapeutic target

ABSTRACT:
Lysosomes are membrane-bound organelles with roles in processes involved in degrading and recycling cellular waste, cellular signalling and energy metabolism. Defects in genes encoding lysosomal proteins cause lysosomal storage disorders, in which enzyme replacement therapy has proved successful. Growing evidence also implicates roles for lysosomal dysfunction in more common diseases including inflammatory and autoimmune disorders, neurodegenerative diseases, cancer and metabolic disorders. With a focus on lysosomal dysfunction in autoimmune disorders and neurodegenerative diseases — including lupus, rheumatoid arthritis, multiple sclerosis, Alzheimer disease and Parkinson disease — this Review critically analyses progress and opportunities for therapeutically targeting lysosomal proteins and processes, particularly with small molecules and peptide drugs.

Introduction:
Discovered in the 1950s by Christian de Duve, lysosomes are membrane-bound vesicles containing numerous hydrolytic enzymes that can break down biological polymers such as proteins, lipids, nucleic acids and polysaccharides1,2. Lysosomes have long been known to have a key role in the degradation and recycling of extracellular material via endocytosis and phagocytosis, and intracellular material via autophagy (reviewed elsewhere2–5) (Fig. 1). The products of lysosomal degradation through these processes can be trafficked to the Golgi apparatus for reuse or for release from the cell through lysosomal exocytosis, which is important in immune system processes. In addition, it has become clear more recently that lysosomes have an important role in other cellular processes including nutrient sensing and the control of energy metabolism3,5–7 (Fig. 1).
Alterations in lysosomal functions, either in the fusion processes involved in the general pathways mentioned above or related to the function of lysosomal enzymes and non-enzymatic proteins, can result in broad detrimental effects, including failure to clear potentially toxic cellular waste, inflammation, apoptosis and dysregulation of cellular signalling8. Such defects have been implicated in many diseases, ranging from rare lysosomal storage disorders (LSDs), which are caused by the dysfunction of particular lysosomal proteins, to more common autoimmune and neurodegenerative disorders5,9,10. Despite some limitations, impressive results have been achieved in treating several LSDs through enzyme replacement therapy (ERT). In addition, substantial efforts have been focused on therapeutically targeting the autophagy processes upstream of lysosomes11–14. However, there has so far been less attention on investigating the potential to directly target lysosomes with small molecules and peptide drugs.
Nevertheless, with recent advances in understanding of lysosomal function and dysfunction in diseases, promising novel opportunities for therapeutic intervention through targeting lysosomes specifically are beginning to emerge. This Review will provide a brief overview of lysosomal biogenesis, structure and function, and describe the role of lysosomal dysfunction in LSDs as well as other, more common diseases. Specifically, the article will focus on organ-specific and non-organ-specific autoimmune diseases, including lupus, rheumatoid arthritis (RA) and multiple sclerosis (MS), as these have not been extensively reviewed elsewhere, but will also briefly highlight neurodegenerative disorders such as Alzheimer disease (AD) and Parkinson disease (PD), to further illustrate the breadth and nature of the emerging therapeutic opportunities. The current ‘toolbox’ of pharmacological agents that modulate lysosomal functions and emerging novel targets and strategies in this set of indications will be highlighted. It should be noted that therapeutic approaches to treat inflammatory and autoimmune diseases aim to inhibit the deleterious excessive lysosomal activity, whereas lysosomal activation would be the goal in the treatment of neurodegenerative diseases. Although beyond the scope of this review, such approaches may have applications in other diseases in which lysosomes may play a role, including cancer, metabolic diseases and ageing (reviewed elsewhere15,16).

Lysosomal biogenesis, structure and function:

The formation of mature lysosomes is a complex process, which involves the fusion of late endosomes that contain material taken up at the cell surface with transport vesicles that bud from the trans-Golgi network5,8,17. These vesicles contain nearly 60 different hydrolytic enzymes (grouped into nucleases, proteases, phosphatases, lipases, sulfatases and others), which are synthesized in the endoplasmic reticulum and delivered to the transport vesicles via diverse systems, such as mannose-6-phosphate tags that are recognized by mannose-6-phosphate receptors (MPRs) at the membrane8,18 or glucocerebrosidase (GCase) that is transported to lysosomes by lysosomal integral membrane protein-2, an ubiquitously expressed type III transmembrane glycoprotein mainly located in endosomes and lysosomes19.

Mature lysosomes have an acidic internal pH, at which the lysosomal hydrolases are active, and a lining known as a glycocalyx that protects the internal lysosomal perimeter from the acidic environment of the lumen5,8,20. This acidic environment is maintained through the activity of a vacuolar-type proton adenosine triphosphatase (v-ATPase), which harnesses energy from hydrolysing ATP to drive the translocation of protons through a V0 membrane domain (reviewed elsewhere5,21). Other key lysosomal proteins include structural proteins such as lysosome-associated membrane protein 1 (LAMP1); proteins involved in trafficking and fusion, such as soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) and RAB GTPases; transporters such as LAMP2A, which has a key role in chaperone-mediated autophagy (CMA); and ion channels such as the chloride channel ClC7 and the cation channel mucolipin 1, a member of the transient receptor potential (TRP) family that is also known as TRPML1 (refs22,23). Most of the proteins are delivered through the clathrin adaptor protein 3-alkaline phosphatase (ALP) pathway, but some proteins are translocated through the lysosome-associated-protein transmembrane-5, a protein that is preferentially expressed in immune cells3,24.

Although the concept still remains controversial, two lysosome species — conventional or secretory — are often distinguished based on their physical, biochemical and functional properties. Catabolism is the main function of conventional lysosomes, and several other lysosome-related organelles (LROs), such as melanosomes, the late endosomal major histocompatibility complex class II (MHCII) compartment (MIIC), lytic granules from neutrophils, eosinophils, basophils, mast cells, CD8+ T cells and platelets, complement these functions8,25–29. Many of the LROs act as professional secretory organelles. LROs share with lysosomes the majority of typical characteristics (acidic environment, lysosomal transmembrane proteins, fusion property to phagosomes and others), in addition to particular properties resulting from their specific cargoes (for example, melanosomes contain melanosome-specific transmembrane glycoprotein, and natural killer cells and CD8+ T cells contain perforins and granzymes). The detailed mechanisms of biogenesis and secretion of LROs remain unclear, although it is known that genetic defects in LROs are involved in rare autosomal recessive disorders characterized by reduced pigmentation, such as Chediak–Higashi disease and Hermansky–Pudlak syndrome30. Secretory lysosomes contain many more proteins in addition to those contained in conventional lysosomes, and they participate in multiple cell functions such as plasma membrane repair, tissue and bone regeneration, apoptotic cell death, cholesterol homeostasis, pathogen defence and cell signalling8.

Lysosomal biogenesis and function are regulated by the basic helix–loop–helix leucine zipper transcription factor EB (TFEB) and the coordinated lysosomal expression and regulation (CLEAR) network4,31,32 (Fig. 2). For example, autophagy, a crucial process in immunity and autoimmunity33, is transcriptionally regulated by TFEB31. Interestingly, lysosomal exocytosis, which is important in many immune functions, also depends on TFEB activation31,32. Moreover, it has been demonstrated that TFEB orchestrates lysosomal Ca2+ signalling34. The fact that multiple lysosomal processes are dependent on TFEB activation strengthens its role as a master regulator in lysosomal functions. Like other transcription factors, TFEB undergoes phosphorylation and dephosphorylation via different cytosolic and lysosomal pathways (Fig. 2), processes regulated by mechanistic target of rapamycin complex 1 (mTORC1), a master controller of cell growth35,36.

Lysosomes are at the crossroads of various degradative pathways, including endocytosis (phagocytosis) and autophagy (Fig. 1). Three main forms of autophagy have been described: macroautophagy (the most extensively characterized form), microautophagy and CMA. At the initiation of macroautophagy, a double-membrane sequestering compartment termed the phagophore, which contains cytoplasmic material, is formed and matures into a vesicle called the autophagosome. The cargo is degraded into vacuoles issued from the fusion of autophagic vesicles and lysosomes (called autolysosomes), and the resulting short products are released back into the cytosol for reuse or, according to sometimes contested observations, possibly

dispatched into the MIIC for ultimate processing and MHCII molecule loading for presentation to CD4+ T cells37,38. In contrast to macroautophagy, microautophagy is characterized by direct lysosomal engulfment of cytosolic material into lysosomes, via the formation of characteristic invaginations of the lysosomal membrane. The third major form of autophagy is CMA, which involves the recognition of substrate proteins containing a KFERQ-like motif by a HSPA8/HSC70-containing complex (Fig. 1b). In CMA, two proteins have a key role: HSPA8 ensures the selectivity of proteins, which will be degraded via the CMA pathway; and LAMP2A translocates the targeted cytosolic proteins across the lysosomal membrane (reviewed elsewhere7). The terminal step of autophagy is called autophagic lysosome reformation, in which tubular proto-lysosomes are extruded from autolysosomes (containing lysosomal membrane components) and mature into functional lysosomes39. This step is not solely a lysosomal biogenesis process; it also includes a series of elements that are tightly correlated with the regulation of autophagy40.
In combination with autophagy, lysosomes are involved in both innate and adaptive immune functions, including foreign material recognition (bacterial, parasitic and viral), activation of pattern recognition receptors (such as Toll-like receptors (TLRs) and nucleotide oligomerization domain-like receptor), antigen processing and presentation, especially in the context of MHCII molecules, T cell homeostasis, antibody production and induction of various immune signals (co-stimulation and cytokine secretion)41. Besides being a degradative organelle, the lysosome has recently been recognized as a cellular signalling platform3,42. It plays an important role in nutrient sensing through mTORC1 and other additional protein complexes, or the so-called ‘lysosome nutrient sensing machinery’. The discovery of a stress-induced lysosome-to-nucleus signalling mechanism through TFEB further supports the key role of lysosomes in cellular signalling36.

Lysosomal storage disorders ::: Lysosome dysfunction in diseases:
LSDs are a heterogeneous group of about 50 inherited metabolic disorders, which have an incidence of ~1 in 5,000 live births44. These disorders and their treatment have been reviewed extensively elsewhere45,46, and so will only be covered relatively briefly here. The mutations responsible for most LSDs have been largely elucidated (Tables 1,2), and many result in the dysfunction of a particular lysosomal hydrolase, leading to the accumulation of the substrate of that hydrolase. For example, in Gaucher disease, the sphingolipid glucocerebroside accumulates in cells (particularly macrophages) and organs, including the liver and spleen, owing to deficiency in the enzyme GCase24,66. In certain LSDs, the resultant pathology can be explained by the nature of molecules that accumulate (Tables 1,2). Thus, the abundance of cerebrosides and gangliosides that deposit in the central nervous system (CNS) of patients with sphingolipid storage disorders, such as type II (acute infantile neuronopathic) Gaucher disease, underlies the severe neurological symptoms of such disorders67,68. In patients with Pompe disease, which is caused by α-glucosidase deficiency, the high levels of non-degraded glycogen that accumulate in muscles could explain the observed myopathy69,70. However, how the undegraded material accumulates and causes the observed cellular and organ pathology in many other LSDs remains unclear.
The accumulation of such undigested macromolecules or monomers in LSDs instigates the formation of secondary products, which ultimately escape from the endosomal–autophagic–lysosomal pathways9,71 and lead to multiple consequences that affect most organs, including the brain, liver, spleen, heart, eyes, muscles and bone (Table 2). Most, if not all, organelles are altered in LSDs, including endosomes, autophagosomes and lysosomes, and their functions in lysosomal formation/reformation and fusion of endosomes or autophagosomes to lysosomes are abnormal. Alterations in several autophagy processes have also been described in LSDs. Thus, deregulated mitophagy, which results in the accumulation of damaged mitochondria, occurs in LSDs, leading to major inflammatory consequences in specific tissues67,72. Perturbations in mitochondrial dynamics are frequently observed, which have been linked to the increased production of reactive oxygen species, ATP production and Ca2+ imbalance. In LSDs, reduced macroautophagy activity (with a decreased autophagic flux) rather than hyperactive autophagy processes, as seen in numerous autoimmune diseases, seems to be responsible for the accumulation of non-degraded cytoplasmic proteins such as α-synuclein, huntingtin (HTT) and others73. Mucolipidosis type IV (Table 2), a disease characterized by severe neurological and ophthalmological abnormalities, is caused by mutations in the MCOLN1 gene and is inherited in an autosomal recessive manner. This gene encodes a non-selective cation channel, mucolipin 1, which has recently been shown to be required for efficient fusion of both late endosomes and autophagosomes with lysosomes74,75. Impaired autophagosome degradation results in the accumulation of autophagosomes in LSDs76. Microautophagy processes that do not involve de novo synthesis of

nascent vacuoles also appear to be impaired in LSDs, and were notably revealed in primary myoblasts from patients with the muscle-wasting condition Pompe disease77. Finally, defective CMA components, such as LAMP2A, could also lead to lysosomal dysfunction. For example, mutations in the LAMP2 gene have been claimed to cause Danon disease (inherited in an X-linked dominant pattern)51. Further investigations are needed to support this assertion.

Autoimmune disorders ::: Lysosome dysfunction in diseases:
Lysosomes are involved in pathways central to the immune system, including the degradation of intracellular and extracellular material, plasma membrane repair, cell death signalling, cell homeostasis and death. Although the direct involvement of lysosomes in immunity is far from fully understood, it has long been expected that lysosome dysfunction will have a major impact in immune diseases (Table 2). Strikingly, however, this field has not been extensively explored. However, elevated levels of lysosomal enzyme activity have been reported to occur in several autoimmune diseases, such as RA, systemic lupus erythematosus (SLE), dermatomyositis and psoriasis3,14,17,18,20–23.
As discussed, autophagosomes formed during the autophagy process must fuse with lysosomes to generate peptide epitopes for further processing, clear possibly deleterious apoptotic debris, fuel the amino acid pool and produce energy (Fig. 1). Any deviation in this complex processing will affect crucial immune cell functions, such as the control of cytokine release, autoimmune cell anergy and programmed cell death of type I (apoptosis) and type II (autophagy). Secretory lysosomes regulate the release of both pro-inflammatory and anti-inflammatory cytokines, in a process that is dependent on the type of stimulation. In addition, lysosomes degrade glucocorticoid receptors, which are essential to bind glucocorticoids, although the reasons are not known78. In this complex system, lysosomes execute anti-inflammatory action via the phospholipase A2 and cyclooxygenase-2 pathways, and also induce inflammation through the IL-1β–caspase-1 pathway. In both conditions (pro-inflammatory and anti-inflammatory), lysosomes act as indirect precursors for autoimmunity. However, induction and suppression of inflammatory signals are stimulus dependent78.
Lysosomal cathepsins have a central role in degrading biological macromolecules in the lysosomes and in the immune response. There are approximately 12 members in this large protease family, most of which are endopeptidases that can cleave peptide bonds of their protein substrates79,80. Cathepsins A and G are serine proteases, cathepsins D and E are aspartic proteases and cathepsins B, C, F, H, K, L, O, S, V, X and W are cysteine proteases. For example, cathepsin S is responsible for the degradation of antigens (and autoantigens) in antigen-presenting cells (dendritic cells, macrophages and B cells), and is therefore involved at an upstream level in the presentation of MHCII–(auto)antigenic peptide complexes to CD4+ T cells81. Cathepsin L preferentially cleaves peptide bonds with aromatic residues in the P2 position and hydrophobic residues in the P3 position. It is central in antigen processing, bone resorption, tumour invasion and metastasis, and turnover of intracellular and secreted proteins involved in growth regulation. Cathepsin L-deficient mice display less adipose tissue, lower serum glucose and insulin levels, more insulin receptor subunits, more glucose transporter type 4 and more fibronectin than wild-type controls82. Cathepsin G is primarily known for its function in killing and digestion of engulfed pathogens83. It is also involved in connective tissue remodelling at sites of inflammation84. Anti-neutrophil cytoplasmic antibodies reacting with cathepsin G have been identified in some patients with SLE85.

Lupus ::: Lysosome dysfunction in diseases:
Abnormal antigen processing and presentation is known to be one of the upstream events that perturb immune responses in SLE86. Because this process is mediated through lysosomes, it was rational to speculate that lysosomal functions could be altered in lupus. Interestingly, hypotheses were raised in the 1960s on the ‘lysosomal fragility’ in lupus, but without much further pursuit87. The composition and fluidity of the lysosomal membrane are effectively crucial in the regulation of lysosomal fusion with other vesicular organelles and for lysosomal uptake of macromolecules. The integrity of the lysosomal membrane also ensures the prevention of release of lysosomal enzymes into the cytoplasm. Some lysosomal enzymes released from ‘fragile’ lysosomes were regarded potentially harmful in lupus88.
Lysosomes are abnormal in splenic B cells from Fas-deficient Murphy Roths Large (MRL)/lpr mice, a mouse model of lupus, compared with B cells from healthy CBA/J mice89. TFEB expression was increased, indicating an enhanced biogenesis of lysosomes, and the lysosomal volume was raised. The expression levels of LAMP1 and cathepsin D were also increased. These

results reinforce previous data showing that the expression and activity of some lysosomal enzymes (such as cathepsins S, L and B) that play important roles in antigen processing are altered in lupus and other autoimmune diseases90,91.
Substantial variations of the acidic endo-lysosomal pH also occur in MRL/lpr mice, being raised by 2 pH units in splenic B cells53,92. This pH change could dramatically influence the activity of soluble lysosomal hydrolases (such as cathepsins) as well as lysosomal membrane proteins (such as LAMPs) that are critical for lysosome activity. pH may also affect the elimination of immune complexes that accumulate in lupus as a result of deficits in complement, lower expression of scavenger receptors, increased expression of Fcγ receptors and other reasons93. These immune complexes, which contain non-selective IgG antibodies or autoantibodies associated with autoantigen (including some apoptotic debris), can initiate inflammation of tissues once deposited (for example, in the kidneys and the skin) and generate a cascade of deleterious effects, such as the release of harmful cytokines and chemokines54.
Recent studies have highlighted the key role of mammalian target of rapamycin complex 2 (mTORC2) in the disruption of lysosome acidification that occurs in this process94. In normal conditions, the regulation of lysosomal acidification requires cleavage of the RAB small GTPase RAB39a, occurring on the surface of phagocytic vesicles by locally activated caspase-194. This finely regulated process requires the association of cofilin with actin that surrounds the vesicle and recruits caspase-11, which then activates caspase-1 (ref.94). In lupus-prone macrophages, chronically active mTORC2 enhances cofilin phosphorylation, thereby hampering its association with actin and affecting the downstream cascade of events leading to the appropriate acidification of lysosomes94. The importance of mTORC1 and mTORC2 has been established earlier in lupus T cells, and in particular, in this context, mTORC1 activity was increased whereas mTORC2 activity was reduced95.
In addition, lysosomal cathepsin K was seen to contribute to the pathological events that develop in Faslpr mice, another model of lupus disease, in part through its activity in TLR-7 proteolytic processing and subsequent effects on regulatory T cells. Cathepsin K-deficiency in Faslpr mice reduced all kidney pathological manifestations (glomerulus and tubulointerstitial scores, glomerulus complement C3 fraction and IgG deposition, chemokine expression and macrophage infiltration) and decreased the levels of potentially pathogenic serum autoantibodies96.
In line with these internal alterations of lysosomes, notably those related to cathepsin functioning, deregulation of autophagy has been reported to contribute to lupus pathology92,97–100. Autophagy failures have been described in the lymphocytes of MRL/lpr mice and (NZBxNZW)F1 mice56,92,97,101 (two spontaneous murine models of systemic autoimmunity of distinct genetic origins and that display different MHC haplotypes) as well as in T and B lymphocytes of patients with SLE97,98,100. Murine and human T cells from the peripheral blood showed a significant accumulation of autophagic vacuoles compared with normal97. The underlying reasons for the dysfunctions in autophagy observed in lupus are not clearly understood, but several independent investigations have identified risk loci spanning autophagy-linked genes in patients with lupus102–106.

Sjögren's syndrome ::: Lysosome dysfunction in diseases:
Recent studies have demonstrated an increase in the level of macroautophagy in salivary gland T lymphocytes and in tears and conjunctival epithelial cells of patients with primary Sjögren's syndrome (SjS)107,108. Alteration of CMA activity was also recently found to occur in the salivary glands of MRL/lpr mice that develop a secondary SjS-like disease56. Lysosomes, which as discussed are mechanistically involved at the downstream level of both macroautophagy and CMA, were found to be altered in salivary glands. Flow cytometry analyses revealed that the mean pH of acidic vesicles in MRL/lpr salivary glands was significantly higher compared with those in mouse control glands and the ATP content was significantly diminished in MRL/lpr salivary gland cells56. Furthermore, amounts of several leukocyte glycosidases and proteases were revealed to be increased in leukocytes of patients with SjS in comparison with healthy controls55. Notably, raised levels of the lysosomal enzymes glucosidase, β-glucuronidase and dipeptidyl peptidase I are involved in the tissue injury in SjS55. Increased expression of lacrimal gland cathepsin S was also reported, which may have application as a diagnostic tool in SjS91. Two members of the RAS oncogene family, RAB3D and RAB27, were found to be implicated in the regulation of cathepsin S secretion levels in SjS109. In vitro studies on lacrimal gland acinar cells suggested further that secreted IFNγ from acinar cells increases cathepsin S expression and that IFNγ stimulated the MHCII-mediated antigen presentation in ocular pathogenesis of SjS110.

Rheumatoid arthritis ::: Lysosome dysfunction in diseases:
Lysosomal cathepsins have important roles in the induction and diagnosis of RA, and levels of several cathepsins (B, D, G, K, L and S) that are present in the serum and synovial fluid of patients have been proposed as a basis for RA diagnosis111–116. Cathepsin S and cathepsin L are highly expressed in synovial macrophages and thymic cortical cells. They each exert essential roles in the positive selection of T cells and antigen presentation, respectively, and participate in the local inflammation and matrix degradation that occurs in joints116. Cathepsin B is involved in collagen degradation, which leads to joint destruction in RA112,117. Expression of cathepsin G, which participates in joint inflammation through its chemoattractant activity, has been shown to be raised in the synovial fluid of patients with RA when compared with individuals with osteoarthritis115. Autoantibodies reacting with cathepsin G were also identified in patients with RA85. Compared with patients with osteoarthritis, cathepsin K expression was found to be elevated in RA113, and genetic deletion of this particular cathepsin was shown to reduce inflammation and bone erosion in RA conditions via TLR mediation118.

Neurological autoimmune diseases ::: Lysosome dysfunction in diseases:
MS, myasthenia gravis, Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP), neuromyelitis optica and neuropsychiatric lupus are neurological diseases induced by abnormal autoimmunity62,119–123. Neurological autoimmunity against various proteins, such as myelin in MS or N-methyl-d-aspartate receptor in neuropsychiatric lupus62,123,124, can affect various structures within the CNS and peripheral nervous system, with diverse consequences. Although the exact cause of amyotrophic lateral sclerosis (ALS) still remains unknown, studies support the existence of autoimmune mechanisms, and ALS is therefore also included in this section. Indeed, autoantibodies against ganglioside GM1 and GD1a, sulfoglucuronylparagloboside, neurofilament proteins, FAS/CD95 and voltage-gated Ca2+ channels have all been reported in patients with ALS (reviewed elsewhere125).
In general, the origin of the breakdown in immune tolerance that occurs in this set of neurological diseases is not known. Only recently have investigations discovered that autophagy processes are altered in some of these diseases59,62,126–130. In MS and in experimental autoimmune encephalomyelitis, an experimental model of MS, upregulation of the protein kinase mTOR has been described, and treatment with rapamycin/sirolimus (an immunosuppressant that inhibits mTOR and consequently stimulates macroautophagy) ameliorates some clinical and histological signs of the disease131. Increased levels of macroautophagy markers were measured in the blood and brain of patients with MS122,132. However, impaired macroautophagy was found in the spinal cord of experimental autoimmune encephalomyelitis mice133. In a rat model mimicking human CIDP, both macroautophagy and CMA processes were found to be hyperactivated in lymphatic system cells and non-neuronal cells (sciatic nerves) of peripheral nervous system cells59. In ALS, current data are conflicted62. Some data suggest an activation of macroautophagy processes with an accumulation of autophagosomes in brain tissues of patients with ALS, or an increase of autophagic vacuoles, aggregated ubiquitin and SOD1 proteins associated with MAP1LC3B-II in motor neurons of mice developing an ALS-like disease134,135. In contrast, other data suggest a reduction of autophagy activity136,137. Mutations in SQSTM1, valosin-containing protein, dynactin (a protein complex that activates the dynein motor protein, enabling intracellular transport) and RAB7 (a member of small GTPases that is important in the process of endosomes and autophagosomes maturation) have also been described in ALS138–141. Further studies are required to better understand the type and extent of autophagy dysfunction in this family of complex diseases.
There are only a few published studies on lysosomal dysfunction in neurological autoimmune diseases (Table 2). These notably include lysosome fragility, which was observed in patients with MS in the white matter of cerebral tissue, an area of the CNS that is mainly made up of myelinated axons142. Lysosome fragility was also suspected in SLE (see above) and other rheumatic autoimmune diseases, albeit in other organs53,58,92. As noted above, significant variations in lysosomal pH have been measured in autoimmune conditions such as lupus and SjS, but to our knowledge such studies conducted in the brain or elements of the peripheral nervous system of patients or animal models with neurological autoimmune diseases have not been published78.
In CIDP, it has been shown that Schwann cells dedifferentiate into immature states and that these dedifferentiated cells activate lysosomal and proteasomal protein degradation systems143,144. Based on these observations, Schwann cells have been claimed to actively participate in demyelinating processes via this dedifferentiation process, but the mechanism involved remains undefined145. In the rat model of CIDP mentioned above, it was shown that LAMP2A expression

was drastically increased in the sciatic nerve macrophages and reduced macroautophagy was observed in Schwann cells and macrophages59.
In MS, studies conducted on white matter demonstrated that lysosomes are involved in myelin sheath degeneration as well as in the fragmented protein formation. Lysosomal swelling was observed near the degenerated materials of astrocytes146, and an accumulation of lipids was found60. It has been hypothesized that lysosomal swelling/permeabilization might cause the release of hydrolases in the cytosol, where they affect native proteins147.
In ALS, patients also show dysfunctions in the endo/lysosomal pathways, which affect both lower and upper motor neurons (Table 2). Cathepsin B was particularly found to be involved in the motor neuron degeneration, whereas cathepsins H, L and D were not significantly affected148. A cDNA microarray analysis on post-mortem spinal cord specimens of four sporadic patients with ALS revealed major changes in the expression of mRNA in 60 genes including increased expression of cathepsins B and D149. Several disease-causing mutations in genes related to autophagy have been identified, such as SOD1, TDP643, FUS, UBQLN2, OPTN, SQSTM1 and C9orf72 (refs61,150), but none of them code for lysosomal proteins. So, a crucial remaining issue is to clearly determine whether the lysosomal abnormalities that are observed are linked to intrinsic defaults of lysosomes or result from upstream dysregulation in autophagosome formation and fusion61,62,151.

Neurodegenerative disorders ::: Lysosome dysfunction in diseases:
Insufficient clearance of neurotoxic proteins by the autophagy–lysosomal network has been implicated in numerous neurodegenerative disorders152. In disorders such as AD, Huntington disease (HD) and PD, modified or misfolded proteins abnormally accumulate in specific regions of the brain. Accumulation of aggregated proteins is also seen in ALS (see above). These abnormal proteins form deposits in intracellular inclusions or extracellular aggregates, which are characteristic for each disease153–155. Although there has been substantial research in this field, it is still unclear why sophisticated ‘quality-control’ systems, such as the lysosome–autophagosome system in particular, fail in certain circumstances to protect the brain against such protein accumulation156.
In AD, one of the most common neurodegenerative disorders, some alterations in the endo/lysosomal pathways have been described (reviewed elsewhere157,158). The amyloid precursor protein (APP) is cleaved by β- and γ-secretases into amyloid-β peptide (Aβ) fragments, particularly Aβ40 and Aβ42 (ref.159). These fragments are found in the amyloid plaques that are one of the hallmarks of AD (the other being neurofibrillary tangles containing phosphorylated tau), and have been widely considered to have an important role in AD pathogenesis159,160. Cell-based experiments have demonstrated that lysosomal cathepsins have a role in the generation of Aβ peptides (through cathepsins D and E) and the degradation of Aβ peptides (by cathepsin B)161. Lysosomal dysfunction has been observed in patients with AD162,163, and accumulation of the Aβ42 fragment in neuronal cells was shown to lead to lysosomal membrane alterations, which cause neuronal cell death63. In this context, it is noteworthy that inhibition of cathepsin D, which is involved in the lysosomal dysfunction and notably in the cleavage of the tau protein into tangle-like fragments, diminishes its hyperphosphorylation in the brain of patients with AD164. In addition, patients with AD with an inherited form of the disease may carry mutations in the presenilin proteins (PSEN1 and PSEN2), APP or apolipoprotein E, resulting in increased production of the longer form of the Aβ fragment (reviewed elsewhere165). Mutation of PSEN1, for instance, leads to direct disruption of the lysosomal acidification due to impaired delivery of the V0A1 subunit of v-ATPase, a proton pump responsible for controlling the intracellular and extracellular pH of cells. The acidification deficit causes excessive release of lysosomal Ca^{2+} through TRPML1 channels, which has numerous deleterious effects166. These findings strongly support the hypothesis that dysfunction of endo/lysosomal pathways is pivotal in AD.
Approximately 15% of patients with PD have a family history of the disorder, although the underlying molecular mechanisms remain unclear. In the context of lysosomal dysfunction, it is notable that the most common of the known PD genetic mutations are in GBA1 (encoding the lysosomal β-GCAse) — the same gene that underlies Gaucher disease — which are present in up to 10% of patients with PD in the United States167. GBA1 mutations are also associated with dementia with Lewy bodies167. Several other genes linked to PD are directly or indirectly related to the endo/lysosomal machinery, such as mutations in SNCA (coding for α-synuclein)63,168. A hallmark of PD is the presence in neurons of protein inclusions called Lewy bodies, which are mainly composed of fibrillar α-synuclein. The α-synuclein protein is normally degraded by the lysosomes through the CMA pathway, but macro-aggregates of α-synuclein mutants, which

display a longer half-life compared with the non-aggregated wild-type protein, are not degraded by this pathway and, rather, would be degraded via the macroautophagy pathway169–172. It was further shown that the mutant proteins bind to LAMP2A and inhibit the translocation of other substrates and, therefore, their final degradation170. Biochemical analyses suggest that α-synuclein is mainly degraded by lysosomal proteases and notably by cathepsin D, rather than by non-lysosomal proteases (for example, calpain I)173,174. Accumulation of α-synuclein was observed in cathepsin D-deficient mice, whereas, conversely, the accumulation of α-synuclein aggregates was reduced in transgenic mice that overexpressed this cathepsin, resulting in protection of dopaminergic neuronal cells from damage175.

HD is a rare autosomal-dominant neurodegenerative disease caused by an aberrant expansion of CAG trinucleotide repeats within exon 1 of the HTT gene, which results in the production of aggregation-prone HTT mutants (mHTT) that are detrimental to neurons176,177. Whereas HTT has a protective role against neuronal apoptosis, accumulation of mHTT, however, induces pathophysiological consequences including lysosomal and autophagy dysfunctions. Thus, mHTT perturbs post-Golgi trafficking to lysosomal compartments by delocalizing the optineurin/RAB8 complex, which, in turn, affects lysosomal function177. Excessive mHTT induces accumulation of clathrin adaptor complex 1 in the Golgi and an increase of clathrin-coated vesicles in the vicinity of Golgi cisternae177. The activity of several cathepsins such as B, D, E, L and Z has also been linked to HD63,80,174,177–179. Cathepsin D is responsible for full degradation of HTT but is less efficient at degrading mHTT, which is processed by cathepsin L180,181. Cathepsin Z also cleaves HTT and elongated polyglutamine tracts182,183. Thus, lysosomal modulators acting on cathepsin activity might have beneficial effects in the treatment of HD. Notably, hyperexpression of cathepsin D (and cathepsin B) was shown to protect primary neurons against mHTT toxicity179. Alterations in macroautophagy, mitophagy and CMA have also been implicated in HD184,185. CMA activity was increased in response to macroautophagy failure in the early stages of HD186, a result supported by the findings that HSPA8 and LAMP2A have important roles in the clearance of HTT187 and that shRNA-mediated silencing of LAMP2A increased the aggregation of mHTT188. Other studies focusing on the HTT secretory pathway revealed that mHTT secretion is mediated by the Ca2+-dependent lysosomal exostosis mechanism via the synaptotagmin 7 sensor in neuro2A cells189. The extracellular release of mHTT was efficiently inhibited by the phosphoinositide 3-kinase and sphingomyelinase inhibitors Ly294002 and GW4869. HD-dependent perinuclear localization of lysosomes was also demonstrated190.

Increasing evidence thus implicates lysosomal (and autophagy) dysfunction in the pathogenesis of neurodegenerative disorders62,63,127,128,130,191,192. TFEB has received particular attention in this regard193–195, with recent data suggesting that TFEB is selectively lost in patients with AD (as well as ALS)196. Increasing TFEB activity might therefore prevent neuronal death and restore neuronal function in certain neurodegenerative diseases, including PD194.

Substrate reduction therapies and small-molecule chaperones ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:

In addition to ERTs for LSDs (Box 1), drug discovery programmes have also focused on alternative small molecule-based approaches, which may be particularly relevant for LSDs that affect the CNS, due to the lack of blood–brain barrier penetration by ERTs283.

Small molecules used in substrate reduction therapies prevent the accumulation of substrates of the defective enzymes in LSDs by inhibiting enzymes involved in substrate production284. Miglustat was the first such drug to be approved in the early 2000s by the US Food and Drug Administration and the European Medicines Agency for Gaucher disease and in 2009 for Niemann–Pick disease type C in Europe. This iminosugar inhibits glucosylceramide synthase (GCS), which catalyses the initial step in formation of many glycosphingolipids. Within cells, glycosphingolipids tend to localize to the outer leaflet of the plasma membrane; they cycle within the cell through endocytic pathways that involve the lysosome. Inhibition of GCS therefore reduces the deleterious accumulation of glycosphingolipids within lysosomes with potential therapeutic benefits in diseases like LSDs. Miglustat also inhibits disaccharidases in the gastrointestinal tract, resulting in diarrhoea as a side effect285. Eliglustat, another GCS inhibitor that does not penetrate the CNS, was also approved for Gaucher disease in 2014. Other GCS inhibitors in clinical development include lucerastat, a miglustat analogue with an improved safety profile that is currently in a phase III trial for Fabry disease (FD)236,286, and ibiglustat, which penetrates the CNS. The latter is in clinical development for FD (phase II), for Gaucher disease type 3 (phase II) and for patients with PD who carry a mutation in GBA (phase II). Recent findings generated in a small number of patients have suggested a possible link between PD and FD287,

which also exists between patients with PD and Gaucher disease who have GBA mutations (see above). Finally, genistein, a pleotropic natural product that inhibits kinases involved in the regulation of proteoglycan biosynthesis and also affects TFEB function, is in a phase III trial for Sanfilippo syndrome288.

Substrate mimetics that inhibit lysosomal enzymes have also been found to stabilize mutated enzymes in LSDs, thereby leading to restoration of some enzyme activity when suitable subinhibitory concentrations are used, as the enzyme remains stable and functional after dissociation of the inhibitor46,283. The pioneering example of this approach is migalastat, described above, that binds to the active site of α-galactosidase A, which is mutated in FD, and stabilizes the mutant enzyme. Other examples of this strategy include afegostat in Gaucher disease (which failed in a phase II clinical trial in 2009 due to lack of efficacy), pyrimethamine in Sandhoff disease and Tay–Sachs disease, and ambroxol in Gaucher disease with neurological symptoms (Table 4). Agents that are at earlier developmental stages include N-octyl-β-valienamine, a competitive inhibitor of β-glucosidase, for Gaucher disease; N-acetylcysteine for Pompe disease; α-lobeline, 3,4,7-trihydroxyisoflavone and azasugar in Krabbe disease; and N-octyl-4-epi-β-valienamine and 5N,6S-(N′-butyliminomethylidene)-6-thio-1-deoxygalactonojirimycin indicated in GM1 gangliosidosis289. The chemical structures of these pharmacological chaperones have been described recently290,291. Finally, an alternative strategy for stabilizing mutant enzymes, by binding away from the active site, is also being investigated. A promising example of this approach is NCGC607, a non-inhibitory small-molecule chaperone of GCase discovered by screening for molecules that improved the activity of the mutant enzyme46,250. Treatment with NCGC607 reduced lysosomal substrate storage and α-synuclein levels in dopaminergic neurons derived from induced pluripotent stem cells from patients with Gaucher disease with parkinsonism46,250. Further testing of NCGC607 in patients with PD and GBA mutations is awaited. Although promising, conflicting viewpoints still remain on the strength of such small molecule-based approaches, primarily because these compounds bind to the catalytic site of enzymes, which may be a risk at high concentrations if they inhibit rather than increase activity291,292. More clinical trials are therefore required in order to analyse the robustness of this approach.

Cathepsin modulators ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:

Robust genetic and pharmacological preclinical investigations have consistently showed that regulating cathepsin activity can favourably improve pathological features in certain autoimmune and inflammatory diseases. Inhibitors of several cathepsins (B, D, L, K and S) have been described174,293 and their activity has been evaluated in rheumatic autoimmune diseases (such as SLE, RA and SjS) and neurodegenerative disorders, notably in AD294 (Table 4). Selective inhibition of cathepsin S with a potent active site inhibitor known as RO5461111 (Roche) mitigated disease in MRL/lpr lupus-prone mice, by reducing priming of T and B cells by dendritic cells, and plasma cell generation262. Promising data have also been generated in murine models, in the context of diabetic nephropathy and cardiovascular diseases295. Further studies based on cathepsin S inhibitors should evaluate the clinical safety and utility of treating patients affected by autoimmune and inflammatory diseases295. Cathepsin K, which is highly expressed by osteoclasts and very efficiently degrades type I collagen, the major component of the organic bone matrix, is also a potential target for modulating lysosomal dysfunction in some of the disorders discussed above, such as SLE96. Yet further investigations with selective cathepsin K inhibitors are required to determine whether this targeted strategy might apply in SLE and other inflammatory conditions in which articular manifestations are a major component (RA, ankylosing spondylitis, psoriatic arthritis and others). It should be noted, however, that various cathepsin K inhibitors have been pursued for postmenopausal osteoporosis, including odanacatib (Merck) which reached phase III trials296. Although odanacatib was effective, its development was discontinued in 2016 due to an increased risk of stroke in treated patients. Other cathepsin inhibitors and their context of clinical evaluation are listed in Table 4.

Despite multiple efforts to develop selective pharmacologic cathepsin modulators, important concerns still remain with regard to off-target effects due to activity against other cathepsins or towards cathepsins present at non-relevant or unwanted sites. Nonetheless, the underlying biology and clinical effects of certain cathepsin inhibitors or activators remain of considerable interest and could guide future therapeutic approaches.

v-ATPase inhibitors ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:
As reported below, v-ATPase, a multisubunit ATP-driven proton pump, is best known for its role in acidification of endosomes and lysosomes. Regulating the function of v-ATPase may impact lysosomal activity and, hence, the acidification of specialized cells and diverse signalling pathways, such as autophagy. v-ATPase inhibitors like bafilomycin A1 and concanamycin A are non-selective compounds (Table 4; Fig. 3) that inhibit both mammalian and non-mammalian v-ATPases, which control the lysosomal pH of acidic vesicles via a manner that is not fully understood (Fig. 2). Through this mechanism, bafilomycin A1 inhibits autophagic flux by preventing the acidification of endosomes and lysosomes297. Bafilomycin and CQ also affect mitochondrial functions, as discovered recently using intact neurons298. Benzolactoneenamides (salicylihalamide A, lobatamides and oximidines; Table 4; Figs 2,3) are much more selective v-ATPase inhibitors299 than bafilomycin A1 and concanamycin A, but also much less potent. Further investigations into v-ATPase regulation of signalling pathways are needed to identify specific and safe molecules that regulate this vital proton pump300.

Ion channel modulators ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:
As discussed above, lysosomal ion channels are master elements of lysosome activity and, thereby, of cell homeostasis. In the family of TRP channels, TRML1 is essential, being widely expressed in late endosomes and lysosomes, and preferentially associates with LAMP1 in the lysosomal membrane22,301,302. Genetic mutations leading to inactivation of TRPML1 cause a rare genetic disorder called mucolipidosis type IV (MLIV). Pharmacological activation of TRPML1 ameliorated some lysosomal functions that are classically associated with MLIV, NPCs and certain LSDs (Tables 2,4; Fig. 2). Thus, the small molecule SF-22 (Fig. 3), which was identified in a screen for TRPML3 activators, was defined as an activator of both TRPML3 and TRPML1 (ref.274), and displayed an additive effect in combination with the endogenous activator phosphatidylinositol-3,5-bisphosphate (PtdIns(3,5)P2)274,303. An analogue of SF-22, in which chlorine on the thiophene had been replaced by a methyl group, showed greater efficacy on TRPML1 activation303,304. Another molecule called ML-SA1 (Figs 2,3), acting as a mucolipin synthetic agonist, also showed an additive effect with endogenous PtdIns(3,5)P2 on TRPML1 channels305. It is important to note that in neurological diseases, as well as in other indications in which lysosomal acidification is defective (see above), interfering with TRML1 may have contraindications.

Phosphatidylinositol kinase modulators ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:
A central modulator of lysosomes is the lipid kinase FYVE finger-containing phosphoinositide kinase (PIKfyve), which converts phosphatidylinositol-3-phosphate into PtdIns(3,5)P2. The latter regulates Ca2+ release from the lysosome lumen and is required for acidification by v-ATPase. Inactivation of PIKfyve leads to many pathophysiological problems including neurodegeneration and immune dysfunction, mostly related to impaired autophagic flux and alteration of lysosomes (trafficking, Ca2+ transport, biogenesis and swelling)306. The small-molecule apilimod (Fig. 3; Table 4) was originally identified as an inhibitor of TLR-induced IL-12 and IL-23, and later found to be a highly specific inhibitor of PIKfyve276. Apilimod was evaluated in clinical trials involving several hundred patients with T helper 1 and T helper 17 cell-mediated inflammatory diseases such as Crohn's disease, RA and psoriasis277,278. It was well tolerated in more than 700 human subjects (normal healthy volunteers and patients with inflammatory disease), but the clinical trials did not meet their primary endpoints and further development was abandoned. Apilimod is currently being evaluated in a clinical trial (NCT02594384) aimed at defining a maximum tolerated dose in patients with B cell non-Hodgkin lymphoma and monitoring safety, pharmacokinetics, pharmacodynamics and preliminary efficacy307. YM-201636 is another selective inhibitor of PIKfyve (Table 4; Fig. 3). This inhibitor contains a FYVE-type zinc finger domain. YM-201636 was found to significantly reduce the survival of primary mouse hippocampal neurons in culture and reversibly impair endosomal trafficking in NIH3T3 cells, mimicking the effect produced by depleting PIKfyve with small interfering RNA. It was also found to block retroviral exit by budding from cells275. From a clinical perspective, although targeting PIKfyve is highly promising, further work is required to pave a way towards a future treatment.

Farnesyl transferase inhibitors ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:
Several molecules with farnesyl transferase inhibitory activity have been developed. However, some earlier compounds were found to have major side effects, and their development was discontinued. Lonafarnib (SCH66336; Eiger Biopharmaceuticals), a synthetic tricyclic halogenated carboxamide, has recently shown some promise in a transgenic mouse, which expresses human tau carrying a P301L mutation282 (Table 4). These mice develop tangles in the hippocampus, amygdala, entorhinal cortex and cerebral cortex by 16 weeks, and about 60% of hippocampal neurons die at about 22 weeks. Compared with untreated mice, mice that received lonafarnib displayed less abnormal behaviour and half of the tangles in the hippocampi and cortices. Treatment also prevented brain atrophy that typically occurs in these transgenic mice, while reducing microgliosis in the hippocampus and tempering astrogliosis in the cortex. Mechanistic studies have shown in lonafarnib-treated mice that substrates were more efficiently delivered to lysosomes, their degradation products disappeared faster and the organelles were more readily degraded, specifically by improving lysosome efficiency. Knowing that lonafarnib is already approved for use in humans for other indications (cancer, and ongoing evaluation for progeria and hepatitis delta virus infection), it might therefore be repurposed for use in patients with tauopathy. In this class of farnesyl transferase inhibitors, tipifarnib (R115777; Johnson & Johnson) might also display interesting therapeutic properties as it has been seen to block lysosomal-dependent degradation of bortezomib-induced aggresomes without inhibition of the early steps of autophagy. Kura-oncology in-licenced tipifarnib in 2014.

Chaperone modulators ::: Pharmacological regulators of lysosomal activity ::: Lysosomes as therapeutic targets:
Molecules targeting chaperone proteins involved in lysosomal function have also been designed for potential therapeutic applications. One of these molecules is VER-155008, a small-molecule inhibitor of HSPA8, a key element of CMA308,309. VER-155008 binds to the nucleotide binding domain of HSPA8 and HSP70, and acts as an ATP-competitive inhibitor of ATPase and chaperone activity. In a mouse model of AD (5XFAD mice), intraperitoneal treatment with VER-155008 reduced the two main pathological features of AD (amyloid plaques and paired helical filament tau accumulation) and improved object recognition, location and episodic-like memory280.
Another molecule, the 21-mer phosphopeptide P140 (Table 4; Fig. 3), was also shown to interact with HSPA8 (Fig. 2)310 and lodge in the HSPA8 nucleotide binding domain92,311. P140 and VER-155008, however, do not have the same mechanism of action, and their effects were not additive312. P140 is a phosphorylated analogue of a nominal peptide that was initially spotted in a cellular screening assay using overlapping peptides covering the whole spliceosomal U1-70K protein and CD4+ T cells collected from the lymph nodes of lupus-prone MRL/lpr mice313. P140 peptide enters B cells via a clathrin coat-dependent endocytosis process to reach early endosomes and then late endosomes/lysosomes92. It affects CMA that is hyperactivated in lupus, likely by hampering the CMA-mediating chaperone HSPA8 (ref.101). P140 peptide reduces the excessive expression of HSPA8 and LAMP2A observed in lupus mice, alters the (auto)antigen presentation by MHCII molecules in the MIIC compartment and, consequently, attenuates the activation of autoreactive T cells92. A significant diminution of MHC molecule expression at the surface of antigen-presenting cells was measured in mice that received the P140 peptide intravenously and on patient's peripheral cells treated ex vivo with the peptide92,101,314. As a downstream consequence, the activation of autoreactive B cells and their differentiation into autoantibody-secreting cells is repressed101,314. T cells from patients with lupus are no longer responders ex vivo to peptides encompassing CD4+ T cell epitopes315. The effect of P140 on CMA was demonstrated in vitro, using a fibroblast cell line that stably expresses a CMA reporter53,92. P140, which selectively targets the CMA/lysosome process and has no effect on mitophagy316, has been evaluated in murine models mimicking other rheumatic diseases with very promising results, notably in mice developing SjS features56, in mice with neuropsychiatric lupus symptoms62 and in rats that develop a CIDP-like disease with disturbance of both CMA and macroautophagy in sciatic nerves59. In clinical trials that included patients with SLE, P140 formulated in mannitol was found to be safe and non-immunogenic after several subcutaneous administrations of peptide312,317,318. P140 showed significant efficacy in a multicentre, double-blind, phase II trial317. This peptide is currently being evaluated in phase III trials in the United States, Europe and Mauritius. In continuation, an open-label trial including several hundred patients with lupus worldwide is planned.

Another peptide has been discovered that, in contrast to P140, activates CMA319. This 24-mer peptide called humanin was originally identified from surviving neurons in patients with AD, and was found to directly enhance CMA activity by increasing substrate binding and translocation into lysosomes. Humanin interacts with HSP90 and stabilizes the binding of this chaperone to CMA cargos as they bind to the lysosomal membrane. These results are important as humanin had been shown to possess some cardioprotective and neuroprotective properties in diseases such as AD, cardiovascular disease, stroke, myocardial infarction, diabetes and cancer320.

Emerging potential lysosomal therapeutic targets ::: Lysosomes as therapeutic targets:
In addition to the targets discussed above, there are a few emerging potential lysosomal therapeutic targets for which there is strong biological validation, but not yet any small molecules in development that target them. An example with likely pharmacological tractability is a lysosomal K+ channel called TMEM175, which is important for maintaining the membrane potential and pH stability in lysosomes321. Deficiency in TMEM175 may play a critical role in PD pathogenicity322. Importantly, the structure of TMEM175 has been recently refined323.
Another target for which ligands have not yet been validated is the KCNQ2/3 channel (also named M-channel or Kv7.2/7.3 channel). It has been shown in NPC1 disease that reduced cholesterol efflux from lysosomes aberrantly modifies neuronal firing patterns324. This disruption of lysosomal cholesterol efflux with decreases in PtdIns(4,5)P2-dependent KCNQ2/3 channel activity may lead to the aberrant neuronal activity. The cholesterol transporter and PtdIns(4,5)P2 floppase, ABCA1, is responsible for the decline in PtdIns(4,5)P2 that consequently modifies the electrical properties of NPC1 disease neurons. Dysfunction in the activity of KCNQ2/3 or altered levels of PtdIns(4,5)P2, due notably to genetic mutations, might also be involved in other neuropathies (for example, some forms of epilepsy, HD, PD, AD, ALS and Friedrich ataxia). Although further experiments are needed to validate the link discovered between hyperexcitability and cell death in NPC1 disease and other neurodegenerative diseases, small molecules such as retigabine, an anti-convulsant drug that keeps KCNQ2/3 channels open, might represent important therapeutic tools324,325. Other channel opener ligands of KCNQ2/Q3 include ICA-069673 and its derivatives.
Another promising therapeutic target is sphingomyelin phosphodiesterase 1 (SMPD1). Defects in the gene encoding SMPD1 cause Niemann–Pick disease type A and type B. SMPD1 converts sphingomyelin to ceramide, and also has phospholipase C activity. Reduced activity of acid sphingomyelinase, associated with a marked decrease in lysosomal stability, has been described in patients with Niemann–Pick disease, a phenotype that was corrected by treating cells with recombinant HSP70326.
Finally, as LAMP2A, a specific lysosomal protein that displays a decisive role in CMA, has been shown to be overexpressed in certain pathological settings such as certain cancers and inflammatory diseases (autoimmune or non-autoimmune), downregulating its expression might be therapeutically beneficial53,327. As mentioned above, however, in other indications there is a defect in LAMP2A. The latter can be due to reduced stability of the CMA receptor and not to decreased de novo synthesis (for example, in ageing)328 or can result from aggregation to the lysosomal membrane of pathogenic proteins such as α-synuclein, ubiquitin carboxy-terminal hydrolase L1 (a deubiquitinating enzyme) and mutant tau, known to amass in neurodegenerative disorders (see above). Targeting LAMP2A therefore remains a challenge, although several strategies may be envisaged, for example by controlling de novo synthesis, by hampering its multimerization into lysosomes (possibly via HSP90 and/or other chaperones) or by regulating the degradation rate of LAMP2A monomers (for reuse) into lysosomes.

Challenges and outlook:
Current research into lysosomal function and dysfunction is revealing novel roles of lysosomes in disease pathogenesis and highlighting new opportunities to treat such lysosomal and autophagy-related diseases. As in the case of autophagy modulation14,56,207, lysosomal activation or inhibition must be investigated with caution, as lysosomal activity can be abnormally reduced or enhanced in some organs or tissues and not in others, and, at another scale, lysosome activity can be altered in certain lysosomes and not in others within the same cell. Biodistribution studies in vivo must be undertaken to avoid accumulation of pharmaceuticals in healthy organs or tissues. There is an obvious requirement for safety, to ensure that a drug used as a lysosome modulator for a particular type of lysosomal disease does not increase vulnerability to another disease.
There is still much to be learned about the intimate working of lysosomes. This is due to the abundance of constitutive elements that comprise these vesicles, the added complexity resulting from their plasticity (ion channels and transporters, acidification and swelling) and the vast

amount of proteins and peptides that are translocated into lysosomes and digested by lytic enzymes. Sensitive analysis methods have allowed important information to be generated about lysosomal membrane proteins, a large majority of which are transporters8. However, many questions remain related to how their expression is regulated and how they regulate their translocator and chaperoning activities. For example, certain cells only contain so-called secretory lysosomes (as in cytotoxic T cells), whereas other cell subsets contain both conventional and secretory lysosomes (as in platelets). Considering the large family of endo-lysosomal vesicles, the whole notion of 'secretory' and 'conventional' lysosomes remains a matter of debate. In many instances, lysosomes act as a basal cell metabolism organelle; whereas in other cases, they assist in the regulation of homeostasis through unconventional secretory pathways, known as lysosomal exocytosis, and different signalling mechanisms.

Although several assays used to measure the activity of lysosomes have been validated worldwide (Box 2; Table 3), they have their limitations, including issues associated with reliability, performance and sensitivity, notably in vivo. Another level of complexity comes from the inherent organelle heterogeneity, which is an issue of tremendous importance. Unfortunately, with the tools and equipment we have in hand today, it is virtually impossible to examine what happens in the lysosomes of an individual patient. The introduction of microfluidic single-cell analysis technologies has enabled cellular populations to be characterized and huge advances to be performed. However, the level of precision has not yet been achieved at the level of lysosomes (0.2–0.5 μm). We know that lysosomes are heterogeneous in nature, composition and activity even in 'normal' settings; they are not all equally competent for autophagy or any other types of activity. Currently, this is obviously the focus of intense research.

Although a certain number of preclinical studies involving lysosomal regulators have been conducted over the years, only a small number of lysosome-targeted therapeutics have so far moved into clinical development. One of the biggest advances in developing such strategies would be the identification of a genetic signature that would allow those patients most likely to respond to a specific therapy to be selected. However, at this stage of our knowledge of specific lysosome-directed drugs and intrinsic lysosomal failures, genetic features that might predict potential responders are still lacking (with the exception of LSDs). Further investigations are required to achieve this level of knowledge, which obviously will also depend on the type of disease, heterogeneity and frequency.

Another issue associated with the development of lysosome-targeted therapeutics relates to delivery. The use of nanovectors represents an attractive delivery method, owing, in particular, to their unique ability to penetrate across cell barriers and, via the endo-lysosomal pathway, to preferentially home in on organelles such as lysosomes. Several nanoscale galenic forms have been developed to serve as vectors or carriers of proteins, peptides or nucleic acids, and a vast literature describes the many advantages of using such nanostructures in nanomedicine. However, safety is a concern as some carbon nanostructures have been claimed to induce nanotoxicity, accompanied by the induction of autophagy and lysosomal dysfunction329–332 (reviewed elsewhere333–336).

The purpose of this Review is to gain awareness of the importance of lysosomes in disease, and to encourage the development of novel lysosomal targeted drugs. However, more research is needed to characterize components that are specifically linked to the lysosome, such as LAMP2A and HSPA8, and to more clearly define their specific involvement in lysosome biogenesis and metabolism. Special attention should be given to the mode of administration of lysosome-targeted medications in order to minimize toxicity and promote specific targeting. It is our hope that a large field of therapeutic applications could emerge from such investigations, encompassing rare and common autoimmune, neurodegenerative and metabolic diseases, as well as cancer, senescence and ageing.