Review

Pharmacological autophagy regulators as therapeutic agents for inflammatory bowel diseases

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Abstract:

The arsenal of effective molecules to treat patients with chronic inflammatory bowel diseases (IBDs) remains limited. These remitting-relapsing diseases have become a global health issue and new therapeutic strategies are eagerly awaited to regulate the course of these disorders. Since the association between autophagy-related gene polymorphism and an increased risk of Crohn's disease development has been discovered, a new domain of investigation has emerged, focused on the intracellular degradation system, with the objective to generate new medicines that are safer and more targeted. This review summarizes the drugs administered to IBDs patients and describes recently emerged therapeutic agents; we compile evidence on the contribution of autophagy to IBDs pathogenesis, give an overview of pharmacological autophagy regulators in animal models of colitis, and propose novel therapeutic avenues based on autophagy components.

Inflammatory bowel diseases, a group of chronic, relapsing disorders depending on environmental, genetic, microbial, and immunological factors

Inflammatory bowel diseases (IBDs) have an increased incidence in developed countries, affecting 0.1% of the western population. They adversely affect several million people worldwide, with the highest rate of incidence in Europe and North America, probably as a result of diet, lifestyle, and sanitation [1–3]. IBDs, which cause inflammation of the lining of the digestive tract, are commonly detected in young people at the age of 18-25 years, and so far, they are non-curable [4]. The patients complain of frequent and chronically relapsing flares, which can lead to abdominal pain, diarrhea, rectal bleeding, fatigue, malnutrition, and weight loss. The major entities of IBDs are typified by Crohn's disease (CD) and ulcerative colitis (UC). Though both share several clinical symptoms, CD and UC have markedly distinct features concerning their pathology and origin [5–7]. CD can affect any part of the gastrointestinal tract most commonly in terminal ileum and colon, and can involve all layers of intestinal tissues. UC affects the large intestine only, and the inflammation is restricted to the mucosal layer [8]. CD and UC are associated with increased intestinal permeability, which involves paracellular passage regulated through tight junctions (TJs) [3,9]. Single nucleotide polymorphisms located in genes encoding interacting TJ proteins and changes in the expression of some TJ proteins have been described [10–14]. Interestingly, several studies have shown the role of autophagy (Box 1) in the defects observed in intestinal TJ barriers that occur in IBDs [15].

The etiology of IBDs involves complex genetic factors and environmental elements. Genome-wide association studies identified more than 200 confirmed genetic risk loci associated with this set of diseases. These loci are notably involved in common, albeit central, cellular pathways, such as autophagy, cytokine signalling, intestinal barrier regulation, and microbial recognition [16–18]. Recent studies hypothesized that IBDs result from chronic abnormal immune responses against enteric bacteria or gut flora that develop in genetically susceptible individuals. IBDs are therefore both a consequence of autoimmune and immune-mediated phenomena. Autoreactive antibodies (Abs) and autoreactive T cells co-exist with cytotoxic leukocytes for colonic epithelial cells and serum Abs against colonic epithelium, which are cross-reactive with *E. coli* antigens, for example. Among the large diversity of Abs occurring in patient's serum, some may serve to differentiate various forms of IBDs and can be used as predictors for disease activity [19–21]. Abnormalities affecting

the innate and adaptive immune system are largely reported in IBDs [6]. A particular subset of dendritic cells (DC) expressing the clusters of differentiation 11b and 103 surface markers appears as a major provider of interleukin (IL)-23 during colitis development [22–25]. The closely related IL-23 and IL-12 cytokines, as well as their major downstream components, including IL-17, play important roles in the regulation of mucosal inflammation, especially in the gut. These cytokines control autophagosome formation and autophagic flux. The **regulatory T cells** (Tregs, see Glossary) compartment is also deeply impacted in IBDs, as discussed elsewhere [5,26,27].

The importance of gut microbiota is central in many vital functions of the body and is not solely directly linked to the intestinal functioning as originally thought. It comprises thousands of diverse microbial species that interact with the host in exerting decisive regulating effects linked to immune, metabolic (e.g., in the regulation of systemic glucose metabolism) and neurological functions. It is only relatively recently that the eminent role of the microbiota-gut-brain axis has emerged and that a connection between the gut microbiome and autophagy has been highlighted [28–31]. In IBDs, it is well documented that the microbial composition, diversity, and richness of microbiota is dramatically altered [32,33]. This microbial imbalance (known as gut dysbiosis) has been reported both at the mucosal and fecal level, and systematic studies have shown that in average, IBDs-affected patients display 25% fewer microbial genes (a reflection of microbiota) than healthy individuals [34,35].

A better knowledge of fundamental aspects involved in the loss of tolerance of immune functions affecting patients with IBDs and a fine understanding of the key elements prevailing in the tropism of organs, tissues or cells that typify these patients, remain a central challenge in our quest of adapted specific treatments and personalized medicine, which would target the causes and not only the symptoms of these complex disorders. A focus is given here on promising ways of intervention linked to autophagy defects that have emerged recently in this context. A brief overview of preclinical data raised with pharmacological autophagy regulators is given, mostly obtained in animal models of IBDs-mimicking colitis.

Animal Models of Inflammatory Bowel Diseases

It has long been recognised that the furtherance of new treatments is closely tied to the pertinence of relevant animal models. In the case of IBDs, more than 65 different animal models have been established, which can be classified as genetically engineered, congenic

mutants, cell-transfer- or chemically-induced models [36]. This multiplicity of experimental *in vivo* models emphasizes the critical point that, in fact, none of them totally satisfies the pure criteria of human IBDs and that it is decisive to adopt several independent experimental animal models to demonstrate the efficacy of a newly-developed treatment or diagnostic tool, or to study IBDs mechanistically.

The first experimental induced colitis model was introduced as early as 1957 in rabbits [37]. Since then, many other genetically-modified animal models have been generated, which have largely contributed to understand the disease pathology and genetic features of IBDs. Genetically-engineered animal models of IBDs include IL-10, signal transducer and activator of transcription (STAT)3, X-box binding protein 1, IL-2R α , IL-23R, transforming growth factor (TGF)- β , and tumor necrosis factor (TNF)- α knockout mice, and TNF superfamily member (SF)-15, IL-7, IL-17 and IL-23-expressing transgenic mice [23,38,39]. Transgenic CEABAC10 mice expressing human carcinoembryonic antigen-related cell adhesion molecule 6, a receptor for some *E. coli* strains, are also commonly exploited [40,41]. Based on manipulated mice, a major role of CXCR1⁺ mononuclear phagocytes-derived TNF-like ligand 1A in driving IL-22 production by a subset of innate lymphoid cells that are central in the regulation of mucosal homeostasis has been demonstrated [39].

Some models are induced by chemicals that generate IBDs-like inflammation in the intestine of normal mice or rats. These models of acute or chronic colitis are notably based on the use of dextran sulphate sodium (DSS), trinitrobenzene sulfonic acid (TNBS), oxazalone and polyI:C. They are commercially available and are routinely used in research [42–44]. They are relatively simple to set up with an appreciable reproducibility and present many similarities with human colitis. Several adoptive transfer colitis models have also been exploited [38,45]. In these models, recipient animals are given an intraperitoneal injection of cells (e.g., CD45RB^{high} T cells or CD62L⁺/CD44⁻ T cells) or monoclonal (m) Abs (e.g., anti-CD40 Ab) and are then evaluated daily (as in the other models of colitis listed above) for survival, body weight, evidence of bloody stool and diarrhoea. Video endoscopy completes this follow-up, providing a daily visual assessment of the severity of colitis and eventually of a possible tracking of mucosal healing after treatment.

Current Treatments of Inflammatory Bowel Diseases

First-line therapies for IBDs

The first drugs used to treat IBDs with some efficacy were immunosuppressants such as aminosalicylates, corticosteroids, and thiopurines (Table 1, and references therein [46-72]. Sulfasalazine, an aminosalicylate, which is a class of anti-inflammatory compounds acting mainly as oxygen scavengers showed some potent effects. This discovery led to the development of a range of drugs falling in this class of compounds, such as mesalazine and others (Table 1). Corticosteroids were also found to be remarkably effective in both CD and UC. However, long-term toxicity, steroid dependency, and refractoriness to treatment occurring in some patients oblige in certain cases to discontinue the medication or restrict their usage [48,68,70]. Continuing research is therefore focused on the repositioning of such molecules that would have less deleterious secondary effects. This already led to the development of several molecules, especially budesonide (Entocort, Mikicort), an oral glucocorticoid, which is quickly metabolized by the liver, thereby reducing corticosteroid-related side effects. It is used in the management of asthma, allergic rhinitis and various skin disorders, and has been extended to CD [47]. Although budesonide appears significantly less effective than conventional steroids (e.g., mesalazine) for inducing remission in active CD, it displays fewer side effects [73]. It was not found to be effective, however, for maintaining remission at 12 months in CD.

Other immunomodulators such as thiopurines, methotrexate, and calcineurin inhibitors were also explored alone or concomitantly with other drugs as treatment options for IBDs (Table 1). Thiopurines get incorporated into nucleotides and suppress T cell function by decreasing the expression of pro-inflammatory cytokines. Methotrexate (e.g., Imeth, Novatrex, Methotrexate Bellon, Metoject) was found to be effective in steroid-dependent CD (effective for induction and maintenance of remission in CD, but not in UC [74], while cyclosporine A and tacrolimus/FK-506 calcineurin inhibitors, which are strong immunosuppressive compounds, were found to decrease pro-inflammatory lymphokine production in UC [63,75].

A recent study including a large cohort of CD patients demonstrated that co-administration of 5-aminosalicylate (5-ASA; mesalazine) and either azathioprine (AZA) or 6-mercaptopurine (6-MP) was not more effective than AZA/6-MP alone in terms of the requirement for rescue medications such as steroids and anti-TNF agents [76]. The cumulative probabilities of hospitalization and intestinal resectional surgery were similar between the groups of patients on either regimen. Thus, though these molecules and peptides were often effective as primary or first-line therapy for IBDs, their long-term use is severely marred by serious ailments such as myelosuppression, multiple infections, pancreatitis, and in some cases sensorineural

hearing loss and tinnitus (Table 1), justifying the introduction of much more selective therapeutic strategies.

The era of therapeutic antibodies and cell modulators for treating IBDs

A number of Abs have been developed against cytokines and adhesion molecules, which are key players in the pathogenesis of IBDs (Table 1; Figure 1). In patients with IBDs, cytokines produced by intestinal mucosa largely contribute to the activation and migration of inflammatory cells such as monocytes and neutrophils [5–7,16]. Cytokines were therefore especially targeted for treating IBDs patients.

Anti-TNF Antibodies

The use of anti-TNF drugs (Box 2) has been a real breakthrough progress in the treatment of IBDs [7,68,77,78]. Several anti-TNF- α Abs are currently approved for treating patients with IBDs. In the case of CD, these include infliximab (Remicade), which is a chimeric human/mouse Ab (and its biosimilars Inflectra, Remsima and Flixabi), adalimumab (Humira), a fully human IgG1 mAb (and its biosimilars Mylan's Hulio, Boehringer Ingelheim's Cyltezo, Samsung Bioepis's Imraldi, Sandoz's Hyrimoz and Amgen's Amgevita), and certolizumab pegol (Cimzia), a humanized antigen-binding fragment (Fab') of a mAb that has been conjugated to polyethylene glycol. For UC, these are infliximab, adalimumab and golimumab (Simponi), a fully human IgG mAb (Table 1). A recent study has demonstrated the efficacy of golimumab in anti-TNF refractory CD patients [79]. At this stage, however, further studies are awaited in CD to formally assess the efficacy of golimumab in a randomized controlled trial and to establish the optimal dosing regimen.

Altogether, TNF-targeting Abs have been claimed to induce a clinical response in about 60% of CD and UC patients, a result that is remarkable in the context of these severe and heterogenous diseases [50,62,77]. It is however important to remind the well-characterized serious adverse effects (SAEs) induced in certain patients by TNF blockers when given for long periods of treatment. Two major concerns with these drugs include the risk of serious infections and malignancies [80–83].

Non-anti-TNF biological therapies

Apart from TNF- α , other cytokines are also used as targets in emerging therapeutic strategies [84]. Ustekinumab (Stelara) is a human IgG1 mAb that targets the p14 subunit of IL-12 and IL-23 by inhibiting their binding to their receptors (Table 1; Figure 1). This mAb, which has

been approved by the American Food and Drug Administration (FDA) in 2016, showed efficacy in CD patients with moderately-to-active disease.

Other biologics are currently evaluated for their potential efficacy. It is the case, for example, of risankizumab (BI-655066 or ABBV-066), brazikumab (AMG 139 or MEDI2070), briakinumab (ABT-874), mirikizumab (LY3074828) which are other Abs that target IL-23 by binding to its P19 subunit (Figure 1; Table 2)[85–94] [72,95–103].

Briakinumab is a human mAb that was initially developed for treating rheumatoid arthritis (RA), MS, and IBDs. In November 2009, completion of a phase-III clinical trial for plaque psoriasis and a phase II clinical trial for MS was announced. A Phase-II clinical trial for CD was underway [104]. Head-to-head comparisons were made with regard to etanercept (Enbrel), a dimeric fusion protein targeting TNF, and placebo, in double-blind trials. The results gained with briakinumab were promising in psoriasis (81–82% of patients under briakinumab, 40–56% under etanercept, and 7% under placebo reached Psoriasis Area Severity Index reduction of at least 75%). On January 2011, the withdrawal of briakinumab application was announced to favour other strategies.

Migration of leukocytes to mucosal lesions is important in the pathogenesis of IBDs, and this trafficking process is actively mediated by integrins. Hence, targeting integrins has emerged as another way for potential therapy. The first attempt in this area was based on natalizumab (Tysabri), a human IgG4 Ab targeting the $\alpha 4$ integrin subunit (Table 1). Its use, however, was preferable for short-term treatment. In some rare cases, due to inhibition of leukocyte migration into central nervous system, it was found to promote reactivation of John Cunningham virus in the brain, resulting in the development of progressive multifocal leukoencephalopathy (PML), a SAE that precluded its indication.

Vedolizumab (Entyvio) is an IgG1 mAb, which also blocks the $\alpha 4\beta 7$ integrin subunit but thanks to its gut selectivity, it was not associated with PML (Table 1). In phase-III clinical trials, vedolizumab was found to be safe and efficient in the induction and maintenance phases of therapy in CD and UC patients. Although patients receiving vedolizumab presented more frequent SAEs and infections compared with placebo, the promising data generated with this Ab led to a growing interest in developing other anti-integrin Abs, such as etrolizumab (rhuMAb $\beta 7$), abrilumab (AMG 181), and PF-00547659, which are currently evaluated in clinical trials (Table 2, and references therein). PF-00547659 is a fully human mAb that binds to human mucosal addressin cell adhesion molecule (MAdCAM), which is

predominantly expressed on the cell surface of high endothelial venules of organised intestinal lymphoid tissues (Peyer's patches, mesenteric lymph nodes). It was found to selectively reduce lymphocyte homing to the intestinal tract. Although compared to placebo, this mAb did not meet the primary endpoint of clinical response in moderate-to-severe CD, it raised great hopes as it presented some appreciable pharmacological effects, which remain to be analysed further [98].

Adverse effects of biologics

Several clinical trials and meta-analyses have verified the efficacy and safety of biologics-based therapies. The risk of SAEs associated with these therapies has been relatively lower compared to other conventional (immunosuppressive) treatments and some biologics have proved to be beneficial in the induction and maintenance of clinical remission and response [105,106]. However, cases of SAEs including hypersensitivity reactions, injection site reactions, skin cancers, drug-induced lupus, psoriasis, reactivation of latent tuberculosis, hepatotoxicity, lymphomas and solid tumour have been reported (Tables 1; Table 2). In addition, the high production cost of therapeutic mAbs remains a hurdle in maintaining the cost-effectiveness of these drugs [107,108].

Another serious issue that is encountered with certain biologics is the generation of anti-drug Abs (ADAs) that makes at least 40% of the patients receiving anti-TNF drugs secondary non-responders. This loss of responsiveness mostly occurs in the case of patients receiving episodic therapy or in the presence of lower levels of ADAs against other anti-TNF agents received earlier (including biosimilars) [109]. According to previous studies, the formation of Abs against infliximab occurs in 61% of patients receiving episodic treatment and 44% of patients losing response to adalimumab were found to have developed Ab to adalimumab. It is a general observation and a source of concern that more and more cases of ADAs are reported in the literature [110–114] influencing the efficacy of treatment and the potential clinical improvement of patients under biotherapy. Sensitive assays have been developed to detect ADAs that are produced early in certain individuals and can dramatically affect the results of clinical trials and the efficacy of current treatments in patients [110,115,116]. A careful follow-up of patients all along their treatment should be performed. High serum concentrations of anti-TNF drugs are generally associated with improved clinical outcomes in UC patients. In contrast low concentrations have been shown to frequently associate with the formation of ADAs [117,118].

Small molecules for treating IBDs

In terms of small molecules, apilimod mesylate (N-[(E)-(3-methylphenyl)methylideneamino]-6-morpholin-4-yl-2-(2-pyridin-2-ylethoxy)pyrimidin-4 amine; formerly STA-5326) that inhibits IL-12/IL-23, was evaluated in clinical trials including patients with CD [119]. Up to 700 subjects have been treated with mild-to-moderate side effects. However, apilimod did not meet the primary end points in phase-II inflammatory disease indications [120]. This molecule is currently evaluated in other indications.

ABX464 (8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine) is a small molecule that induces IL-22 production in macrophages, which may have great interests in acting on intestine inflammation. Laquinimod (ABR-215062 or TV-5600; developed by Active Biotech and Teva) is another oral drug that has inhibitory effects on antigen-presenting cells and T cells, resulting in reduced pro-inflammatory cytokine production. In randomised controlled trials laquinimod was efficacious for CD (Table 2). Head-to-head studies with existing treatments and longer term safety data are however needed at this stage of investigation.

The JAK (Janus kinase)-STAT pathway is a major signalling cascade downstream from the cytokine and growth factor receptors, and hence JAK inhibition has been shown to be potentially therapeutic in IBDs. Tofacitinib (Xeljanz) is a pan-JAK inhibitor currently available for treatment of UC [121]. Other molecules such as filgotinib and upadacitinib (JAK1 inhibitors) are under clinical trials for CD and UC.

Mongersen (GED-0301), an oral oligonucleotide drug containing an anti-SMAD7 (mothers against decapentaplegic homolog 7) oligonucleotide has proved to be able to restore signalling by mucosal anti-inflammatory cytokine TGF- β 1. Although positive results were obtained in phase II trials for CD with a clinical remission rate of 72% after two weeks of treatment, this drug was withdrawn from clinical studies in November 2017 due to disappointing results from an interim analysis of a phase-III study [89,102].

Small molecules targeting leukocyte trafficking are also currently investigated. One of them is the α 4-integrin antagonist AJM300, an oral phenylalanine derivative, which is presently evaluated in phase-III clinical trials for UC (investigated in a small cohort of patients until now; Table 2). Amiselimod (MT1303; Biogen), ozanimod (RPC1063; Celgen) and etrasimod (APD334; Arena Pharmaceuticals) are other molecules that act as sphingosine-1-phosphate receptor (S1P) modulators, which lead to lymphocyte sequestration in lymph nodes and reduce the migration of lymphocytes to gastrointestinal tract. The development of

amiselimod, which was in phase II-clinical trials for CD has been halted, as Biogen is currently focusing on other drugs from its portfolio. A phase-III clinical trial of ozanimod in patients with moderate-to-severe UC is ongoing. Etrasimod is also being tested in a phase-II trial in UC [96].

Pros and Cons: how can we progress?

Although the advent of all these therapeutic options greatly helps maintaining middle-term remission and improving the IBDs patients' quality of life to a certain extent, we must recognize that they remain mostly symptomatic and do not address the root genetic causes [4–6,17,68]. Besides, their high cost, severe impacts and SAEs of some of these treatments in the long-term, call for the development of cost-effective small molecule drugs that are disease-specific. In this context, several elements of the autophagy pathway might be key targets for novel therapeutic options.

Autophagy, an Emerging Element in IBDs Regulation and a Novel Therapeutic Option

IBDs and autophagy

Autophagy is a crucial intracellular pathway that continuously degrades, recycles and clears unnecessary or dysfunctional cellular components (e.g., damaged organelles, proteins abnormally folded or produced in excess). It is a finely gene-regulated and evolutionary-conserved process. Autophagy is prominent in the adaptation of cells to their environment and to maintain cell homeostasis, especially under stress conditions (nutrient deprivation, hypoxia, oxidative stress, changes of intracellular level of calcium). It is thus a central actor in cellular processes, such as development, lineages differentiation, and immunity.

Among the pathways that have been associated with so far identified IBDs risk loci, autophagy seems to be a significant one. Since the identification of the autophagy-related gene (ATG) 16L1 ([Box 3](#)) as a major hit in IBDs genetics in 2006 [122], various studies have repeatably established a link between IBDs, ATG16L1 and the process of autophagy [123–130]. It has been shown that ATG16L1 modulates ubiquitination of the adaptor protein sequestosome 1 (SQSTM1)/p62 through the neddylation of cullin-3 (a core element of a complex known as an E3 ubiquitin ligase), leading thus to the suppression of IL-1B signaling [131]. Animal models with mutations in *Atg16l1* (e.g., *Atg16l1*/T300A knockin mice and especially *Atg16l1*/XbpΔIEC mice) have been engineered and used to reinforce our understanding of the importance of this *Atg* in the development of IBDs [132,133]. Other ATGs such as genes encoding **immune-related GTPase M** (IRGM) [134–137], **leucine-rich**

repeat kinase 2 (LRRK2) [138], and **nucleotide-binding oligomerization domain (NOD)2/CARD15** [125,139], have also been closely associated with CD and UC [140–142].

Some alterations -either upregulation or downregulation- in several autophagy pathways, including macroautophagy, chaperone-mediated autophagy (CMA; [Box 1](#)), mitophagy and other forms of autophagy, have been implicated in numerous (auto)immune and inflammatory disorders, such as SLE, Sjögren's syndrome (SjS), RA, psoriasis, some neuroinflammatory and neurodegenerative diseases including MS, chronic inflammatory demyelinating polyneuropathies, amyotrophic lateral sclerosis (ALS), and Huntington's, Alzheimer's, and Parkinson's diseases [126,141,143–151]. Hence components of this central metabolic system recently emerged as a particularly attracting, and even key therapeutic target in many of them [152–159]. A previous study has shown, for example, that an MTOR inhibitor molecule ([Figure 2](#)), namely an haloacyl aminopyridine-based molecule called P2281, was efficient in a murine model of DSS-colitis by inhibiting T cell function [160]. Another report, in patients, also concluded that rapamycin/sirolimus, a macrocyclic triene antibiotic, which binds to the cytosolic 12-kDa tacrolimus-binding protein (FKBP12) and also inhibits MTOR pathway, could represent a good candidate to treat CD patients [161]. In this retrospective analysis of patients treated with rapamycin, 5 of the 11 UC patients and all of the 3 CD patients achieved clinical remission. An additional 2 UC patients achieved clinical response. The remaining 4 UC patients did not respond to rapamycin treatment. Mucosal healing was achieved in 5 of 11 UC patients and 2 of 3 CD patients. Clinical response to treatment occurred at least 2 weeks after treatment was started. The only significant side effect reported was minor gastrointestinal distress [161]. This report confirms some data generated in TNBS-treated mice, the intestinal inflammation and colitis was ameliorated by rapamycin and also by trehalose [162]. P2281, rapamycin and trehalose all affect the macroautophagy pathway ([Figure 2](#)). Besides their pathophysiological interest, the aforementioned results present potential pharmacological evidence supporting that targeting autophagy using small molecules can be robust for future treatment.

The autophagy pathways: novel options to treat patients with IBDs

As described above, autophagy plays multiple roles in IBDs pathogenesis. Under the control of genes such as *ATG16L1*, *IRGM*, *LRRK2*, and *NOD2/CARD15*, the expression of which appears deregulated in susceptible patients, several vital functions assumed by autophagy processes are severely altered. As a matter of consequences, intracellular bacterial killing, antimicrobial peptide secretion by Paneth cells, goblet cell functions, proinflammatory

cytokine production by macrophages, antigen presentation and processing by antigen-presenting cells (DCs, B cells, macrophages), and endoplasmic reticulum stress response in enterocytes are deregulated (Figure 3) [4,5,17,142,144]. The first line of defence for pathogenic infection and many other aspects of the innate and adaptative immune response are profoundly unbalanced. Therefore, elements of autophagic pathways represent targets of choice to probe in novel therapeutic options of IBDs patient's treatment.

The multiplicity of targets of current anti-autophagy regulators

Developing an effective treatment targeting specific components of autophagic pathways requires to identify what are the elements of the said pathways that are crucial, whose expression is specifically modified (activated or repressed) as a result of the pathophysiological context, and for which we possess tools (existing drugs or newly developed molecules) that reach their target (preferably one) without generating side effects in unrelated metabolic circuits. In general, small molecules possess several pharmacological receptors, and targeting some of them may generate unwanted reactions that hamper or limit their use due to their deleterious influence on vital functions.

A number of activators and inhibitors of autophagy have been described (Figure 2) [153–155,157,158,163–168], and a growing list of patents have been filed. They notably include phytochemicals and anti-oxidants (e.g., polyphenol, curcumin, resveratrol) [169]. In almost all cases, their fine specificity is not known. Few of them, if any, interact with one single target of a specific autophagic process (e.g., macroautophagy, microautophagy, CMA, mitophagy, lipophagy and others), and the fact that pharmacologic molecules develop several effects, *via* distinct cell receptors and intracellular pathways, can render them harmful to health. An illustrative example is **hydroxychloroquine** (HCQ; Plaquenil, Axemal, Dolquine, Quensyl). HCQ a potent autophagy inhibitor, which affects the lysosomal pH (Figure 2) but also, for example inhibits CXCL12/CXCR4 signaling resulting in reduced phosphorylation of extracellular signal-regulated kinases (ERK) and signal transducer and activator of transcription 3 (STAT3). The main concern regarding HCQ is its toxicity with regard to retina that imposes a regular ophthalmic follow-up to evaluate the extent of eventual retinopathy in susceptible individuals [156,170]. Identifying the retinal target of HCQ should help in generating a class of molecules, which keep their primary efficacy without developing their secondary deleterious effects.

Rapamycin that was discussed above, is another example of a molecule that exerts potent effects on different targets. It has been shown to acutely inhibit MTORC1 whereas chronic exposure to rapamycin can also inhibit MTORC2, two functionally distinct complexes in mammalian cells [171]. These complexes share some protein components, but their distinctive activities are defined by their unique components, namely Raptor (MTORC1), and Rictor and mSin1 (MTORC2). Rapamycin forms a tripartite complex with MTOR and FKBP12 (see above) that leads to MTORC1 inhibition. The complexity of its effects on MTORC2 is not completely resolved [172].

Another example of this complexity emerges with metformin (Glucophage, among other names; [Box 4](#)), a synthetic derivative of guanidine which acts as an inducer of autophagy but also displays several other effects via different targets. Several independent studies have shown that metformin ameliorates IBDs [173–175]. In an experimental model, administration of metformin reduced inflammation through the inhibition of p-STAT3, IL-17 and p-MTOR expression and the increased expression of p-AMPK and Foxp3 [173]. It was also demonstrated that metformin limits DSS-induced intestinal barrier disruption by a mechanism involving the inhibition of c-Jun N-terminal kinase activation *via* an AMPK α 1-dependent signaling pathway [174].

Selective regulators of autophagy

These recent years, a great deal of effort was done to identify more selective drug's targets, in particular, based on interactome or metabolomics studies, and accordingly to redesign some molecules that were discarded to render them more selective of the chosen target. This kind of investigation is especially important in IBDs to adapt treatment in a frame of personalized and precision medicine that aims at optimizing treatment practices and at significantly reduce SAEs.

Example of this new family of molecules is the peptide LR12 [176] of sequence H-LQEEDTGEYGCY-NH₂ that inhibits the triggering receptor expressed on myeloid cells 1 (TREM-1). LR12 was shown to correct the severity of colitis clinically, endoscopically and histologically in a DSS-induced mouse model of colitis [177]. TREM-1 is expressed on the majority of innate immune cells and to a lesser extent on parenchymal cells. The frequency of TREM-1-expressing neutrophils and recruited macrophages has been found to be higher in inflamed than in non-inflamed biopsies from patients with UC and CD [178]. Injection of LR12 peptide in DSS-induced model mice generated a significant increase of

macroautophagy (ATG1/ULK-1, ATG13, ATG5, ATG16L1, and MAP1LC3-I/II) and CMA (HSPA8 and HSP90AA1) protein expression. This impressive effect of LR12 peptide was confirmed genetically using *Trem-1* knockout mice [177]. TREM-1 inhibition prevented dysbiosis.

To our knowledge, the cell-permeable trans-activator of transcription (TAT)-coiled-coil, moesin-like BCL2-interacting protein (BECLIN)-1 peptide construct (YGRKKRRQRRRGGTNVFNATFEIWHDGEFGT) [179], which has shown some promise in several neurological, infectious and tumoral settings and is now commercialized, has not been evaluated in experimental models of IBDs. TAT-BECLIN peptide might have interesting applications since it has been proposed that BECLIN-1 regulates TJ barrier *via* endocytosis of occludin (a 65-kDa tetraspan integral membrane protein) in an ERK- and a MTORC2-dependent way [180].

P140 (Rigerimod, IPP-201101) is another peptide that selectively targets autophagy processes, and more especially CMA. This 21-mer phosphopeptide corresponding to the sequence H-RIHMOVYSKRSGKPRGYAFIEY-OH (residues 131-151) was described in 2003 [181]. It was initially spotted in a cellular screening assay using overlapping peptides covering the whole spliceosomal U1-70K protein and CD4⁺ T cells collected from MRL/lpr mice lymph nodes. A number of analogues have been produced and the one that possesses the most favorable properties identified nowadays contains a phosphoserine residue in position 140, which is crucial for its activity and stability [182–185]. P140 is not immunogenic [185], it is safe and displays no immunosuppressive activity in mouse and human settings [186–190]. It was shown to directly interact with HSPA8 [182,191] and to inhibit the chaperone activity of the latter [182,183]. It also alters HSPA8 shuttling between the cytoplasm and the nucleus/nucleoli in case of stress [192]. It has been shown *in vitro* and *in vivo* that P140 enters MRL/lpr spleen B cells *via* a clathrin-dependent pathway and accumulates into lysosomes [148]. Notable effects associated with different components of the autophagy process were identified after treating cells and autoimmune mice with P140. The levels of HSPA8 and LAMP2A, which are overexpressed in MRL/lpr B cells, are corrected after P140 treatment. P140 has no direct effect on B cell receptor signaling in memory, naive, mature, transitional, or B1 human cells, suggesting that it does not alter B cell survival and maturation in these B cell subsets [190]. However, likely as a matter of consequence resulting from its interaction with HSPA8, it strongly reduces the overexpression of major histocompatibility complex (MHC) class II molecules on lupus B cells acting as antigen-

presenting cells and hampers peptide–MHC molecule loading in late lysosomal vesicles [143,148,183,190]. This impressive effect shown both in mice and in human settings decelerates the complex signaling cascade leading to the final production of pathogenic autoAbs. P140 effectively down-regulates T cell activation [187] and consequently reduces the differentiation of human B cells into plasma cells and IgG secretion [190]. Altogether, these results indicate that by interfering with overactivated autophagy processes, P140 peptide efficiently affects the processing of endogenous (auto)antigen, the peptide loading to MHCII molecules, and the entire down-stream deleterious proinflammatory events. It must be emphasized that the normal immune system is not affected in this scheme, and that experimental MRL/lpr mice are still capable of developing cellular and humoral immune reactions towards a pathogen [187]. In a multicenter, randomized, placebo-controlled phase-IIb study for lupus, P140/Lupuzor was found to be safe and met its primary efficacy endpoints, confirming pre-clinical data generated in MRL/lpr lupus-prone mice [189]. Lupuzor is currently evaluated in phase-III clinical trials in the US, Europe, and Mauritius. An open-labeled trial including several hundred lupus patients worldwide is planned. It is also evaluated in the context of other autoimmune or inflammatory conditions and showed some promises in preclinical studies including experimental animal models [143,150,151]. Preliminary data tend to show that P140 could also be a highly valuable tool for treating IBDs. Future investigations based on chemically- and genetically-induced murine models, organoids, and cells collected from patients with CD and UC are warranted to determine if P140 could be exploited as a potent drug in affected patients.

Concluding remarks

Research for therapeutic options to treat IBDs identified new compounds targeting elements involved in maintaining intestinal homeostasis and identified biomarkers allowing detection of inter- and intra-personal variations in patients (see [Clinician's Corner](#)). In the pipeline of new possible treatments, elements of the autophagy process are particularly indicated. In this context, a crucial aspect that has largely hampered the clinical applications of autophagy-based therapeutic strategies is that until now, among the large set of existing activators/inhibitors molecules, very few are strictly selective to one autophagy pathway and one target. In general, molecules such as rapamycin, HCQ, trehalose, metformin, perifosine (inhibitor of protein kinase B or AKT), minocycline (a semi-synthetic tetracycline derivative with dual properties on autophagy processes), or niclosamide (inhibitor of MTORC1) interact with several targets and receptors [193]. This favors SAEs and therefore limits their use as

478 drugs. Intense research is therefore devoted to identification of small molecules and peptides
479 to precisely up- or down-regulate specific autophagy processes that are pathologically
480 defective without interfering with the other autophagy processes.

481 Another aspect that further complicates the design of new strategies based on deregulated
482 autophagy is that in a single individual, autophagic activity can be raised in certain organs or
483 tissues, and diminished in others [143,194], in an order that can vary from patient to patient
484 [194]. More research is therefore eagerly needed to understand the interplay between the
485 different autophagic pathways that are supposed to protect cells and ensured cell homeostasis,
486 and the effects of counterbalance between them in the same organ, and in cells of different
487 organs (see Outstanding Questions). We should not have a reductionist definition of
488 phenomena, and just claim that autophagy, as a whole, is exacerbated or compromised in a
489 particular illness. Instead, we would be well advised to more precisely define the type of
490 autophagy pathway, and in which organ and cell subtype these defaults occur [168]. Further
491 investigation is also needed to discover valid predicting markers of drug responsiveness. This
492 whole set of information is crucial to direct rescuing molecules to specific sites of autophagy
493 dysregulation, and to design more personalized and safe therapeutic options. Finally, new
494 directions taking into account the specific infectious facet of IBDs-affected patients should
495 lead to new precision medicine development based on molecules that selectively target
496 xenophagy, which would contribute to eliminating invading pathogens and therefore an
497 innovative segment that deserves most attention.

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506 **Conflict of interest**

507 SM has the following conflicts of interest to disclose: Research funding (paid to institution)
508 and past consultant for ImmuPharma; co-inventor of CNRS-ImmuPharma patents on P140

509 peptide. The authors declare that the research was conducted in the absence of any
510 commercial or financial relationships that could construed as a potential conflict of interest.

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Figure Legends

Figure 1: Targets of the major medications indicated for the treatment of IBDs. Existing drugs and compounds under development commonly target two large areas of regulation, namely inhibition of cytokine signalling pathways (left) and inhibition of leukocyte trafficking to the gut mucosa (right). The target of therapeutic antibodies (green) and small molecules (violet) are shown. Most if not all of these regulating compounds are inhibiting/blocking agents. Abbreviations: $\alpha 4\beta 1/7$, integrin alpha 4 beta 1/7; IL, interleukin; JAK, Janus kinase; TYK, tyrosine kinase; MadCCAM1, mucosal addressin cell adhesion molecule 1; TGF- β , transforming growth factor beta; TNF, tumor necrosis factor; S1P-R, sphingosine-1-phosphate receptor; SMAD7, mothers against decapentaplegic homolog 7.

Figure 2: Signalling pathways that control the macroautophagy machinery and sites of action of past and novel autophagy-regulating pharmacological tools. Autophagy can be activated by inhibitors of MTOR (rapamycin, torin1, P2281, niclosamide, temsirolimus/CCI-779), AKT (perifosine), TREM-1 (LR12 peptide) as well as by activation of AMPK (metformin, minocycline, trehalose). Inhibitors of autophagy include PI3K inhibitors (3-MA, wortmannin, LY294002, SAR405, VPS34-IN1, spautin, PT21, CH5132799, GDC-0941), initiation inhibitors (MRT67307, MRT68921, SBI-0206965, NSC185058, edaravone, cardiac glycosides), a phagophore elongation inhibitor mdivi-1 and autophagosome-lysosome fusion inhibitors (e.g. bafilomycin A, vinblastine, nocodazole, monensin). The final degradation step of autophagy can also be inhibited by compounds such as HCQ, CQ, bafilomycin A, lys05, leupeptin, pepstatin, E64d, mefloquine, and NH_4Cl . In lupus, administration of P140 peptide significantly decreases the overexpression of LAMP2A and HSPA8, which are key factors of CMA. Abbreviations not used in the text: 3-MA, methyladenine; Mdivi-1, mitochondrial division inhibitor. P2281, LY294002, Lys05, SAR405, VPS34-IN1, PT21, CH5132799, GDC-0941 MRT67307, MRT68921, NSC185058, SBI-0206965, NSC185058 are compound codes used in the literature.

Figure 3: The role of autophagy in the pathogenesis of IBDs. Genetic predispositions in autophagy-related genes (*ATG16L1*, *IRGM*, *LRRK2*, *NOD2/CARD15*) are largely reported in IBDs. Upon environmental triggers, such as pathogens and unhealthy diet, defects in multiple steps of autophagy may lead to altered functions of the intestinal barrier and abnormal intestinal immune responses, which can collectively manifest as IBDs. Abbreviations: TJ, tight junctions; APC, Antigen-presenting cells; ER, endoplasmatic reticulum.

1020 **Table 1: Therapeutic strategies currently approved and/or in current use for the**
1021 **treatment of IBDs**

Drug		Mechanism of action /target	Efficacy (significant results)	Serious Adverse Effects	Clinical status	Refs.
Small molecules (corticoids and immunosuppressants)						
Amino salicylates (sulfasalazine, mesalazine, 4-aminosalicylic acid, balsalazide, olsalazine)		Free radical scavengers, 5-lipoxygenase inhibition, effects on leucocyte function and production of cytokines	Clinical remission rates of 40–70% have been reported with mesalazine over 6–8 weeks in UC	Nephrotoxicity, agranulocytosis, alveolitis, pancreatitis, abdominal pain, flatulence, nausea, dyspepsia	Common use	[57,59]
Prednisone, 6-methylprednisolone, budesonide MMX		Binds to high affinity intracellular cytoplasmic receptors	Clinical remission at 8 weeks (17.4%-Budesonide MMX 9 mg vs. 4.5%-placebo) in UC	Diabetes, osteoporosis, moon face, and acne, growth retardation in children, psychosis, hepatic steatosis	Common use	[47,48, 68,70]
Thiopurines (6-mercaptopurine, azathioprine)		Incorporates into nucleotides	Maintain remission in moderate to severe CD/UC	Myelosuppression, non-Hodgkin lymphoma	Common use	[61]
Methotrexate		Inhibits enzymes in folic acid metabolic pathway (for high doses used in oncology/ hematology; mode of action is unknown for low doses used in CD)	Clinical remission at 16 weeks (39%-25 mg vs. 19%-placebo; 65%-15 mg vs. 39%) in CD	Dyspnoea, nausea, vomiting, and neutropenia	Common use	[63,64]
Cyclosporine		Calcineurin inhibitor	Cyclosporine (4 mg/kg) showed 82 % response rate vs. placebo (P < 0.001) in 7 days in UC	Renal failure, bacterial pneumonia, Pneumocystis jiroveci pneumonia, venous catheter infections	Common use	[46]
Tacrolimus		Calcineurin inhibitor	Clinical remission at 2 weeks (9.4% vs. 0.0%-placebo) in UC	Tremor, paraesthesia, insomnia, hot flush, alopecia, hyperglycaemia, hypomagnesaemia, hypertension, hepatotoxicity, nephrotoxicity	Common use	[56]
Therapeutic antibodies						
Generic name	Trade name/ synonym					
Infliximab	Remicade	TNF- α antagonist	Clinical remission at 30 weeks (35.8%-10 mg vs.	Drug-induced lupus, infusion reactions, hypersensitivity	Approved by FDA since 2007 for CD/UC	[49,65]

			15.7%-placebo) in UC. Clinical remission at 10 weeks (57.5%) in CD	reactions, demyelination, reactivation of latent tuberculosis, Non-Hodgkin's lymphoma		
Adalimumab	Humira	TNF- α	Clinical remission at week 56 (36%-400 mg eow, 41%-400 mg weekly, vs. 12%-placebo) in CD	Congestive heart failure, lupus-like syndrome, lymphoma, cytopenia, multiple sclerosis/neurological disease, pancytopenia	Approved by FDA since 2007 for CD/UC	[62]
Certolizumab Pegol	Cimzia	TNF- α	Clinical response at 10 weeks (52.8%-400 mg vs. 30.1%- placebo) in CD	Injection site reaction, infections, lupus-like syndrome	Approved by FDA since 2008 for CD	[50,51, 54]
Golimumab	Symponi	TNF- α	Clinical remission at 54 weeks (27.8%-100 mg vs. 15.6%-placebo) in UC	Erythema, tuberculosis, rectal, thyroid, and lung adenocarcinoma	Approved by FDA and EMA since 2003 for UC	[69,72]
Ustekinumab	Stelara	IL-12/ IL-23	Clinical remission at 44 weeks (53.1%-90 mg every 8 weeks and 48.8%-90 mg every 12 weeks vs. 35.9%-placebo) in CD	Nasopharyngitis upper respiratory tract infections, diverticulitis, cellulitis, pneumonia	Approved by FDA since 2016 for CD	[53,58, 66,71]
Natalizumab	Tysabri	$\alpha 4$ integrin	Clinical remission at 8 weeks (26%-300 mg vs. 16%-placebo) in CD	Pharyngitis, urinary tract infection, urticaria, cephalgia, arthralgia, PML	Approved by FDA since 2004 for CD	[52]
Vedolizumab	Entyvio	$\alpha 4\beta 7$ integrin	Clinical remission at 52 weeks (44.8%-300 mg vs. 29.1%-placebo) in CD	Gastrointestinal and respiratory tract infections, hepatic steatosis	Approved by FDA since 2014 for CD/UC	[55,60, 67]
Other small molecules						
Tofacitinib	Xeljanz	Pan-JAK inhibitor	Clinical remission at 8 weeks (18.5%-10 mg vs 8.2%-placebo) in UC	Herpes zoster infection, upper respiratory tract infections, headache, diarrhea, nasopharyngitis	Approved by FDA since 2018 for UC	[121]

1022 Abbreviations not used in the text: vs., versus

1023

1024 **Table 2: Therapeutic strategies currently under clinical evaluation for IBDs**

Drugs		Mechanism of action /target	Efficacy (significant results)	Serious Adverse Effects	Clinical status	Refs.
Generic name	Trade name/ synonym					
Therapeutic antibodies						
Golimumab	Symponi	TNF-α	Retrospective analysis in 115 CD patients: Clinical response 55.8% in 4 months	Infections, drug-induced lupus	No formal trials have been done for CD	[72,95]
Risankizumab	BI-655066 or ABBV-066	IL-23(p19)	Clinical remission at 12 weeks (31% vs. 15%-placebo) in CD	Nausea, worsening of underlying CD	Phase III trials for CD	[94]
Brazikumab	AMG 139 or MEDI2070	IL-23(p19)	Clinical remission at 8 weeks (49.2% vs. 26.7%-placebo) in CD	Headache, nasopharyngitis	Phase II trials for CD	[99]
Briakinumab	ABT-874	IL-12/23(p40)	Clinical remission at 24 weeks (48%-400 mg, 57%-700 mg vs. 29%-placebo) in CD	Respiratory tract infection, nausea, abdominal pain, headache, cardiovascular events	Phase II trials for CD	[85,90]
Mirikizumab	LY3074828	IL-23 (p19)	Clinical remission at 12 weeks (31% vs. 4.8%-placebo) in UC	No SAEs reported	Phase II trials for CD/UC	[103]
Etrolizumab	rhuMAb β7	β7 subunit of integrins α4β7 and αEβ7	Clinical remission at 10 weeks (21%-100mg vs. 10%-placebo) in CD	Exacerbation of UC, headache, fatigue, abdominal pain, dizziness, nasopharyngitis, nausea, arthralgia, urinary tract infection	Phase III trials for CD/UC	[88,92]
	PF-00547659	MAdCAM-1	Clinical remission at 12 weeks (16.7%-22.5 mg vs. 2.7%-placebo) in CD	No SAEs reported	Phase II trials for CD/UC	[98,101]
Abrilumab	AMG181	α4β7 integrin	Clinical	Upper respiratory	Phase II	[87]

			remission at 12 weeks (30.8%-210 mg vs. 17.6%-placebo) in CD	tract infection, headache	trials for CD/UC	
Small molecules						
Filgotinib	GLPG0634	JAK-1	Clinical remission at 10 weeks (47% vs. 23%-placebo) in CD	No SAEs reported	Phase III trials for CD/UC	[100]
Upadacitinib	ABT-494	JAK1	Clinical remission at 16 weeks (27%-6 mg vs. 11%-placebo) in CD	Headache, non-melanoma skin cancer	Phase II trials for CD/UC	[97]
Ozanimod	RPC1063	sphingosine-1-phosphate receptor	Clinical remission at 32 weeks (21%-1 mg, 26%-0.5 mg vs 6%-placebo) in UC	Headache, anemia, nasopharyngitis, urinary-tract infections	Phase III trials for UC	[96]
Mongersen	GED 0301	TGF- β 1	Clinical remission at day 15 (55%-40 mg, 65%-160 mg vs 10%-placebo) in CD	No SAEs reported	withdrawn after interim analysis of a phase III trial for CD	[89,102]
	AJM300	α 4 integrin	Clinical remission at 8 weeks (23.5%-960 mg vs. 3.9%-placebo) in UC	Potential risk of PML	Phase III trials for UC	[91]
Laquinimod	ABR-215062 or TV-5600	Inhibitory effect on antigen presenting cells and T cells	Clinical remission at 8 weeks (48.3%-0.5 mg vs. 15.9%-placebo) in CD	Headache, CD exacerbation	Phase II trials for CD	[86]
	ABX464	Triggers IL-22 secretion in macrophages	50 mg, daily for 2 months, clinical remission 35% vs 11% placebo in UC	Headaches, nausea and vomiting (not considered as treatment-limiting effects)	Phase II trial for UC	[93]

1025 Abbreviations not used in the text: vs., versus.

Box 1: Autophagy

Autophagy is a vital cellular process in which a cytoplasmic cargo is delivered to lysosomes for degradation and recycling. This evolutionary-conserved intracellular pathway is finely-regulated by a large family of genes [195,196]. It continuously clears unnecessary or dysfunctional cellular components (damaged organelles, abnormally folded proteins or proteins produced in excess). Autophagy is crucial for cell adaptation to the environment and maintenance of cell homeostasis, especially under stress conditions (nutrient deprivation, hypoxia, oxidative stress, changes of intracellular level of calcium). Three main forms of autophagy have been described: **macroautophagy**, **microautophagy** and **chaperone-mediated autophagy** (CMA). Besides these defined types, other forms of selective autophagy are operating, e.g., mitophagy, that selectively disrupts damaged mitochondria by autolysosomal degradation, **xenophagy** and others [195]. One of the major mechanisms by which autophagy affects the pathogenesis of IBDs is the regulation of pathogen clearance. Autophagy in Paneth cells, macrophages, and goblet cells in the intestinal wall targets invading pathogens for degradation or helps in secretion of antimicrobial peptides. In addition to pathogen clearance, autophagy plays a critical role in adaptive immune response through MHC class II-dependent antigen presentation, as substrates of autophagy can be loaded onto MHC. Mice lacking ATG5 in thymic epithelium develop severe colitis implicating the requirement of autophagy in adaptive immune response in protection against IBDs [123,124].

Macroautophagy: In this canonical autophagy process, which is highly genetically-controlled, a double-membrane sequestering compartment termed “phagophore” is formed and expands in encapsulating cytoplasmic cargos. The resulting sealed, double-membrane vacuoles termed “autophagosomes,” subsequently fuse with hydrolytic enzyme-rich lysosomes to form vacuoles called “autolysosomes” in which the cellular cargos that have been engulfed are degraded. The resulting compounds that are cleaved by hydrolases are released back into the cytosol for reuse (recycling).

Microautophagy: This dynamic form of autophagy is characterized by direct engulfment of cytoplasmic cargos in lytic organelles (lysosomes in mammals and vacuoles in plants and fungi).

Chaperone-mediated autophagy: CMA is a selective form of autophagy that, in contrast to macroautophagy and microautophagy, does not involve vesicles, but instead implicates

chaperone proteins to directly target specific proteins to the lumen of lysosomes. CMA-targeted cargos are soluble cytoplasmic proteins, which contain a KFERQ-related pentapeptide motif that is recognised by HSPA8. Once docked on the outside of the lysosomal membrane, the targeted protein begins to unfold before it is internalized into the lysosomal lumen with the help of other chaperone and co-chaperones, including lysosomal HSPA8 and HSP90 proteins. LAMP2A plays a crucial role in the translocation process. Proteases and hydrolases that optimally function at low pH in the lysosome lumen degrade the selected unfolded cargo and recycle critical amino acid residues.

Xenophagy: This selective autophagy process is used to eliminate invading pathogens. Intracellular pathogens that are either inside the cytosol or in pathogen-containing vacuoles are surrounded by isolation membranes, engulfed into autophagosomes, and degraded inside autolysosomes.

Box 2: Mechanisms of action of anti-TNF antibodies

The mechanism of action of anti-TNF Abs can be many, including simple neutralisation of TNF- α ligand, modulation of the immune system, outside-to-inside signalling and the induction of direct or indirect apoptosis [197,198] (and references in Table 1). Infliximab, which was the first anti-TNF- α mAb reported to be successful for the treatment of IBDs in 1993, binds with high affinity to soluble and transmembrane TNF- α but not to lymphotoxin- α (also called TNF- β). Adalimumab neutralizes the activity of TNF- α by inhibiting its interaction with p55 and p75 cell surface TNF- α receptors. Its clinical efficacy was proven for maintaining remission in moderate to severe CD through 56 weeks. Its safety and efficiency were also demonstrated in patients with secondary loss of response. As infliximab and adalimumab, certolizumab pegol binds and neutralises TNF- α . However, thanks to the fact that it does not contain an Fc region, an important structural difference with regard to infliximab and adalimumab, certolizumab pegol does not mediate complement-dependent cytotoxicity and Ab-dependent cell-mediated cytotoxicity and hence emerges as an attractive alternative anti-TNF drug. Although observed in a small proportion of patients, anti-TNF therapy displays wide-ranging effects on the immune system resulting in a spectrum of potential adverse events. Many efforts are developed to minimize these complications [80,81,83].

Box 3: ATG16L1, an autophagy-related gene that is associated with IBDs risk

ATG16L1 is an essential component of autophagy [199]. It undergoes self-multimerization and forms a heterocomplex with ATG5 and ATG12, which acts as a scaffold to MAP1LC3 for lipidation. Among the nine genetic variants of ATG16L1 that have been associated to CD, the variant rs2241880, comprising a missense mutation resulting in threonine to alanine substitution at the amino acid position 300 has been associated to an increased risk to develop the disease. The T³⁰⁰A mutation is located in the cleavage site of caspase 3, and this mutation enhances the degradation of ATG16L1 by caspase 3, and hence diminishes autophagy. Loss of function of ATG16L1 inhibits autophagy in intestinal Paneth cells, resulting in a decreased production of antimicrobial peptides. Besides, ATG16L1 risk variants are defective in the generation of MHCII antigen-specific CD4⁺ T cell responses in DCs.

Box 4: Metformin, a molecule that displays pleiotropic effect on autophagy

Metformin (or 1,1-dimethyl biguanide) is a drug which is widely given to patients with type 2 diabetes. Recent investigations led to the discovery that this synthetic derivative of guanidine displays a larger spectrum of properties than expected and could, therefore, be advantageously used in other indications, as notably autoimmune diseases, certain cancers (breast, ovarian, glioblastoma), and in aging [200,201]. Metformin is an inducer of autophagy that acts in an AMP-activated protein kinase (AMPK)-dependent manner, which phosphorylates the Unc-51 like autophagy activating kinase-1 (ULK-1/ATG1) and BECLIN 1. Metformin interacts with several receptor molecules and, directly or indirectly, interferes with several cellular pathways that are vital in cell metabolism and regulation, notably in immune cells. The mitochondrial respiratory-chain complex 1 (OCT1) is presented as the primary target of metformin [202]. The preferential action of metformin in hepatocytes is due to the predominant expression of OCT1, which has been shown to facilitate cellular uptake of metformin. It seems that metformin does not directly target AMPK but would activate AMPK in a process that would be secondary to its effect on the mitochondria, the primary target of the drug. Regarding the effect of metformin on insulin receptor, it was found that it could act through the inhibition of PTP-1B, a phosphatase that inhibits the insulin receptor tyrosine kinase activity.

Clinician's Corner

- Genetic susceptibility, environmental factors, microbial flora, and alterations affecting both the innate and adaptive immune systems are common components that are emphasised as major contributors to the complex set of inflammatory bowel diseases (IBDs). Most

1121 importantly, the cytokine imbalance of pro-inflammatory and favourable regulating cytokine
1122 responses is thought to be critically involved. The current strategies consider these elements
1123 and some immunosuppressive drugs, corticoids and biologics have shown efficacy in
1124 reducing, at least transiently, the disease progression.

1125
1126 ● Some of the current drugs display harmful effects that can generate even more dramatic
1127 health status. Thus, deciphering further the molecular and cellular elements giving rise to
1128 IBDs is necessary.

1129
1130 ● The objective of novel therapeutic strategies is to replace disease-modifying medications
1131 by mechanism-driven therapies, which will be much more targeted and specific than the
1132 current ones and should therefore prove to be safer for the patients. Such targeted therapies
1133 could be personalised if appropriate biomarkers of responsiveness can be identified, avoiding
1134 thus the use of medications that are ineffective in individual patients.

1135
1136 ● Due to the diversity of symptoms in IBDs, and the extent and location of inflammation, it is
1137 unlikely that a single drug will correct all the defaults in the millions of patients affected by
1138 CD and UC. Combination therapy, or polytherapy, should help to control these aspects. In
1139 these cases, however, a particular caution should be taken to avoid administration of selective
1140 molecules with opposite properties that can adversely occur in certain individuals.

Glossary

HCQ (hydroxylated analogue of chloroquine): this potent autophagy inhibitor prevents lysosomal acidification, thereby interfering with a key step in the autophagic process. Also acts as a TLR7/9 inhibitor.

IRGM: belongs to the p47 immunity-related GTPase family. Is implicated in autophagy induction and autophagosome maturation. Reduced expression of IRGM increases the survival of the CD-associated adherent-invasive *E. coli* strain, correlated with decreased autophagy-mediated bacterial clearance. Multiple CD-associated polymorphisms have been found in the IRGM locus affecting the protein expression and splicing. A CD-associated exonic synonymous single nucleotide polymorphism alters the binding of microRNA-196 to the IRGM risk variant. MicroRNA-196 is overexpressed in inflamed intestinal tissue resulting in the loss of regulation of IRGM expression levels and hence defective autophagy-mediated clearance of adherent-invasive *E. coli* bacteria.

LRRK2: a multifunctional kinase localising to endolysosomal compartments and specific membrane microdomains. Is thought to be a regulator of macroautophagy and CMA. Its expression level is higher in colon biopsy specimens of patients with CD.

NOD2/CARD15: an intracellular pattern-recognition protein expressed in intestinal Paneth cells and monocyte-derived immune cells. Around one-third of patients with CD harbour NOD2 mutation with a 17-fold increased risk of the disease. Three mutations within the leucine-rich repeat region have been associated with CD. Acts as a muramyl peptide sensor, which activates the NF- κ B pathway in response to bacterial infections. Interacts with ATG16L1 to induce an autophagic response against bacteria. Its stimulation leads to autophagy-dependent upregulation of MHCII molecules and generation of antigen-specific CD4⁺ T cell responses.

Tregs: a subset of CD4⁺ Th cells that express the transcription factor Foxp3 and potently suppress many immune responses.

Figure 1

Figure 3