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## The future of inhalation therapy in chronic obstructive pulmonary disease

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### ABSTRACT

The inhaled route is critical for the administration of drugs to treat patients suffering from COPD, but there is still an unmet need for new and innovative inhalers to address some limitations of existing products that do not make them suitable for many COPD patients. The treatment of COPD, currently limited to the use of bronchodilators, corticosteroids, and antibiotics, requires a significant expansion of the therapeutic armamentarium that is closely linked to the widening of knowledge on the pathogenesis and evolution of COPD. The great interest in the development of new drugs that may be able to interfere in the natural history of the disease is leading to the synthesis of numerous new molecules, of which however only a few have entered the stages of clinical development. On the other hand, further improvement of inhaled drug delivery could be an interesting possibility because it targets the organ of interest directly, requires significantly less drug to exert the pharmacological effect and, by lowering the amount of drug needed, reduces the cost of therapy. Unfortunately, however, the development of new inhaled drugs for use in COPD is currently too slow.

### 1. Introduction

Inhalation therapy is central when treating patients with chronic obstructive pulmonary disease (COPD), with bronchodilators and anti-inflammatory drugs being recommended by international treatment strategy documents at any stage of the disease (Halpin et al., 2021; Boulet et al., 2019). In order to deliver drugs by inhalation it is essential to use inhaler devices, the choice of which is therefore as important as that of the drugs (Donner et al., 2018).

Regrettably, the development of an inhaler device is very often unrelated to the drug(s) to be administered using that inhaler (Cazzola et al., 2020a). Unquestionably, it is easier, quicker, and cheaper for drug companies to innovate treatment through technological modifications of inhaler devices using drugs that have become generic than to research and develop new pulmonary drugs to be delivered with a new inhaler (Cazzola et al., 2020a).

As knowledge about the pathogenesis and evolution of COPD has expanded, it has become increasingly obvious that the treatment of COPD cannot be limited to the use of classical inhaled bronchodilators ( $\beta_2$  agonists and muscarinic antagonists) and corticosteroids (Cazzola and Matera, 2021). Some new drugs are in development (Cazzola et al., 2019a; Rogliani et al., 2020; Matera et al., 2021a, 2021b, 2021c; Ora

et al., 2020a), but it is difficult to predict which ones will be approved for clinical use because the translation to humans of interventions that are effective in animal models is always not easy and often unreliable (Cazzola and Matera, 2021).

Nevertheless, many researchers have a real interest in developing effective drugs capable of interfering in the natural history of the disease. Administering such drugs by inhalation can be extremely important because by this route they are delivered directly into the affected organ, which means that it is possible to reduce the quantity of the drug to obtain the same therapeutic effect, with a consequent great economic advantage related to the reduction of the cost of therapy (Cazzola et al., 2020a).

However, designing and then synthesizing inhaled drugs are extremely difficult tasks as they are dependent on many factors to be considered simultaneously, such as the nature of the target (intracellular vs. extracellular, receptor vs. enzyme), the mode of action (agonist vs. antagonist, activator vs. inhibitor), the desired length of the action and how to achieve it (pharmacokinetics vs. receptor or enzyme binding kinetics, limited solubility vs. soluble compounds) the desired onset of action, and the systemic risk (is a systemic 'spill over' level tolerated?) (Pasqua et al., 2021).

In any case, regardless of all other considerations, the constant

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requirement for any new drug is to ensure that it reaches the site of action as a free form in solution in sufficient quantity and for enough time to exert the desired biological effect (Pasqua et al., 2021).

The aim of this narrative review is to examine and report on the literature concerning the future of inhalation therapy in COPD with a particular focus on new therapies that can be used by inhalation and have already been tested in humans.

## 2. Search strategy

A literature review was performed via PubMed and Scopus databases to identify English-language studies related to our stated purpose for publications available up to October 2021. Thereafter, evaluation of the references of the selected articles identified other relevant publications. All authors participated in evaluation of literature.

## 3. Evolution in pulmonary drug delivery devices

There has been a substantial evolution in inhaled medicine dispensers also because pharmaceutical aerosol technology has developed rapidly in recent years (Cazzola et al., 2020a). The last two decades have seen not only an increase in the delivery capacity of inhalers, but also the refinement of drug formulations (Rogliani et al., 2017). Furthermore, there are numerous technical innovations under development (Cazzola et al., 2020a).

Improvements in inhaler devices and drug formulations are beginning to allow for a more personalized approach to inhaler therapy (Cazzola et al., 2020a). Unfortunately, however, we are still far from having an inhaler that can be deemed perfect. Consequently, considering the advantages and disadvantages of any inhaler device, we must choose among the various options available, taking into account the difficulties that may be encountered especially by frail patients, those who are older, and/or are suffering from comorbidities, such as arthritis or visual, hearing, and cognitive impairment, which may affect the proper use of the device (Cazzola and Rogliani, 2015).

There is clearly an unmet need for new inhalers with substantial innovations to address at least some of the existing limitations (Cazzola et al., 2020a). It was suggested that there are potentially three main areas in which we can expect to see future innovations in inhaler therapy: device engineering and design; chemistry and formulations; and digital technology associated with inhalers (Biddiscombe and Usmani, 2018).

The first area is extremely complex and technical. The reader is therefore referred to the specific literature on it (Longest et al., 2019; Hickey, 2020; Ari and Fink, 2020).

However, the use of nanotechnologies, incorporating liposomes, niosomes, nanoparticles, nanoemulsions, nanosuspensions and exosomes into inhalers, induces targeted effects (Gulati et al., 2021). It allows the maximum dose to reach the lungs with improved drug solubility and distribution and prevents hepatic first-pass metabolism, improves patient compliance, and reduces drug side effects (Gulati et al., 2021). However, part of the drug can still be found in the systemic circulation because it is absorbed through the gastrointestinal tract. In any case, although in general this may predispose to the occurrence of inhaled drug side events, it is advantageous when there is a need to target inhaled therapy beyond the lungs to interfere with COPD comorbidities (Mortensen and Hickey, 2014).

The use of smart delivery systems will soon be a likely advance. Indeed, by taking advantage of the integration of microprocessors into inhaler devices, it will help to understand how the patient breathes and thus capture those small changes that the he/she does not perceive but are important in evolution of the disease (Biddiscombe and Usmani, 2018). This will allow the dose of drug delivered to the lungs to be tailored to the patient's needs. Environmental information will also be recorded, which will identify potential triggers. A wide application of digital technology will certainly facilitate the fundamental interaction between patient and physician, providing also important information on

treatment adherence, and will allow a more correct self-management by the patient (Biddiscombe and Usmani, 2018). However, there is a need for additional studies focused on specific aspects of inhaled drug delivery, including cost-effectiveness analysis (Cazzola et al., 2020a).

### 3.1. Novel inhaled drugs for treating COPD

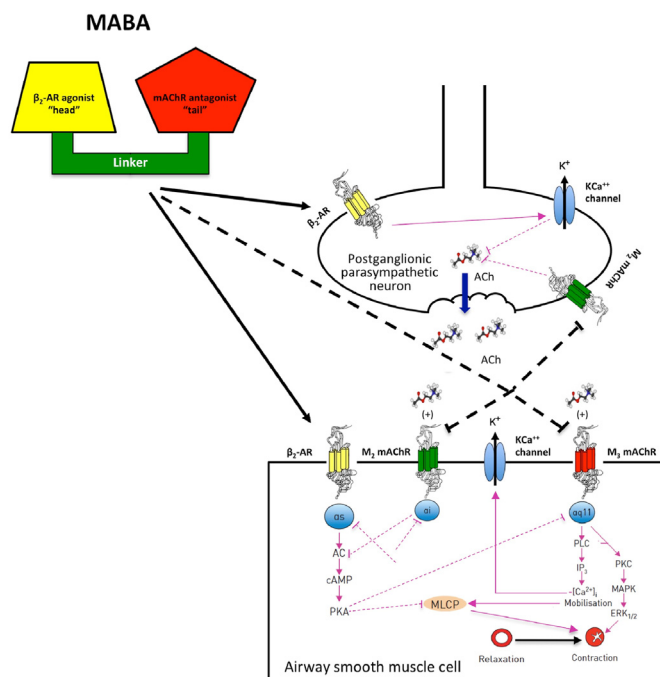
At present, the search for new drugs to treat COPD remains largely focused on molecules capable of inducing relaxation of airway smooth muscle and on those that elicit anti-inflammatory effects in some way (Matera et al., 2021c). However, the development of drugs that can also act on the possible infectious component of COPD is not neglected.

### 3.2. Novel inhaled bronchodilators

Although bronchodilators are still the pivotal drugs in the treatment of COPD, critical aspects such as their inability to modify the course of the disease, the possible adverse events they may induce, and the loss of efficacy over time especially of  $\beta_2$  agonists make the development of new classes of bronchodilators an absolute need (Cazzola et al., 2019a).

An attractive innovation is to develop bifunctional drugs, those capable of exerting two distinct but complementary primary pharmacological actions (Page and Cazzola, 2014). Indeed, some bifunctional bronchodilators and bifunctional bronchodilator/anti-inflammatory drugs are in different stages of preclinical or even clinical development (Page and Cazzola, 2017).

MABAs (muscarinic antagonist- $\beta_2$  agonist) that combine muscarinic antagonism and  $\beta_2$  agonism into a single molecule (Hughes and Jones, 2011) (Fig. 1) represent the main innovation currently under investigation in the field of bronchodilation. However, their clinical development is extremely slow, although there are several MABAs (batefeneterol,



**Fig. 1.** MABA dual pharmacology molecule concept of combining the mAChR antagonist and  $\beta_2$ -AR agonist moieties into a single molecule and synthesis of the cross-talk between the two moieties. AC: adenylyl cyclase; ACh: acetylcholine; cAMP: cyclic adenosine monophosphate; ERK: extracellular signal-regulated kinase; GR: glucocorticoid receptor; IP<sub>3</sub>: inositol-3-phosphate; KCa<sup>2+</sup>: calcium-activated potassium channel; mAChR: muscarinic ACh receptor; MAPK: p38 mitogen-activated protein kinase; MLCP: myosin light chain phosphatase; PKA: protein kinase A; PKC, protein kinase C; PLC: phospholipase C;  $\beta_2$ -AR:  $\beta_2$ -adrenoceptor. Solid line, activation; dotted line, inhibition.

navafenterol, CHF6366, AZD8999/LAS190792, AZD2115, and THRX200495) that are in clinical development (Ora et al., 2020b).

Batefenterol or GSK961081 was found to be effective, safe, and well tolerated in COPD and will likely be the first to enter clinical practice (Wiolders et al., 2013). The documentation that at doses  $\geq 150$   $\mu\text{g}$  it induced improvements in lung function comparable to those achieved with umeclidinium/vilanterol 62.5/25  $\mu\text{g}$  once daily in patients with COPD and  $\text{FEV}_1 \geq 30\%$  and  $\leq 70\%$  predicted makes this drug appealing (Crim et al., 2019). Navafenterol or AZD8871/LAS191351 at a dose of 400  $\mu\text{g}$  caused significant and sustained bronchodilation that was superior to that induced by placebo, and at a dose of 1800  $\mu\text{g}$  was more effective than 150  $\mu\text{g}$  indacaterol or 18  $\mu\text{g}$  tiotropium, without eliciting the occurrence of adverse events (Singh et al., 2020a). Furthermore, at a dose of 600  $\mu\text{g}$  once daily it provoked  $\text{FEV}_1$  changes in patients with COPD similar to those observed with umeclidinium/vilanterol 62.5/25  $\mu\text{g}$  once daily (Singh et al., 2021). CHF6366 is another interesting MABA. In an experimental setting using dogs, it, but neither batefenterol nor formoterol, induced complete bronchoprotection for 24 h without causing adverse effects (Carnini et al., 2017). In male healthy volunteers, CHF6366 was well tolerated, with a safety profile similar to that of placebo, and very modest systemic exposure at all doses tested (Kots et al., 2021).

The use of MABAs simplifies the pharmacological approach with the dual bronchodilation that is always complicated when different drugs that often have dissimilar pharmacokinetic profiles and formulations must be combined in the same inhaler (Cazzola et al., 2013). Nevertheless, the pharmacodynamic profile over time of the two activities in MABAs can be dissimilar and thus there will be molecules with a predominance of antagonist activity and others with agonist predominance. This must be understood as a real limitation to the dosing flexibility of MABAs as the ratio of the two different pharmacological activities cannot be adjusted as needed (Cazzola et al., 2013). However, the use of MABAs will also simplify the possibility to deliver three different complementary pharmacological activities at the same time, the so-called triple therapy. There is documentation that a single high dose of batefenterol/fluticasone furoate reduced exposure to fluticasone furoate compared with the administration of fluticasone furoate alone (Ambery et al., 2019).

One of the possible attempts to create bifunctional bronchodilator/anti-inflammatory drugs is to develop molecules capable of simultaneously inhibiting phosphodiesterase (PDE)3, whose blockade elicits direct airway smooth muscle relaxation and amplifies the effect induced by  $\beta_2$  adrenoceptor stimulation, and PDE4, which is present in many of the cells involved in the pathogenesis of COPD (Abbott-Banner and Page, 2014) (Fig. 2). Several molecules with these characteristics induce, at least in vitro, bronchodilatory and anti-inflammatory effects that are far greater than those observed after the block of PDE3 or PDE4 alone (Matera et al., 2021c). They also increase mucociliary clearance.

Currently, ensifentrine or RPL554 is the only PDE3/PDE4 inhibitor

that is under clinical development for the treatment of asthma, COPD, and cystic fibrosis (Cazzola et al., 2019b). In experimental studies that used isolated human airways, it relaxed smooth muscle and synergistically amplified the inhibitory effects induced by muscarinic receptor antagonists (Calzetta et al., 2013). This effect translated into significant bronchodilation when patients with asthma or COPD inhaled this PDE3/PDE4 inhibitor (Franciosi et al., 2013). In addition, in healthy volunteers, ensifentrine elicited anti-inflammatory actions and reduced the inflammatory cell infiltration in induced sputum triggered by lipopolysaccharide (LPS).

In asthmatic patients, a comparison between single therapeutic escalating doses of salbutamol and those of ensifentrine, both delivered by nebulizer, showed that the dose-dependent bronchodilation induced by the two drugs was quite similar, but ensifentrine did not exhibit the characteristic systemic effects of  $\beta_2$  agonists (Bjermer et al., 2019). In patients with COPD, the add-on of ensifentrine to salbutamol 200  $\mu\text{g}$  or ipratropium 40  $\mu\text{g}$  amplified bronchodilation and reduction of hyperinflation compared with the effects of the two bronchodilators in monotherapy (Singh et al., 2018). Furthermore, nebulized ensifentrine added to tiotropium for 4 weeks in COPD patients presenting with symptoms and impaired lung function produced clinically important improvements in functional parameters and quality of life with a safety profile like placebo (Ferguson et al., 2021).

Unfortunately, the relative scarcity of clinical data does not allow us to define the therapeutic role of ensifentrine in asthma and COPD (Cazzola et al., 2019b). Above all, there is no solid demonstration that it is effective in exerting anti-inflammatory activity in these pathological disorders (Cazzola et al., 2019b).

Identifying and developing new classes of bronchodilators is difficult, expensive and without any certainty that the possible incremental improvement that such drugs might induce justifies the time and money spent (Cazzola et al., 2021a). Nevertheless, intensive research has led to the identification of at least eight new classes of bronchodilators that have the potential for clinical development: 1) bitter-taste receptor (TAS2R) agonists; (2) E-prostanoid receptor 4 agonists; (3) Rho kinase inhibitors; (4) calcilytics; (5) agonists of peroxisome proliferator-activated receptor- $\gamma$ ; (6) agonists of relaxin receptor 1; (7) soluble guanylyl cyclase activators; and (8) pepducins (Cazzola and Rogliani, 2015; Matera et al., 2020). In our opinion, three of these eight classes, TAS2R agonists, Rho kinase inhibitors, and pepducins, deserve consideration (Cazzola et al., 2021a). Unfortunately, there is still no evidence that any of the molecules in these classes can be developed for inhalation administration.

### 3.3. Novel inhaled anti-inflammatory drugs

The persistent inflammation that characterizes COPD and critically affects its natural course with an apparent impact on the extent of its symptoms, is the reason why there is abundant research aimed at finding

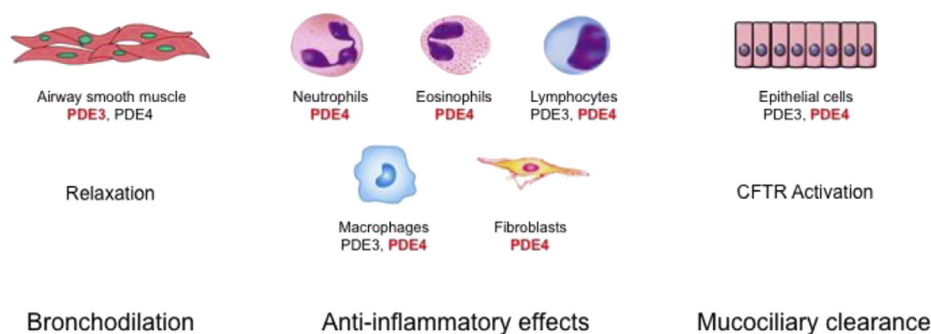


Fig. 2. Combined inhibition of phosphodiesterase (PDE)3 and PDE4 has additive and synergistic anti-inflammatory and bronchodilatory effects versus inhibition of either PDE3 or PDE4 alone. Furthermore, it increases mucociliary clearance. In red, the main PDE involved in the activity of the specific cell (Matera et al., 2021c). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

molecules that can regulate the inflammatory process.

Indeed, preclinical research, which is using animal models of COPD, has identified a number of anti-inflammatory drugs that, at least experimentally, reduce or block the recruitment and/or activation of inflammatory cells implicated in COPD or, alternatively, target inflammatory mediators that are thought to be important in the recruitment or activation of these cells or are released by them (Cazzola and Matera, 2021; Matera et al., 2021b, 2021c). At present, only very few of these anti-inflammatory drugs have been tested in humans and even fewer have been administered by inhalation.

Among drugs that inhibit the recruitment and activation of cellular components of inflammation, PDE4 inhibitors are certainly the most widely studied. At present, only roflumilast has been approved for clinical use and, in any case, it is burdened by side effects that considerably reduce patient compliance (Cazzola et al., 2016). The inhalation route could be used to reduce the detrimental effects of PDE4 inhibitors (Matera et al., 2014, 2021d). However, the development of almost all inhaled PDE4 inhibitors was discontinued because they produced only modest clinical benefits in patients with COPD. In fact, when administered by inhalation, PDE4 inhibitors are not able to influence systemic inflammation, in contrast to when these drugs are administered orally (Phillips, 2020). Nevertheless, it has been reported that in patients with COPD the addition of tanimilast (CHF6001), an inhaled PDE4 inhibitor, to triple inhalation therapy is capable of amplifying the anti-inflammatory effects as indirectly demonstrated by a further reduction in exacerbations (Singh et al., 2020b).

The phosphatidylinositol 3-kinase (PI3K) pathway is very important in COPD. PI3K, by catalyzing the production of phosphatidylinositol-3,4,5-triphosphate (PIP<sub>3</sub>) initiates several cytosolic events that are involved in cell growth, proliferation, metabolism, and survival (Cazzola et al., 2012). A number of these events are proinflammatory, essential in the activation of macrophage and neutrophils (Gupta et al., 2016) (Fig. 3). The PI3K pathway also has a major influence on angiogenesis (Cazzola et al., 2012).

The  $\delta$  isoform of PI3K is not only involved in neutrophil trafficking and directional movement but also influences the function of histone deacetylase 2, which affects corticosteroid sensitivity (Gupta et al.,

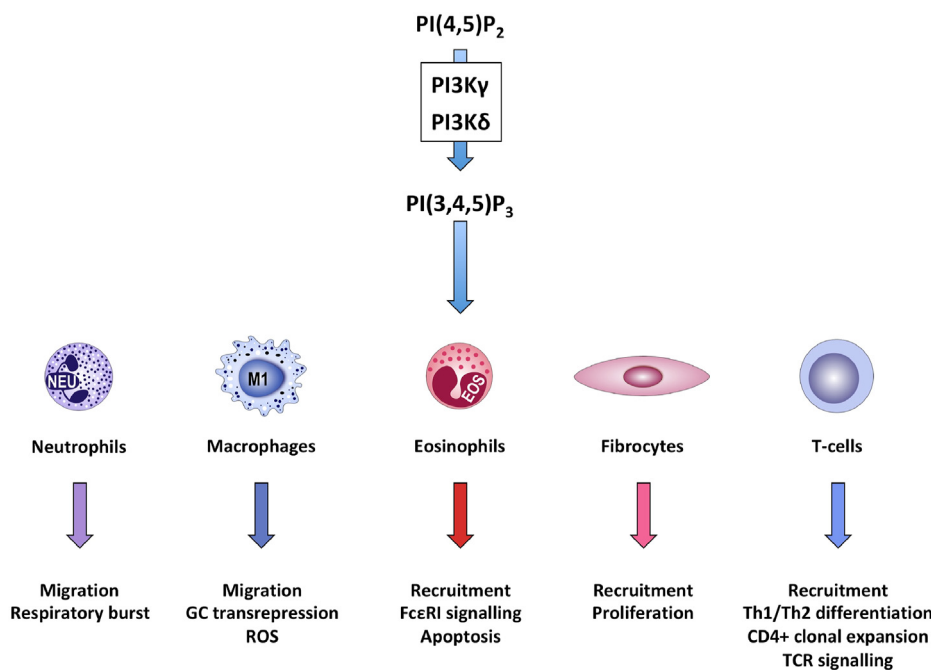
2016). Increased expression and activation of the PI3K $\delta$  pathway has been demonstrated in neutrophils and lung specimens from patients with COPD, and correlates closely with disease severity. These findings explain why the various isoforms of the PI3K enzyme, particularly PI3K $\delta$ , are considered important targets in the treatment of COPD (Cazzola et al., 2012).

In patients with an acute exacerbation of COPD (AECOPD), the addition of inhaled nemiralisib, one of the most selective PI3K $\delta$  inhibitors (Begg et al., 2019), to standard care for 3 months not only improved lung function but also reduced the rate and severity of further AECOPDs (Cahn et al., 2017). However, another study showed that nemiralisib 1000  $\mu$ g via DISKUS or 700  $\mu$ g via ELLIPTA induced changes in the neutrophil migration phenotype in stable COPD but not in AECOPD, despite an improvement in lung function in the latter group (Begg et al., 2021).

Also AZD8154, a dual PI3K $\gamma/\delta$  inhibitor, is under clinical development. An initial study in healthy volunteers demonstrated that AZD8154 has a good safety profile, prolonged pulmonary retention and a half-life that supports daily dosing (Sadiq et al., 2021).

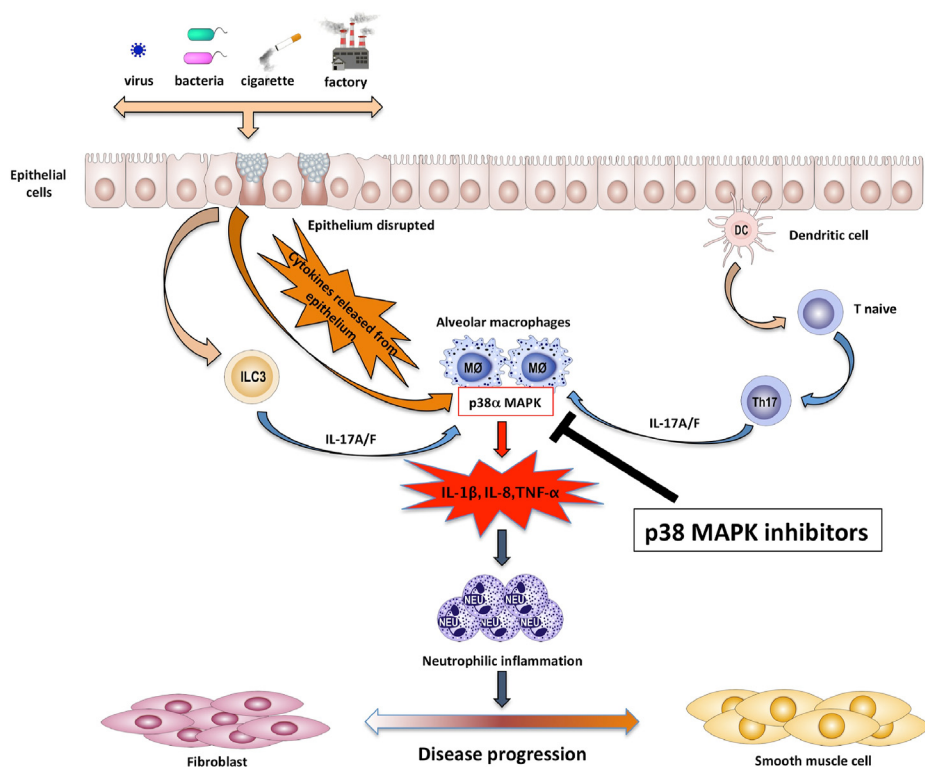
The p38 mitogen-activated protein kinases (MAPK), includes the p38 kinases, p38 $\alpha$ , p38 $\beta$ , p38 $\gamma$ , and p38 $\delta$ , which interact with the final effectors (Martínez-Limón et al., 2020). Activation of p38 MAPKs, especially the  $\alpha$  isoform that is the most abundant in inflammatory cells, controls apoptosis of macrophages and neutrophils. It also induces an increase in the biosynthesis in, and release from, macrophages and neutrophils of several cytokines and chemokines that play a critical role in the pathophysiology of COPD being involved in the development, maintenance, and amplification of chronic pulmonary inflammation (Cazzola et al., 2012; Pelaia et al., 2020) (Fig. 4). This occurs through phosphorylation of numerous substrates including downstream kinases, transcription factors, and transcriptional regulators.

All of these effects explain the importance of pharmacologically blocking p38 MAPKs in COPD. Indeed, there is elegant documentation that, in contrast to inhaled fluticasone propionate 500  $\mu$ g, PH-797804, an oral selective inhibitor of p38 $\alpha/\beta$  MAPK, and PF-03715455, which shows some selectivity for p38 $\alpha$  over p38 $\beta$  and is administered by inhalation, reduced neutrophilic airway inflammation induced by LPS stimulation in



**Fig. 3.** Schematic representation of the roles of phosphoinositide 3-kinase (PI3K) $\gamma$  and PI3K $\delta$  signaling in selected cells important or potentially important in COPD. PI(4,5)P<sub>2</sub>: phosphatidylinositol (4,5)-bisphosphate; PI(3,4,5)P<sub>3</sub>: phosphatidylinositol-3,4,5-triphosphate; TCR: T-cell receptor; ROS: reactive oxygen species; GC: glucocorticoid.





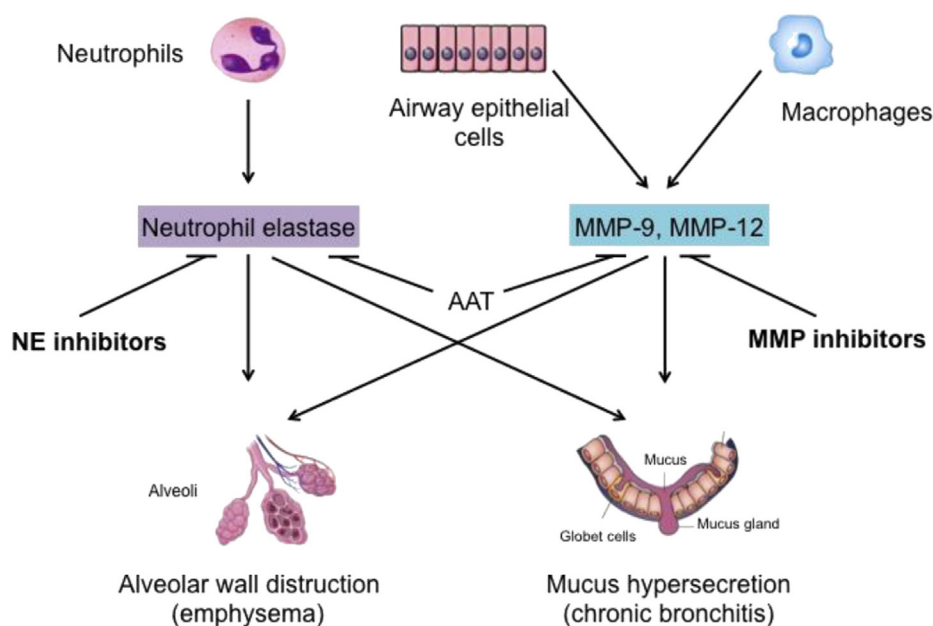
**Fig. 4.** The p38 MAPK pathway, which is activated by a wide variety of stimuli, is crucial in inflammation, cell death and cell proliferation, and therefore has been implicated in many cellular events relevant to COPD pathophysiology. In particular, p38 signaling pathway induces the release of proinflammatory cytokines and chemokines (IL-1 $\beta$ , IL-8, TNF- $\alpha$ ), which together with other mediators (IL-17A and IL-17 F) produced by Th17 and ILC3 cells contribute to recruitment and activation of neutrophils. These findings support the pharmacological rationale for targeting p38 MAPK.

healthy subjects (Singh et al., 2015). However, PH-797804 had a greater effect on sputum neutrophils and supernatant cytokines than PF-03715455 probably because more consistent anti-inflammatory effects are obtained with oral administration as it induces greater systemic exposure.

RV568, a selective p38 $\alpha/\gamma$  inhibitor, administered for 14 days at doses of 100  $\mu$ g and 50  $\mu$ g did not induce significant pre-bronchodilator FEV<sub>1</sub> improvements compared with placebo in patients with COPD and this was regardless of whether they were using ICS or not (Charron et al.,

2017). In contrast, the reduction in sputum malondialdehyde levels induced by RV568 was significantly different from that induced by placebo, although sputum cell counts did not change. Even a 12-week treatment with RV568 400  $\mu$ g did not improve lung function and patient reported outcomes despite signs indicating a reduction in cough and sputum (Robinson et al., 2016).

AZD7624, a specific p38 $\alpha/\beta$  MAPK inhibitor, administered by inhalation for >1 year showed no benefit in patients with COPD (Patel et al., 2018). Although it was shown to block LPS-induced release of interleukin



**Fig. 5.** Neutrophil elastase (NE) and metalloproteinases (MMP-9 and MMP-12) are implicated in COPD so that blocking a single enzyme with a NE inhibitor or a MMP inhibitor may not have a major therapeutic effect. A1-Antitrypsin (AAT) inhibits NE and suppresses MMP-12 production by macrophages (Matera et al., 2021c).

(IL)-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) from alveolar macrophages, AZD7624 did not delay time to first moderate or severe AECOPD nor did it reduce the number of early dropouts compared with placebo.

As already mentioned, attempts can be made to antagonize the cellular products of inflammation. It is well known that both neutrophil elastase (NE) and metalloproteinases (MMP-9 and MMP-12) play a critical role in pathogenesis of COPD, so that blocking a single enzyme with a NE inhibitor or a MMP inhibitor may not have a major therapeutic effect (Matera et al., 2021c) (Fig. 5). Furthermore,  $\alpha$ 1-antitrypsin (AAT) inhibits NE and suppresses MMP-12 production by macrophages (Matera et al., 2021c).

To the best of our knowledge there is no inhaled MMP inhibitor that has been tested or is in clinical development. Instead, there are two inhaled NE inhibitors, POL6014 and CHF6333, in early clinical development.

POL6014, which when inhaled produces high pulmonary concentrations with very low systemic exposure, has been shown to block NE in sputum of subjects with cystic fibrosis (CF) even after inhalation a single dose (Barth et al., 2020). The inhibitory effects on NE of CHF6333 were assessed in the bronchoalveolar lavage fluid (BALF) obtained from patients with CF and compared with those measured in the BALF of patients with non-CF bronchiectasis (McElvaney et al., 2018). The potency of inhibitory activity on NE was far greater in the BALF of patients with non-CF bronchiectasis. In a second study focused on CF samples, CHF6333, AAT and BAY85-8501, an oral NE inhibitor, have been found to similarly inhibit NE in BALF, spontaneous sputum and induced sputum samples (McElvaney et al., 2018).

Since AAT reaches the lung in a relatively inactive state when administered intravenously (Cazzola et al., 2020b), it is possible that the inhaled route is a viable option to overcome this limitation. Indeed it directs the delivery of AAT straight to the organ of interest, requires much less substance to inhibit NE and reduces the cost of therapy. However, this route must access the alveolar space that is destroyed in emphysema. Furthermore, there is still doubt as to whether inhaled AAT is capable of exerting an inhibitory action on NE that is adequate to affect the course of bronchiectasis and emphysema (Cazzola et al., 2020b).

Kamada's inhaled AAT (80 mg/day or 160 mg/day) delivered by eFlow mesh nebulizer for 12 weeks in AAT-deficient subjects produced mean AAT concentrations in endothelial lining fluid (ELF) that were 2-5-fold higher than those found in normal individuals (Brantly et al., 2017). AAT was found in the plasma of all patients treated with inhaled AAT. In addition, compared with placebo, anti-proteolytic (NE) and anti-inflammatory (% of neutrophils, but not pro-inflammatory cytokines) biomarkers were significantly increased by the inhaled AAT.

However, Kamada's inhaled AAT administered for 50 weeks to patients with severe AAT deficiency (PI\*ZZ), severe COPD, and frequent exacerbations did not significantly delay the time to first exacerbation or reduce the mean rate of annual exacerbations (Stolk et al., 2019). Furthermore, patients treated with inhaled AAT experienced more treatment related adverse events compared with placebo. However, there was a real improvement in the safety profile after changes were made to the handling of the AAT and nebulizer device, with the safety profile of the AAT treatment group that became like that of the placebo group.

Inhaled AAT was also tested in CF patients. Administered for 21 days by eFlow (Kerem et al., 2009) or 28 days by AKITA jet nebulizer (Gaggar et al., 2016), it induced a reduction in neutrophils and NE in sputum and was always safe and well tolerated.

It has been highlighted that protein characteristics (particle size, density, lipophilicity, and charge) still need to be optimized to achieve AAT formulation stability (Usmani, 2020). Furthermore, a suitable drug delivery system must be used to ensure that the delivered dose of inhaled AAT reaches and maintains protective levels in lung tissue.

Heparin, due to its ability to bind and neutralize inflammatory mediators and enzymes released during an inflammatory process and with several mechanisms, such as neutralization of cationic mediators, inhibition of adhesion molecules, and the inhibition of heparinase, that are

potentially relevant because involved in leukocyte recruitment into tissues, can modulate the inflammatory response (Lever and Page, 2012). As this anti-inflammatory activity suggests a potential benefit in the management of COPD, we tested the effects of inhaled unfractionated heparin at 75,000 IU and 150,000 IU twice daily for 21 days in patients with moderate to severe COPD (Shute et al., 2018). The results of this study showed that when administered by inhalation, regular treatment with heparin not only induced clinically significant improvements in lung function that were maintained long after treatment was discontinued, but also a reduction in air trapping with a substantial improvement in exercise capacity and a reduction in dyspnea.

### 3.4. Inhaled monoclonal antibodies

Several monoclonal antibodies (mAbs) have been approved for use in severe asthma (Matera et al., 2019) and have been tested or are currently under clinical evaluation for the treatment of COPD (Cazzola et al., 2021b). They can be divided into those targeting specific pro-inflammatory and pro-neutrophilic cytokines and chemokines, such as TNF- $\alpha$ , IL-1 $\beta$ , C-X-C Motif Chemokine Ligand 8, IL-17A and IL-1 $\beta$ , and those targeting T2-mediated inflammation, by blocking IL-5 and/or its receptor, preventing IL-4 and IL-13 signaling, affecting the IL-33 pathway, and blocking thymic stromal lymphopoietin (TSLP), respectively (Cazzola et al., 2021b).

MABs are large molecules that are administered systemically and only reach the lung in a small percentage of the administered dose (Matera et al., 2019). This explains, at least in part, their low clinical efficacy while exposing the rest of the body to potential toxicity and serious adverse effects. It is hypothesized that their administration by inhalation may increase the proportion of active drug in the lung, while limiting its passage into the bloodstream (Matera et al., 2016). However, technological issues such as stability during mAb delivery complicate the use of this route of administration (Mayor et al., 2021). In addition, there are important formulation challenges that need to be addressed. In particular, it is necessary to ensure that the mAb particles are not moved away from the lungs, but rather remain there for a long time to maximize the therapeutic effect (Respaud et al., 2014; Matera et al., 2021e).

As mAbs often require the delivery of high quantities of drug in a constant volume, nebulizers with mesh technology (vibrating micro-perforated membrane) should be preferred also because they better preserve the molecular integrity of proteins, as there are less stringent chemical and physical restraints (Respaud et al., 2014). The molecular integrity, on which the pharmacological activity of mAbs during nebulization also depends, is facilitated by the addition of surfactant (Cazzola et al., 2020a). In any case, just mAbs whose doses have a very high potency are suitable for administration by the lung route because only small volumes of liquid can be delivered by inhalation (Matera et al., 2019).

GSK1995057, a mAb that selectively blocks TNF- $\alpha$  receptor 1 (TNFR1), administered by nebulizer to healthy subjects, even after a single dose reduced pulmonary neutrophilia and inflammatory cytokine release in BAL and serum samples and also signs of endothelial injury induced by an LPS challenge (Proudfoot et al., 2018).

In any case, if the dose to be delivered is not too high, the use of a dry powder inhaler (DPI) may also be considered. The advantage of the dry powder formulation is the potential to achieve stability at room temperature, which makes it possible to overcome instability of mAb nebulizer solutions or degradation of reconstitutable formulations during freezing and thawing cycles (Hickey and Stewart, 2021).

Inhalation administration of abrekimab (formerly VR 942), an anti-IL-13 monoclonal antibody in dry powder form, to asthmatic patients reduced FeNO levels very rapidly and for a long time and was generally well tolerated, although a few subjects developed bronchospasm and reductions in FEV<sub>1</sub> (Burgess et al., 2018).

CSJ117, a potent antagonist Ab fragment that belongs to the immunoglobulin G1/ $\lambda$  isotype subclass and binds to TSLP, was inhaled once daily for 12 weeks by patients with mild atopic asthma via a DPI; it

inhibited the early and late allergic response to the allergen compared to placebo and was well tolerated (Gauvreau et al., 2020). A phase 2 study in patients with COPD who are subject to exacerbations and symptomatic at baseline despite being on regular triple therapy is evaluating the efficacy, pharmacokinetics, and safety of two doses of CSJ117 administered for 12 weeks compared with placebo (ClinicalTrials.gov Identifier: NCT04882124).

#### 4. Attempts to interfere with the possible infectious component of COPD using the inhalation route

Bacterial colonization of the lower airways with consequent local inflammation and infection can modulate characteristics and frequency of AECOPDs. For this reason, several clinicians believe that long-term prophylactic antibacterial treatment is needed to prevent AECOPDs at least in those patients with COPD who are frequent exacerbators and do not respond favorably to standard care (Uzun et al., 2014).

We are totally against the prophylactic use of antibiotics mainly because there are differences in the composition of the bacterial community, depending on the bronchus in which it is sampled even within the same lung. Furthermore, many external factors including cigarette smoking, disease severity, the presence of an AECOPD, and even subsequent antibiotic and steroid use can alter the composition of the pulmonary microbiome (Cazzola et al., 2017). In any case, the possible emergence of antibiotic resistance and the appearance of specific adverse effects are not of minor importance.

Nevertheless, the available evidence supports a protective role of influenza vaccination (Bao et al., 2021) and the routine use of 13-valent or 23-valent polysaccharide pneumococcal vaccines (Papi et al., 2020) in patients with COPD. Besides, when antibiotic treatment is necessary during AECOPD, short duration of treatment should possibly be preferred, together with de-escalation therapy, in order to shorten hospitalization time and to cut healthcare costs (Di Pasquale et al., 2020) and also, even more importantly, to reduce the risk of bacterial resistance.

##### 4.1. Inhaled vaccines

Most vaccines are administered intramuscularly, although the majority of airborne viral and bacterial infections occur at mucosal surfaces of lungs and upper respiratory tract, and mucosal immune responses constitute the first line in protection against most infections (Longet et al., 2018). These findings explain why inhalation-based vaccination platforms have gained particular attention for protection against these airborne infections.

Inhalation of vaccines provides the opportunity to target all regions of the respiratory tract (Heida et al., 2021) and, furthermore, has the potential to be used as a needle-free alternative. The vaccine can be delivered directly at the front door for many pathogens, where it can elicit a local immune response supported primarily by secretory IgA antibodies, which are mainly produced by bronchus-associated lymphoid tissue (BALT) (Tonnis et al., 2012) and are thought to be cross protective against different subtypes of pathogens. Vaccine administration to the mucosa of the respiratory tract also induces systemic IgG-mediated and cell-mediated responses, which are of utmost importance for the immune defense of the respiratory tract (Heida et al., 2021).

To be used by inhalation, the vaccine must fulfill some characteristics (Hellfritzsch and Scherließ, 2019). First, the antigen must be presented in a particulate form, and always co-administered with an adjuvant with mucosal efficacy because of its poor immunogenicity when used alone. Next, the formulation must be capable of being delivered through a suitable device that allows deposition at the target site and interaction with immune cells.

Inhaled vaccines are basically available in liquid and powder formulations (Heida et al., 2021). Those in aqueous solution often degrade. When properly dried, powder formulations are more stable and can be stored at ambient conditions, thus circumventing the requirement for a

cold chain and, furthermore, they weigh less than liquid solutions, which facilitates their transport. Dry powder formulations are typically dispensed using disposable DPIs to avoid reuse of the inhaler, unintentional contamination and humidity-induced degradation. These DPIs allow the delivery of reproducible doses with one or a few inhalations compared to liquid formulations.

Over the past two decades, several research groups have successfully created dried vaccines, suitable for inhalation delivery, with formulations that maintain in vivo efficacy. There are also patented attempts to formulate microbial antigens in a dry powder (Kaur, 2017), but there is a lack of solid clinical documentation probably because we still do not really know what is the fate of the antigens after pulmonary administration and how both the type of formulation and inhalation device must be characterized to induce protection.

##### 4.2. Inhaled antimicrobial agents

The penetration of antimicrobial agents into the lung parenchyma during respiratory infections may be modest when they are administered parenterally or orally. This is not only a limitation for clinical success but also a danger for the possible emergence of antibiotic resistance, the rate of which is gradually increasing. Administration of inhaled antibiotics greatly increases the proportion of drug available at the site of respiratory infections and reduces systemic side effects (Rubin and Williams, 2014). Furthermore, targeted administration minimizes systemic exposure and associated toxicity.

Antibiotics with concentration-dependent effects such as aminoglycosides and fluoroquinolones should always be preferred because they achieve high concentrations in the respiratory tract that may allow maximization of antibacterial action (Restrepo et al., 2015). There are many inhaled antimicrobial agents in clinical development that are also currently used as off-label drugs (Debnath et al., 2021).

Inhaled antibiotics appear to be an effective and safe treatment in COPD patients who are frequent exacerbators and in those with chronic bronchial infection sustained by any potentially pathogenic microorganism, regardless of the presence of bronchiectasis (De la Rosa Carrillo et al., 2021).

There are innovations in development. In particular, the use of liposomal formulations of antibiotics that are capable of acting on infected macrophages and biofilms appear promising (Maselli et al., 2017). The use of polymers, which through permeabilization by hydrolysis of the shell and dissolution of the medium may allow for controlled drug release, also seems to be an attractive option. Furthermore, there are many inhaled antimicrobial agents that have been studied using animal models (Debnath et al., 2021) but for which clinical evidence that may support their use is still lacking.

## 5. Conclusion

There is no doubt that the inhaled route of drug delivery to treat patients with COPD is extremely useful. Inhaler devices, which are crucial in the management of COPD, have improved in their efficiency over the years, and still continue to improve in terms of technical design, chemistry and formulations, and associated digital technology.

In parallel, a continuous expansion of our knowledge about the pathogenesis and evolution of COPD is leading to the identification of new targets potentially useful for the development of novel drugs to be used in the treatment of COPD. Numerous molecules have been synthesized, but it must be admitted that only a few have reached the initial stages of clinical development and even fewer are being evaluated for inhaled administration.

At present, we cannot yet predict which new inhaled medications will be introduced into the COPD therapeutic armamentarium. However, we are quite optimistic for the future because there is a palpable ongoing effort to provide physicians and patients with new molecules that are effective when administered correctly, and offer maximum benefit with



minimal risk to the patient. Nevertheless, it must be highlighted that the development of new inhaled medications for use in COPD still seems to be too slow.

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Given Mario Cazzola's role as an Associate Editor and Luigino Calzetta's role as the Editor-in-chief, we had no involvement in the peer review process and have no access to information regarding its peer review. Full responsibility for the Editorial process of this article was delegated to Cynthia Koziol-White.

### Declaration of competing interest

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