Journal Pre-proof

Regenerative Therapeutics for Chronic Obstructive Pulmonary Disease

Luke van der Koog, Henry Showell, Dyan Nugraha, Mareike Lehmann, Thomas M. Conlon, Ali Önder Yildirim, Rocío Fuentes-Matéos, Hoeke Baarsma, John-Poul Ng-Blichfeldt, Barbro N. Melgert, Antonella F.M. Dost, Janette K. Burgess, Stacy Yam, Irene H. Heijink, Sidrah Ahmed, Margherita Paschini, Eva Jansen, Wouter J. Hinrichs, Jill R. Johnson, Xinhui Wu, Anika Nagelkerke, Henderik W. Frijlink, Carla F. Kim, Reinoud Gosens

PII: S0031-6997(26)00012-8

DOI: https://doi.org/10.1016/j.pharmr.2026.100124

Reference: PHARMR 100124

To appear in: *Pharmacological Reviews*

Received Date: 21 November 2025

Accepted Date: 29 January 2026

Please cite this article as: van der Koog L, Showell H, Nugraha D, Lehmann M, Conlon TM, Yildirim AÖ, Fuentes-Matéos R, Baarsma H, Ng-Blichfeldt JP, Melgert BN, Dost AFM, Burgess JK, Yam S, Heijink IH, Ahmed S, Paschini M, Jansen E, Hinrichs WJ, Johnson JR, Wu X, Nagelkerke A, Frijlink HW, Kim CF, Gosens R, Regenerative Therapeutics for Chronic Obstructive Pulmonary Disease, *Pharmacological Reviews* (2026), doi: https://doi.org/10.1016/j.pharmr.2026.100124.

Regenerative Therapeutics for Chronic Obstructive Pulmonary Disease

Luke van der Koog[1,2,3,4], Henry Showell[1,4], Dyan Nugraha[1,4,5], Mareike Lehmann[6,7,8], Thomas M Conlon[8], Ali Önder Yildirim[8,9], Rocío Fuentes-Matéos[1,4], Hoeke Baarsma[1,4], John-Poul Ng-Blichfeldt[10], Barbro N. Melgert[1,4], Antonella F.M. Dost[11], Janette K. Burgess[12,4], Stacy Yam[12,4], Irene H Heijink[12,4], Sidrah Ahmed[13,14], Margherita Paschini[13,14], Eva Jansen[2], Wouter J. Hinrichs[2], Jill R. Johnson[15], Xinhui Wu[16], Anika Nagelkerke[17], Henderik W. Frijlink[2,3], Carla F Kim[13, 14], Reinoud Gosens[1,4*]

[1] Department of Molecular Pharmacology, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, the Netherlands;

[2] Department of Pharmaceutical Technology and Biopharmacy, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands;

[3] MimeCure B.V., Eelde, the Netherlands;

[4] Groningen Research Institute for Asthma and COPD (GRIAC), University of Groningen, University Medical Center Groningen, the Netherlands.

[5] Department of Pharmacy, Faculty of Health, Sari Mulia University, Banjarmasin, Indonesia

[6] Institute for Lung Research, Philipps-University Marburg, Member of the German Center for Lung Research (DZL), Marburg, Germany.

[7] Institute for Lung Health (ILH), German Center for Lung Research (DZL), Giessen, Germany;

[8] Institute of Lung Health and Immunity (LHI), Helmholtz Munich, Comprehensive Pneumology Center (CPC-M), Member of the German Center for Lung Research (DZL), Munich, Germany;

[9] Institute of Experimental Pneumology, Ludwig-Maximilians University (LMU), Munich, Germany;

[10] Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge Biomedical Campus, Cambridge, United Kingdom;

[11] Hubrecht Institute for Developmental Biology and Stem Cell Research-KNAW & University Medical Centre Utrecht, Utrecht, Netherlands;

[12] University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, The Netherlands;

[13] Stem Cell and Regenerative Biology Program, Department of Pediatrics, Divisions of Hematology/Oncology and Pulmonary Medicine, Boston Children's Hospital, Boston, MA, USA;

[14] Department of Genetics, Harvard Medical School, Boston, MA, USA

[15] School of Biosciences, College of Health and Life Sciences, Aston University, Birmingham, United Kingdom;

[16] Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, Canada;

[17] Department of Pharmaceutical Analysis, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands.

[*] Corresponding author

Reinoud Gosens, PhD

Department of Molecular Pharmacology,

University of Groningen,

Antonius Deusinglaan 1,

9713 AV Groningen,

The Netherlands

+31503638177

r.gosens@rug.nl

Manuscript page numbers: 146

Abstract: 216 words

Figures: 8

54 **Tables: 1**

55 **References: 691**

List of Abbreviations

A1AT:	α_1-antitrypsin
AATD:	α_1-antitrypsin deficiency
AD-MSCs:	Adipose derived MSC
AGE:	Advanced glycation end product
ALK:	Activin receptor-like kinase
all-trans-RA:	All-trans-retinoic acid
AMs:	Alveolar macrophages
APC:	Adenomatous Polyposis Coli
AT:	Alveolar Type
ATMP:	Advanced therapy medicinal product
BALF:	Bronchoalveolar lavage fluid
BASC:	Bronchioalveolar stem cell
BMMC:	Bone marrow mononuclear cell
BM-MSC:	Bone marrow-derived mesenchymal stromal cell
BMP:	Bone morphogenetic protein
cAMP:	Cyclic adenosine monophosphate
CK1α:	Casein kinase 1 alpha
COPD:	Chronic Obstructive Pulmonary Disease
CRABP2:	Cellular retinoic acid binding protein 2
CRP:	C-reactive protein
CS:	Cigarette smoke
CT:	Computed tomography
CYP:	Cytoplasmic Cytochrome P450
DAMP:	Damage-associated molecular pattern
DKK1:	Dickkopf-1
DLCO:	Diffusing capacity for carbon monoxide

DPI:	Dry powder inhaler
DPP-I:	Dipeptidyl peptidase I
DVL:	Dishevelled
D+Q:	Dasatinib and quercetin
EC:	Endothelial cell
ECM:	Extracellular matrix
EMA:	European Medicines Agency
EP:	E prostanoid
Epac:	Exchange protein directly activated by cAMP
EPC:	Endothelial progenitor cell
EV:	Extracellular vesicle
FDA:	U.S. Food and Drug Administration
FGF:	Fibroblast growth factor
FGFR:	Fibroblast growth factor receptor
FZD:	Frizzled receptor
GLS-1:	Glutaminase-1
GPCR:	G protein-coupled receptor
GR:	Glucocorticoid receptor
GRE:	Glucocorticosteroid responsive element
GSK-3β:	Glycogen synthase kinase-3β
HAPLN1:	Hyaluronan and proteoglycan link protein 1
HDAC:	Histone deacetylase
HGF:	hepatocyte growth factor
ICS:	Inhaled corticosteroids
IFN-γ:	Type II interferon
IL:	Interleukin
IMs:	Interstitial macrophages
iBALT:	Inducible bronchus-associated lymphoid tissue

iPSCs:	Induced pluripotent stem cells	
IPF:	Idiopathic pulmonary fibrosis	
KGF:	Keratinocyte growth factor	
LABA:	Long-acting β2-adrenoceptor agonist	
LAMA:	Long-acting muscarinic antagonists	
LEF:	Lymphoid enhancer factor	
LGR:	Leucine-rich repeat containing GPCR	
LiCl:	Lithium chloride	
LMSC:	Lung resident MSC	
LNP:	Lipid nanoparticle	
LRAT:	Lecithin:retinol acetyltransferase	
LRP:	Lipoprotein-related receptor	
LTβR:	Lymphotoxin-β receptor	
LTi:	Lymphoid tissue inducer	
MCID:	Minimal clinically important difference	
MMP:	Matrix metalloproteinases	
mRNA:	Messenger ribonucleic acid	
MSC:	Mesenchymal stem/stromal cell	
NCOR:	Nuclear receptor corepressor	
NCOA:	Nuclear coactivator	
NE:	Neutrophil elastase	
NIK:	NF-κB-inducing kinase	
NPY:	Neuropeptide	Y
OGN:	Osteoglycin	
PDE4:	Phosphodiesterase-4	
PIGF:	Placental growth factor	
PKA:	Protein kinase A	
PM2.5:	Particulate matter	

139	PP2A:	Protein phosphatase 2A
140	PPARγ:	Peroxisome proliferator-activated receptor gamma
141	PPRE:	Peroxisome proliferator response element
142	PR1P:	Prominin-1-derived peptide
143	PRP:	Platelet-rich plasma
144	RA:	Retinoic acid
145	RAGE:	Receptor for advanced glycation end product
146	RALDH:	Retinaldehyde dehydrogenase
147	RAR:	Retinoic acid receptors
148	ROCK:	Rho-associated coiled-coil kinase
149	ROS:	Reactive oxygen species
150	RSPO:	R-spondin
151	RTK:	Receptor tyrosine kinase
152	RXR:	Retinoid X receptor
153	SA-β-gal:	Senescence-associated β-galactosidase
154	SABA:	Short-acting β2-adrenoceptor agonist
155	SASP:	Senescence-associated secretory phenotype
156	SFTPC:	Surfactant protein C
157	siRNA:	Small interfering ribonucleic acid
158	SIRT:	Sirtuin
159	SLPI:	Secretory leukocyte protease inhibitor
160	sFRP:	Secreted frizzled-related protein
161	sm-α-actin:	Smooth muscle α-actin
162	STRA6:	Surface receptor stimulated by retinoic acid 6
163	TGF-β:	Transforming growth factor beta
164	TCF:	T-cell factor
165	TIMP:	Tissue inhibitors of metalloproteinase
166	TNFα:	Tumor necrosis factor alpha

VEGF: Vascular endothelial growth factor

VEGFR: Vascular endothelial growth factor receptor

Table of Contents

Abstract

Chronic obstructive pulmonary disease (COPD) is one of the most common lung diseases worldwide, characterized by an accelerated loss of lung function. A key problem underlying COPD is increased tissue destruction in combination with defective lung tissue repair. As current therapies do not modify the progression of the disease, new therapies aimed at restoring lung tissue repair in COPD need to be developed.

In an attempt to address this major unmet need, there has been a surge in both preclinical and clinical studies, aiming to identify key mechanisms underpinning defective lung repair and the ability to inhibit or even reverse this defect. This includes small molecules such as retinoids, as well as advanced therapy medicinal products such as cell therapies or therapies with cell-derived products such as extracellular vesicles, or secreted proteins. The results of these endeavors have been variable with failures as well as successful proof-of-concepts.

In this review, we provide an overview of the current state of the field, including modes of action of the therapeutics that are or have been considered for lung regeneration, including a discussion on the reasons for failure where relevant. In addition, we discuss hurdles in the clinical development of regenerative therapeutics for COPD including clinical outcomes, route of administration and formulation as these are pivotal considerations moving forward.

Significance statement:

Chronic obstructive pulmonary disease is characterized by progressive alveolar destruction and defective epithelial regeneration. Targetable mechanisms, including cellular senescence, altered mesenchymal-epithelial signaling, and chronic inflammation, impair progenitor function and niche integrity. Therapeutic strategies that restore epithelial repair, including small molecules, biologics, and cell-based approaches, represent a promising path toward disease modification and long-term lung function restoration.

Keywords: lung repair, small molecules, cell therapy, biologics exacerbation, translational medicine

1. INTRODUCTION

1.1 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a major global health challenge, affecting over 300 million people and is ranked as the fourth leading cause of death worldwide, according to the World Health Organization [1, 2]. The disease is characterized by a progressive and largely irreversible loss of lung function and airflow limitation, leading to symptoms such as chronic cough, dyspnea, excessive mucus production, and, in some cases, wheezing and chest tightness [3-5]. These symptoms significantly impact the daily functioning and quality of life of patients with COPD. COPD primarily affects individuals over 60 years old and is mainly caused by chronic exposure to airborne toxic substances, including tobacco smoke, air pollution, and occupational exposures to dust or wood particles [3, 4, 6-8]. Additional risk factors include lung infections, abnormal lung development, and genetic predisposition. Globally, the burden of COPD is expected to rise further due to continued population aging and increasing exposure to risk factors such as air pollution and smoking in low- and middle-income countries [1, 9].

COPD is a heterogeneous disease with two primary pathological components: chronic bronchitis and emphysema. Chronic bronchitis is characterized by persistent airway inflammation, mucus hypersecretion, small airway wall fibrosis, and epithelial remodeling [3-5, 10]. Emphysema involves the destruction of alveolar structures, reducing the surface area available for gas exchange and leading to progressive respiratory impairment [3-5]. In addition to these changes, small airway disease (SAD) has emerged as a key driver of early COPD and a major contributor to airflow limitation. SAD involves inflammation, fibrosis, and luminal narrowing of terminal bronchioles (<2 mm in diameter), leading to obstruction well before emphysema becomes radiographically apparent [11, 12, 13]. By contrast, emphysema typically develops later and predominates in advanced stages, when alveolar destruction and impaired

306 gas transfer become more pronounced [12, 13]. Chronic exposure to harmful substances induces persistent
307 inflammation and oxidative stress, driving tissue destruction and abnormal tissue repair. These
308 processes contribute to small airway obstruction, alveolar wall damage, and ultimately to progressive
309 lung function decline [3, 4, 6-8]. It is important to emphasize that lung function decline in COPD is
310 multifactorial, and the result of both inflammation, bacterial and viral infections, small airways disease,
311 emphysema development, and mucus obstruction. A critical and relatively recent insight into COPD
312 progression is that lung function decline does not occur in a steady, linear manner but rather in episodic
313 phases, partially due to exacerbations [14]. Many patients with COPD experience these exacerbations,
314 which are defined as an acute worsening of COPD symptoms beyond normal day-to-day variation that
315 requires additional treatments [15, 16]. Recurrent bacterial and viral infections account for approximately
316 50% of the total accelerated lung function loss throughout the life of a patient with COPD [16-18].
317 Furthermore, mucus plugging appears to play a crucial role as longitudinal analysis of chest CTs of
318 patients with COPD indicated that those patients without notable mucus plugs on chest CT or those
319 with resolvable mucus plugs have similar rates of lung function decline, whereas patients with persistent
320 presence of mucus plugs have substantially accelerated decline of lung function [19]. This implicates that
321 targeting these pathological processes in COPD has the potential to slow down disease progression.
322
323 At a population level, the only proven interventions to slow lung function decline in patients with COPD
324 are tobacco control measures and reductions in air pollution [20, 21]. For individual patients,
325 pharmacological treatment primarily focuses on symptom relief, exacerbation prevention, and quality-
326 of-life improvement, rather than modifying the underlying disease. Current pharmacological options
327 include bronchodilators, inhaled corticosteroids, phosphodiesterase-4 (PDE4) inhibitors, systemic
328 corticosteroids, biologics, and antibiotics [21-23]. Bronchodilators are the cornerstone of COPD
329 management, with short-acting and long-acting beta-agonists or muscarinic antagonists used to relieve
330 airway constriction and improve lung function. Long-acting bronchodilators are preferred for most
331 patients due to their superior efficacy in symptom control and exacerbation reduction [14, 21]. Inhaled
332 corticosteroids are added for selected patients, particularly those with elevated eosinophil counts, to

further reduce exacerbation risks. During acute exacerbations, systemic corticosteroids and antibiotics are commonly used to manage airway inflammation and secondary infections [14, 21]. In addition to pharmacological therapy, several non-pharmacological interventions play a crucial role in COPD management [24, 25]. Pulmonary rehabilitation, which includes exercise training, nutritional guidance, and patient education, has been shown to improve physical function and quality of life [25]. Long-term oxygen therapy is indicated for patients with chronic hypoxemia, while non-invasive ventilation can be beneficial for individuals with respiratory failure [25]. In advanced COPD, surgical interventions such as lung volume reduction surgery, endobronchial valves and coils, or lung transplantation may be considered, but these invasive treatments are only suitable for a highly select group of patients [25-30].

Despite the availability of both pharmacological and non-pharmacological treatments, no currently approved intervention can reverse established COPD or fundamentally slow disease progression [31-33]. The underlying challenge lies in the chronic imbalance between tissue injury and insufficient repair, particularly in the alveolar compartment. This imbalance stems from dysregulated interactions between inflammation, progenitor cell dysfunction, and aberrant tissue remodeling. As a result, the long-term prognosis for patients with COPD remains poor. Alternatively, a pharmacological strategy that reactivates endogenous lung repair mechanisms could offer a scalable and non-invasive solution to modifying disease progression. Such a therapy could modify the disease trajectory and improve long-term outcomes for patients with COPD [32, 33]. Given the increasing burden of COPD and the limitations of current treatment approaches, the development of regenerative pharmacological therapies represents a crucial research priority [33, 34].

To advance development of a pharmacological strategy with the capacity to reactivate endogenous lung repair mechanisms it is first necessary to understand the central elements that have key roles in this process in health and disease. In the next section a summary of current knowledge is provided.

1.2 Alveolar epithelial repair

The long-standing belief that the adult human lung lacks regenerative capacity is challenged by emerging evidence, including case reports, showing lung regrowth following surgical resection [35, 36]. While the lung exhibits a low level of structural turnover during homeostasis, it possesses significant repair capacity after injury [37]. Although the cellular mechanisms of adult lung regeneration remain incompletely understood, studies in animal models and human lung tissue have identified multiple stem and progenitor cell populations capable of responding to injury and facilitating repair [37-39]. *In vivo* lineage-tracing studies have demonstrated that mature lung epithelial cells act as regionally restricted progenitors, maintaining and repairing tissue following mild injury in animal models [40]. In the proximal airways, basal cells within the pseudostratified epithelium act as multipotent progenitors that self-renew and give rise to both secretory and ciliated epithelial cells [41-44]. In the terminal bronchioles, respiratory airway secretory cells function as progenitors for alveolar type (AT)2 cells, which are essential for maintaining and regenerating the alveolar niche [45, 46]. In addition, bronchioalveolar stem cells are crucial for bronchioalveolar epithelial repair and are suggested to contribute to the regeneration of both proximal and alveolar epithelial cell types after injury [46, 47]. Within the alveoli, AT2 cells play a central role in repair, serving as progenitors capable of self-renewal and differentiation into AT1 cells, which are critical for gas exchange [48, 49]. Following lung injury, apoptosis of AT1 cells triggers the activation and proliferation of AT2 cells, which then differentiate into new AT1 cells to restore alveolar integrity [48, 49]. Functional epithelial regeneration requires both the proliferation of progenitor cells to replace lost cells and their differentiation into mature cell types, including surfactant-producing AT2 cells and barrier-forming AT1 cells. Recent studies have identified distinct AT2 subpopulations with specialized roles in regeneration. One such subpopulation, $Axin2^{+}$ alveolar epithelial progenitors, is quiescent during homeostasis but proliferates rapidly following injury [48, 50]. Another population is the distal lung progenitor (integrin $\alpha6/\beta4^{+}$, surfactant protein C^{-}), which replenish the AT2 cell pool after lung injury in mice [51]. Additionally, a quiescent, immature AT2 subpopulation marked by PD-L1 expands after pneumonectomy in mice and has also been identified in humans [52]. Moreover, emerging data indicate that Club cells, secretory cells in the bronchiolar airways, act as facultative progenitor cells during alveolar repair. In murine models and in vitro 3-D culture systems, club cells ($Scgb1a1^{+}$) have been shown to proliferate and differentiate into AT2- and AT1-like cells, forming alveolar-like structures [53].

Furthermore, lineage-tracing studies identified a subpopulation of H2-K1^{high} club-derived progenitors that mobilize after bleomycin injury and contribute directly to alveolar cell lineages [54]. More recently, airway secretory-cell derived p63^{+} progenitors (within the club/secretory cell compartment) were shown to enter distal alveolar regions and aid repair in severe lung injury [55]. These findings suggest that under significant injury, club cells may act as a facultative alveolar progenitor pool, potentially compensating when classical alveolar progenitors are compromised. Together, these findings reveal an unexpectedly dynamic and plastic epithelial progenitor population within the distal lung, capable of coordinating repair after injury.

In COPD, disruption in the natural processes of alveolar epithelial repair, particularly in emphysema, results in an imbalance between tissue injury and the reduced capacity of alveolar progenitor cells to support repair [56, 57]. It is believed that repetitive injury in COPD leads to the depletion of the stem and progenitor cell pool, thereby limiting the regenerative capacity of the remaining epithelial progenitor cells [56]. While progenitor cell populations may survive in the lungs of patients with COPD, they may develop abnormalities that compromise their function. For instance, telomere shortening has been observed in both smokers with and without COPD, in addition to increased AT2 cell senescence in individuals with emphysema [58-60]. Furthermore, the cellular composition of the alveolar compartment is altered in emphysema, with an increased proportion of AT2 cells and a reduction in AT1 cells, suggesting that many AT2 cells fail to undergo proper differentiation. On one hand this shift includes a numerical decline in functional epithelial cells, whilst on the other hand there is an accumulation of aberrant or arrested AT2 cells, which further impair regeneration [61-63]. This suggests that chronic insults may push progenitor cells toward senescence or exhaustion, undermining their ability to support repair. Furthermore, critical signaling pathways that govern epithelial progenitor activity are dysregulated in COPD. For example, Wnt signaling, a key pathway regulating AT2 cell behavior, is reduced in AT2 cells derived from patients with COPD, indicating impaired regenerative signaling [64, 65]. These alterations may further limit progenitor cell function and contribute to the progressive failure of alveolar repair observed in the disease.

Although much of the current research focuses on alveolar repair, it is increasingly recognized that regeneration of the small airways is equally important for restoring lung function. Early COPD is dominated by SAD, characterized by airway wall fibrosis, luminal narrowing, and loss of terminal bronchioles [11, 12]. Regeneration in this compartment will require both resolution of peribronchiolar fibrosis and re-epithelialization of the distal conducting airways, processes that remain poorly understood but represent critical targets for future repair-focused therapies [66].

In summary, the alveolar epithelium contains several progenitor populations with significant reparative potential, but in COPD, these progenitors are impeded by cumulative damage, cellular senescence, and disrupted signaling. Understanding and reversing these dysfunctions is essential to restoring alveolar structure and function in affected individuals.

1.2 The alveolar progenitor niche

Alveolar epithelial progenitor cells do not function in isolation but reside within a specialized microenvironment, the alveolar progenitor niche, that orchestrates their behavior. This niche supports progenitor cell survival, regulates their proliferation and differentiation, and integrates repair signals following injury [38, 67, 68]. It is composed of various stromal cells and immune cells, microvascular endothelial cells (ECs), and the extracellular matrix (ECM), which together regulate progenitor cell behavior through complex cellular and molecular interactions [67, 68]. In healthy lungs, the niche provides the structural and biochemical cues necessary for epithelial regeneration and homeostasis. In COPD, however, these niche interactions are disrupted, impairing repair capacity and contributing to progressive alveolar damage [57, 69, 70]. Dissecting the composition and function of this regenerative microenvironment, both in health and disease, offers critical insight for developing targeted therapies (Figure 1). The following subsections explore the major components of the alveolar niche, including proinflammatory signals, macrophages, ECs, mesenchymal cells, and the ECM, and examine how each contributes to or hinders epithelial regeneration in COPD.

1.3.1 Pro-inflammatory cytokines

COPD is characterized by persistent, sterile inflammation, with an increased presence of immune cells and elevated levels of pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-8, IL-17, tumor necrosis factor alpha (TNFα), and type II interferon (IFN-γ) [71-77]. In addition, patients with COPD are more susceptible to infections, which can trigger inflammatory spikes that frequently lead to disease exacerbations [15, 78]. While these cytokines are well known for their roles in modulating immune cell activity and sustaining inflammation, their direct effects on lung regeneration remain poorly understood. Elucidating how pro-inflammatory cytokines interfere with alveolar repair is critical for developing therapies that can effectively restore damaged alveolar tissue.

Several cytokines elevated in COPD, including IL-1β [79], IL-6 [67, 80], and TNFα [79] are typically viewed as inflammatory drivers, but emerging evidence suggests that their effects on epithelial regeneration are complex and context-dependent. In short-term assays such as organoid cultures, these cytokines promoted alveolar cell proliferation and survival [67, 79]. Moreover, transient IFN-γ exposure has been found to be necessary for effective epithelial repair following acute infection [81]. Additionally, a recent study that modeled the complex inflammatory environment of a COPD exacerbation using a cytokine cocktail found increased epithelial proliferation under these conditions [82].

In contrast, these same cytokines can also disrupt epithelial repair processes. TNFα has been linked to alveolar dysfunction and impaired epithelial barrier integrity [83]. IFN-γ, while beneficial in low doses, has been associated with emphysema development in overexpression models and can induce apoptosis in alveolar epithelial cells in both human and murine models [84-87]. Chronic IL-1β exposure has been shown to reprogram fibroblasts towards a pro-inflammatory state, diminishing their capacity to support epithelial growth in co-culture organoid models [88]. Additionally, IL-1β can drive AT2 cells to a transitional state between AT2 and AT1 identities, preventing full differentiation when exposure is prolonged [89]. Notably, the previously mentioned cytokine exacerbation cocktail that enhanced proliferation also altered progenitor cell differentiation trajectories [82].

Together, these findings underscore that the impact of COPD-associated cytokines on lung regeneration is complex and highly context-dependent. The same cytokine may exert either supportive or detrimental effects depending on its concentration, duration of exposure, and the affected cell type. This duality may help explain why COPD lungs often display disordered epithelial differentiation and accumulation of aberrant cell types [45, 90]. Consequently, therapies aimed solely at stimulating epithelial proliferation are unlikely to achieve functional repair fully. Instead, coordinated therapeutic strategies must aim to restore the critical balance between progenitor cell proliferation and differentiation within an appropriate alveolar niche.

1.3.2 Macrophages

While various immune cells are present in the alveolar progenitor niche, lung macrophages occupy a central and dual role, being capable of both driving emphysema or promoting epithelial repair depending on their activation state. Historically, activated macrophages were described as polarized toward either pro-inflammatory (M1) or reparative (M2) phenotypes. However, several studies have demonstrated that alveolar macrophages from COPD patients exhibit a mixed or aberrant activation profile, with features of both M1- and M2-like phenotypes rather than a simple polarization toward one subtype [70, 91-96]. This complex activation state is often described as dysfunctional or reprogrammed, with impaired phagocytic and efferocytic capacity, altered protease–antiprotease balance, and ineffective inflammatory resolution [97]. Consequently, the use of strict M1/M2 terminology is now discouraged in human studies, as macrophage activation represents a continuum rather than two discrete phenotypes [98].

Lung macrophages are broadly categorized into alveolar and interstitial macrophages based on their anatomical location [99, 100], with each population differing in origin, mode of replenishment, and contribution to inflammation and repair processes. Alveolar macrophages are derived from fetal liver progenitors and are long-lived, self-renewing cells that maintain surfactant balance and homeostasis in the lung under steady-state conditions [101, 102]. Upon injury, they are often depleted and can be replaced through local proliferation or by recruited alveolar macrophages originating from circulating monocytes

[103]. These monocyte-derived alveolar macrophages are highly plastic and can adopt either pro-inflammatory or reparative phenotypes [104]. While fetal-derived alveolar macrophages are linked to homeostatic and anti-inflammatory functions, monocyte-derived alveolar macrophages are thought to contribute most to COPD pathology and emphysema development [105, 106]. Interstitial macrophages, initially derived from yolk sac progenitors, are largely replaced postnatally by bone marrow-derived cells maintained by circulating monocytes [107-109]. Located within the lung parenchyma or bronchial niches, interstitial macrophages contribute to immune regulation through antigen presentation and constitutively produce chemokines and immunosuppressive cytokines[110]. Interstitial macrophages have been reported to be quantitatively and phenotypically altered in COPD and engage in immune-regulatory crosstalk with epithelial cells [111]. Human and experimental data indicate that interstitial macrophages may protect against emphysema, positioning interstitial macrophages as modulators of chronic inflammation and tissue remodeling rather than passive bystanders [112], a perspective echoed by recent reviews calling for compartment-specific analysis of macrophage function in COPD [113].

Although both alveolar macrophages and interstitial macrophages contribute to COPD pathology, most studies have focused on alveolar macrophages without distinguishing their developmental origin. alveolar macrophages were found to contribute to alveolar destruction through the release of proteolytic enzymes and oxidative mediators[114-116]. Although neutrophils also participate in this process, the number of alveolar macrophages correlates more strongly with emphysema severity[117]. Moreover, animal studies demonstrated that emphysema development critically depends on macrophages and macrophage-derived matrix metalloproteinase 12 (MMP12), but not on neutrophils [118-120].

More recent studies highlight that alveolar macrophages are also key regulators of lung repair after injury [121, 122], a property that could be therapeutically exploited. A critical aspect of their unction in the alveolar niche is crosstalk with alveolar epithelial progenitor cells [123]. As mentioned before, macrophage function and phenotype are undeniably altered in COPD [124, 125], but how this impacts repair is less understood. Aging and chronic exposure to noxious stimuli like cigarette smoke can induce macrophage senescence, skewing their phenotype toward a pro-inflammatory state [126-128]. In COPD, macrophages

also exhibit impaired phagocytic and efferocytic function, leading to accumulation of apoptotic cells and persistent inflammation [129]. This failure to clear debris not only delays resolution of injury but also alters epithelial-macrophage signaling, ultimately disrupting the fate and proliferation of alveolar type 2 progenitor cells. For instance, alveolar macrophage peroxisomes support AT2 self-renewal via lipid and mitochondrial regulation, and peroxisomal dysfunction through excessive inflammation was shown to result in progenitor cell dysfunction [122]. Alveolar macrophages also secrete factors like placenta-expressed transcript 1, which directly stimulate epithelial proliferation and barrier restoration [121]. The importance of macrophage-epithelial interactions in lung regeneration was also highlighted by Rochford and colleagues, who showed that enhancing cyclic adenosine monophosphate (cAMP) signaling in pro-inflammatory monocyte-derived alveolar macrophages via PDE4b inhibition restored reparative capacity and resolved lung injury in mice [130]. These findings suggest that therapeutic strategies aimed at reprogramming macrophages, such as through modulation of cAMP-PDE4b signaling or enhancement of peroxisomal function, could be used to restore proper epithelial-macrophages interactions and support alveolar repair in COPD.

1.3.3 The microvascular endothelium

The alveolar capillary network is a vital component of the progenitor niche, supporting epithelial homeostasis and repair through both structural support and paracrine mechanisms. Alveoli are lined by a thin layer of pulmonary microvascular ECs, which form capillary networks tightly associated with the alveolar epithelium [131]. These ECs are separated from the epithelium by a basement membrane, a specialized ECM layer that maintains the structural integrity of the blood-air barrier and enables efficient gas exchange [131]. While ECs are often considered passive conduits for oxygen and nutrient delivery, they also function as dynamic regulators of lung homeostasis and epithelial regeneration [131-133].

Endothelial cells contribute to lung repair by interacting with alveolar epithelial progenitor cells to regulate their proliferation, self-renewal, and differentiation [131-136]. For instance, co-culturing primary

human AT2 cells with pulmonary ECs significantly enhanced alveolar organoid formation, demonstrating the role of endothelial-derived regenerative cues in epithelial repair [137]. Additionally, studies have shown that intravenous administration of pulmonary ECs ($CD45^{-}VE\text{-}cadherin^{+}CD31^{+}$) stimulated epithelial proliferation and differentiation and reversed elastase-induced emphysema in mice [138]. A crucial mechanism through which ECs support lung repair is the secretion of angiocrine factors, which are signaling molecules that regulate epithelial progenitor activity [131-133]. For example, in pneumonectomy models, ECs produced regenerative signals that drove epithelial progenitor expansion. One of these angiocrine factors, bone morphogenetic protein (BMP)4, and BMP6 have been identified as a key regulator of alveolar progenitor cell activity [135, 139]. Additionally, endothelial-derived hepatocyte growth factor (HGF) played a role in epithelial cell differentiation during lung development and repair, further reinforcing the importance of endothelial–epithelial cross-talk [140].

In COPD, alveolar ECs become dysfunctional, which contributes to impaired epithelial repair and disease progression. Indeed, microvascular EC loss has been observed before alveolar destruction in patients with emphysema, suggesting that vascular dysfunction may be an early driver of disease rather than a secondary consequence [137, 141]. Furthermore, exposure of human pulmonary ECs to CS reduced their ability to support alveolar organoid formation, indicating impaired regenerative signaling [137]. This endothelial dysfunction likely deprives the progenitor niche of key angiocrine signals, compounding epithelial injury.

Endothelial injury contributes to COPD and emphysema, yet the roles of distinct endothelial cell (EC) populations remain unclear. Single-cell RNA sequencing has revealed significant EC heterogeneity in the lung, including macrovascular (maECs), microvascular (miECs), Car4-high ECs, and $Atf3^{+}$ capillary ECs [134, 142]. Following H1N1-induced injury, both Car4-high and other ECs proliferate, while $Atf3^{+}$ ECs expand, supporting alveolar repair through genes regulating angiogenesis, migration, and development; endothelial-specific loss of Atf3 impairs regeneration, causing alveolar endothelial loss and emphysema-like changes. Car4 ECs form close contacts with AT1 cells across a thin, pericyte-free basement membrane and are lost after epithelial Vegfa deletion, leading to alveolar enlargement despite

normal myofibroblasts [143]. In COPD patients, endothelial progenitor cells (EPCs, $CD34^{+}KDR^{+}$) are reduced and inversely correlated with emphysema, whereas circulating endothelial cells (CECs) remain largely unchanged but track with microvascular dysfunction [144]. These findings highlight EC heterogeneity and indicate that impaired repair, rather than uniform loss, drives emphysema-related vascular pathology.

While the role of ECs in lung regeneration is increasingly recognized, it remains incompletely understood. Further research is needed to explore whether endothelial-targeted therapies could enhance lung regeneration in COPD. Beyond restoring endothelial function, therapeutic strategies that harness angiocrine signaling pathways may offer novel opportunities to stimulate epithelial repair and improve clinical outcomes.

1.3.4 Mesenchymal cells

Among the various components of the alveolar progenitor niche, lung-resident mesenchymal cells form a critical supportive element, particularly in regulating and fine-tuning epithelial development and repair [145]. These cells, including various types of fibroblasts and lung mesenchymal stromal cells, are important for the production and maintenance of ECM that orchestrates tissue repair upon injury [146]. They also secrete growth factors, inflammatory mediators, and extracellular vesicles (EVs), thereby providing paracrine cues to the surrounding endothelium and epithelium [145, 147-149]. Stromal fibroblasts are activated upon the release of transforming growth factor beta (TGF-β) from injured epithelial cells and differentiate into ECM-producing myofibroblasts that proliferate and deposit ECM proteins [148]. Meanwhile, fibroblasts also initiate paracrine signaling with AT2 cells and ECs through gaps in the basement membrane [150, 151]. The multidirectional interactions among fibroblasts, AT2 cells, and the endothelium guide immune cells from capillaries into interstitial space, and eventually across the alveolar epithelium to arrive in the alveolar airspace [152-154]. In the small airways, similar fibroblast-driven fibrotic remodeling underlies airway wall thickening and luminal loss. Preclinical and pathological studies show that small-airway narrowing and loss precede emphysematous changes and

correlate strongly with lung-function decline [11, 155]. Targeting myofibroblast activation, TGF-β signaling, or aberrant extracellular-matrix cross-linking can partially reverse peribronchiolar fibrosis and reopen obstructed airways [156]. Understanding how to re-establish a reparative rather than fibrotic fibroblast phenotype may be key to restoring small-airway patency in early COPD.

Fibroblasts derived from lung tissue from patients with COPD exhibit reduced proliferative capacity, diminished responsiveness to injury signals, increased senescence, and a pro-fibrotic phenotype [146, 147, 157-162]. Evidence suggests that extensive exposure to CS may permanently alter the fibroblast responsiveness in COPD, where MSCs and fibroblasts exhibit functional deficiencies such as a reduction in growth factors (FGF2; VEGF; HGF) secretion [147, 163-166]. In some instances, COPD fibroblasts release more transforming growth factor beta 1 (TGF-β1) but exhibit dysregulation of the TGF-β/Smad pathway and blunted transcriptional/ECM responses, leading to an impaired ability to produce ECM components [161, 167]. In addition to reduced secretion of key growth factors, COPD fibroblasts display an aberrant response to TGF-β stimulation, characterized by diminished proteoglycan production, impaired ability to support epithelial organoid formation in vitro, and a shift towards senescence or pro-inflammatory fibroblast phenotypes in response to matrix degradation [167-169]. Consequently, the reduced growth factor secretion and altered growth factor signaling result in a dysregulated repair program characterized by excessive ECM deposition, impaired epithelial regeneration, and the development of emphysematous lesions [145, 158].

Senescence markers, such as laminin-B1, cyclin dependent kinase 1A (p21), cyclin dependent kinase 2A (p16), and SA-β-gal are elevated in fibroblasts from patients with COPD and this is associated with resistance to apoptosis and increased secretion of growth factors and pro-inflammatory cytokines. The latter are part of the senescence-associated secretory phenotype that amplifies inflammation and tissue remodeling [158, 159] Cross-talk between fibroblasts and epithelial cells is important for maintaining homeostasis in lung tissue [170]. In COPD, dysfunctional fibroblasts may propel epithelial-to-mesenchymal differentiation, with subsequent migration through a fragmented reticular basement membrane [171]. This leads to a loss of epithelial–mesenchymal contact and impaired leukocyte clearance,

631 contributing to leukocyte accumulation in the interstitial space and further disrupting alveolar repair [152].
632 Together, these findings highlight mesenchymal dysfunction as a central contributor to impaired
633 progenitor activity and regenerative failure in COPD.

634

635 **1.3.5. The extracellular matrix**

636 In addition to cellular components, the ECM forms an integral part of the alveolar progenitor niche. The
637 ECM is a network of proteins and other supporting molecules that provide structural and biochemical
638 support to the surrounding cells, which dictates the tissue integrity and lung function [172, 173]. A crucial
639 role of ECM is providing a scaffold that supports the lung architecture. Beyond its structural
640 architecture, the ECM also serves as a dynamic growth factor reservoir and signaling interface that
641 modulates immune cell migration, activation, and retention within lung tissue [174, 175]. Altered matrix
642 degradation and composition in COPD ECM expose cryptic fragments and modify chemokine
643 gradients, thereby dysregulating leukocyte trafficking and contributing to the perpetuation of chronic
644 inflammation [176, 177]. This is primarily attributed to ECM proteins such as collagens, elastin, and
645 proteoglycans (decorin, perlecan, biglycan, and veriscan), which together provide tensile strength,
646 elasticity, and facilitate fiber assembly and signaling, respectively [173, 178-181]. The alveolar structural and
647 functional integrity relies on appropriate arrangement of these ECM proteins [181-183]. In COPD, extensive
648 ECM remodeling contributes to small airway fibrosis and narrowing. While this review focuses on
649 alveolar repair, resolving aberrant ECM deposition and restoring elastic recoil in the small airways will
650 be equally important for functional regeneration of the distal lung [180, 184]. In emphysema, alveolar
651 destruction results from progressive damage to the ECM network in the lung parenchyma [185]. The loss
652 of collagen type I and elastin correlate with a reduction of tissue stiffness, which makes the structure
653 more susceptible to external forces applied during normal expiration that result in alveolar
654 overexpansion, wall rupture, less oxygen exchange and collapse [178, 185, 186].

655

656 Numerous ECM alterations are observed in lung tissue from patients with COPD, including increased
657 ECM degradation, especially elastin and collagen I, along with dysregulated ECM turnover and

abnormal remodeling [71, 187]. There are variable reports of ECM changes in COPD, possibly reflecting different tissue sampling and staining protocols. A recent study described altered signatures of ECM expression profiles in COPD parenchyma, including lumican and collagen type 6α1, with these changes correlating with disease severity [184]. In parallel, decreased elastin levels in COPD ECM may result from CS-linked elastase/anti-elastase imbalance. This imbalance leads to the formation of dysfunctional elastic fibers which greatly reduce elasticity of the lung tissue [188]. Moreover, decreased decorin levels in the parenchyma disrupt the regulation of collagen fibrillogenesis and inhibit cellular responses to inflammatory cytokines [146, 167, 189, 190]. Elevated levels of versican in the parenchyma may further inhibit the assembly of elastic fibers and contribute to impaired matrix organization [147]. In contrast, Annoni et al reported a proportional reduction in elastin and versican in the distal parenchyma of patients with COPD [178], highlighting the heterogeneity of ECM alterations across disease stages and lung regions. Taken together, these findings highlight that ECM remodeling in COPD not only reflects structural disintegration but also contributes to progenitor cell dysfunction and impaired alveolar repair.

1.4 Summary and outlook

In summary, COPD is characterized by progressive alveolar destruction and impaired tissue repair. Although the alveolar epithelium contains progenitor populations with regenerative capacity, this potential is disrupted in COPD due to chronic inflammation, cellular senescence, and dysfunctional niche signaling. The alveolar progenitor niche, which includes immune cells, ECs, fibroblasts, and the ECM, plays a central role in coordinating epithelial repair (Figure 1). In COPD, alterations across all these components converge to create a non-permissive (i.e. inhibitory) environment for regeneration. A better understanding of these interactions will enable the development of regenerative therapies aimed at restoring alveolar structure and improving long-term outcomes for patients with COPD. Given the multifactorial nature of repair failure in COPD, a wide range of therapeutic strategies are currently being explored. These include small molecules that directly stimulate epithelial regeneration, compounds that inhibit processes contributing to regenerative dysfunction, cell-based therapies, and emerging approaches involving EVs or cell-derived proteins. The following sections will discuss these

pharmacological and biological strategies in detail, highlighting their mechanisms of action, regenerative potential, and current stage of development.

2. REGENERATIVE THERAPEUTICS FOR COPD

2.1 Small molecules that directly activate regeneration

2.1.1 cAMP-based drugs

Cyclic adenosine monophosphate (cAMP) is a key intracellular second messenger involved in various physiological processes, including lung homeostasis, inflammation, and metabolic regulation. Given its anti-inflammatory, bronchodilatory, and potential pro-regenerative properties, pharmacological strategies aimed at increasing intracellular cAMP levels have emerged as promising therapeutic avenues for COPD. In this context, PDE4 inhibitors and other cAMP-modulating compounds have been extensively investigated in both preclinical and clinical settings [191].

cAMP can be activated by a wide range of extracellular and intracellular stimuli, triggering downstream signaling effectors such as protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac). Through the activation of these key effectors, cAMP exerts regulatory effects on inflammation and energy metabolism [191] (Figure 2).

2.1.1.1 β-adrenoceptor agonists

β2-adrenoceptors are highly expressed in the alveolar walls, endothelium, pulmonary arteries, tracheal smooth muscle, and bronchial epithelium, where they contribute to airway tone and fluid clearance [192]. Activation of the β2-adrenoceptor stimulates adenylyl cyclase via G proteins, thereby increasing intracellular cAMP levels [191]. Consequently, β2-adrenoceptor agonists have become essential components of pharmacological therapy for asthma and COPD [192]. These agents induce bronchodilation via cAMP-mediated activation of PKA [193], while also promoting mucociliary clearance and attenuating inflammation [194].

Short-acting β2-adrenoceptor agonists (SABAs), such as salbutamol, pirbuterol, and terbutaline, provide rapid symptom relief, while long-acting β2-adrenoceptor agonists (LABAs), including formoterol, salmeterol [192], indacaterol [195], olodaterol [196], and vilanterol [197], offer sustained bronchodilation, although formoterol is now also used as a reliever medication based on its fast-acting properties. LABAs are commonly used in combination with long-acting anticholinergics based on the observation that dual bronchodilation is more effective than single bronchodilation and more effective than the combination of LABAs with inhaled corticosteroids [198, 199]. Beyond their bronchodilatory effects, LABAs have been shown to reduce airway smooth muscle proliferation, enhance ciliary function, and decrease the release of inflammatory mediators and neutrophil activation [200].

Regarding regenerative potential, β2-adrenoceptor agonists have demonstrated beneficial effects on epithelial repair in models of acute respiratory distress syndrome [201] and bovine bronchial epithelial cell wound healing [202]. The potential role of β2-adrenoceptor agonists on inflammation and epithelial repair in acute respiratory distress syndrome is reviewed in detail in [203]. However, recent findings suggest that β2-adrenoceptor agonists may impair airway epithelial regeneration via cAMP-independent mechanisms, specifically through enhanced activity of protein phosphatase 2A (PP2A) [204]. Indeed, Epithelial β1 Integrins play a role in alveolar homeostasis and restitution through the regulation of alveolar epithelial cell inflammation [205]. Similar inhibitory effects have been observed on wound healing in keratinocytes [206, 207] and corneal epithelial cells [208]. These findings raise concerns regarding the long-term impact of β2-adrenoceptor agonists on epithelial repair in chronic respiratory diseases, where delayed wound healing could increase susceptibility to infections and disease progression. With respect to mucus plugging, which is known to accelerate lung function decline [19], β2-adrenoceptor agonists may have beneficial effects as they increase ciliary beat frequency, enhancing mucociliary transport [209, 210]. However, this is less effective if mucus is very thick or infection/inflammation dominates and consequently the impact of LABAs on lung function decline may be present, but is not overwhelming [211].

2.1.1.2 Phosphodiesterase inhibitors

Intracellular cAMP levels are tightly regulated by PDE activity. Under physiological conditions, cAMP suppresses pro-inflammatory responses. However, in COPD, increased PDE activity leads to excessive hydrolysis of cAMP into its inactive form, 5'AMP, resulting in diminished cAMP signaling and exacerbated inflammation [191].

There are 11 known PDE families in mammals, comprising over 50 isoforms, some of which exhibit tissue-specific expression patterns. Among them, PDE3, PDE4, and PDE7 are particularly enriched in the lungs [212, 213]. PDE4, the predominant isoform responsible for cAMP degradation in pulmonary tissues, has garnered significant interest as a therapeutic target in COPD due to its upregulation, particularly in macrophages from patients with COPD [214].

PDE4 inhibitors prevent cAMP breakdown, thereby enhancing its signaling effects. Roflumilast, a selective PDE4 inhibitor approved for use in COPD, has demonstrated efficacy in reducing moderate and severe exacerbations by 12% and 16%, respectively [215, 216]. However, its widespread clinical use has been limited by systemic side effects such as nausea, diarrhea, weight loss, and abdominal discomfort [217].

Ensifentrine is a novel dual PDE3/PDE4 inhibitor with the PDE3 inhibition responsible for bronchodilation being the most potent activity, that was recently approved for the treatment of COPD. It enhances lung function, quality of life and reduces the rate of exacerbations in patients with COPD, which is relevant to COPD progression [218]. The impact of ensifentrine on lung regeneration is not clear, but it does inhibit injury of human microvascular endothelial cells and alveolar epithelial cells in response to methicillin-resistant S. aureus *in vitro* [219]. It will be of interest to explore the impact of ensifentrine on lung regeneration further, because of its dual action, which broadens its mode of action, affecting both structural cells such as fibroblasts and epithelial cells as well as inflammatory cells [220].

Apremilast, another oral PDE4 inhibitor approved for inflammatory conditions like psoriasis and psoriatic arthritis, also holds potential for COPD management, especially in patients prone to recurrent lung infections due to its anti-infective and anti-inflammatory properties. Efforts to formulate apremilast for inhaled administration via aerosolized nebulization are currently underway [221]. In a rat

model, apremilast has shown the capacity to reduce lung inflammation and promote airway repair upon LPS stimulus [222].

Tanimilast (CHF6001) is a next-generation inhaled PDE4 inhibitor developed to enhance therapeutic efficacy within the lungs while reducing systemic exposure and associated side effects [223]. Unlike selective inhibitors, Tanimilast targets all four PDE4 isoforms (A–D) without isoform preference. It has shown broad anti-inflammatory activity across various human immune and structural cell types, including neutrophils, eosinophils, macrophages, dendritic cells, lymphocytes, and bronchial epithelial cells. These effects have also been demonstrated in human lung explants [224] and precision-cut lung slices [225]. By elevating intracellular cAMP levels, Tanimilast suppresses the production and release of a wide array of inflammatory mediators, while also reducing chemotactic responses and reactive oxygen species (ROS) production [223, 226]. In preclinical models of acute and sub-chronic pulmonary inflammation, Tanimilast has been effective in reducing neutrophil recruitment and overall inflammatory burden [227-229]. It is currently under evaluation in two Phase III clinical trials (NCT04636801 and NCT04636814) as an add-on therapy to inhaled corticosteroids (ICS), LABAs, and long-acting muscarinic antagonists (LAMAs) in COPD and chronic bronchitis patients who continue to experience symptoms despite triple therapy.

2.1.1.3 Prostanoids

Prostanoids are a family of lipid mediators derived from the arachidonic acid cascade, including PGD2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A2. Among these, PGE_2 and PGI_2 have garnered particular interest in chronic respiratory diseases such as asthma, COPD, and idiopathic pulmonary fibrosis (IPF) [230]. PGE_2 is ubiquitously produced by various lung cell types, with epithelial cells and macrophages being its principal sources [230, 231], whereas PGI_2 is predominantly produced by ECs [230].

Prostanoids exert both autocrine and paracrine effects through binding to G protein-coupled receptors (GPCRs). PGE_2 interacts with four distinct E prostanoid (EP) receptors (EP1–EP4), while PGI_2 signals via the IP receptor. The downstream signaling outcome depends on the specific receptor subtype engaged. EP1 activates phospholipase C, leading to protein kinase C activation and an increase in

cytosolic calcium, while EP3 inhibits adenylyl cyclase, thereby reducing cAMP levels. In contrast, EP2, EP4, and IP receptors stimulate adenylyl cyclase and increase intracellular cAMP. As such, the net effect of PGE_2 signaling is determined by the expression pattern of its receptors on the target cell [230].

The role of PGE_2 and PGI_2 signaling in lung repair in COPD remains controversial. Elevated PGE_2 levels and increased expression of EP2/EP4 receptors and PGI_2 have been detected in fibroblasts [161, 232] and airway secretions [233, 234] from patients with COPD. This has led to the hypothesis that enhanced prostanoid signaling may contribute to defective repair mechanisms and emphysema progression. However, several preclinical studies challenge this notion. Administration of a PGI_2 analogue conferred significant protection against CS extract-induced emphysema in rats [235], while treatment with stable analogs of PGE_2 (16,16-dimethyl prostaglandin) and iloprost (a PGI_2 analog) promoted epithelial regeneration and alveolar differentiation in lung organoid models exposed to CS extract [236]. Furthermore, selective EP2 and EP4 receptor agonists have shown potential in inhibiting fibroblast-to-myofibroblast differentiation following TGF-β stimulation[237], and even in reversing it [238], suggesting a role in mitigating fibrosis and preventing mesenchymal exhaustion.

2.1.1.4 Adenosine

Adenosine is a purinergic signaling molecule that accumulates extracellularly in response to tissue stress or injury. Elevated levels of adenosine have been reported in both healthy smokers and patients with COPD, showing a negative correlation with FEV_1% and increasing as disease severity progresses [239]. These changes include the up-regulation of CD73, which converts 5'-AMP into adenosine, and a down-regulation of adenosine deaminase activity leading to reduced breakdown [240]. Adenosine signaling can elicit pro-inflammatory or anti-inflammatory responses, as well as tissue-destructive or regenerative effects, depending on the receptor subtype and cellular context, which complicates its therapeutic targeting.

Adenosine exerts its effects via four GPCR subtypes: A_1, A_{2A}, A_{2B}, and A_3 receptors. While A_1 and A_3 receptors inhibit adenylyl cyclase and reduce intracellular cAMP levels, A_{2A} and A_{2B} receptors activate adenylyl cyclase, leading to increased cAMP production [241]. Interestingly, the therapeutic strategies

differ by receptor: antagonists of A_1, A_{2B}, and A_3 receptors, and agonists of A_{2A} receptors, have shown potential benefit in the treatment of asthma and COPD [241]. Specifically, A_{2A} receptor agonism has demonstrated anti-inflammatory effects across various animal models of airway disease [242-244]. In humans, A_{2A} receptors are expressed in bronchial and alveolar epithelial cells, as well as in smooth muscle and ECs [245]. Despite encouraging preclinical data, clinical transition has been proven challenging. For example, the selective A_{2A} agonist UK432,097 was discontinued after failing to demonstrate efficacy in a Phase II trial in COPD (NCT00430300).

Conversely, A_{2B} is the adenosine receptor with the lowest affinity but is highly inducible under inflammatory conditions. Its activation has been associated with airway inflammation and tissue remodeling [246, 247], playing a key role in fibrosis development [248]. Inhibiting A_{2B} with the selective antagonist CVT-6883 has been shown to reduce these effects in murine models [249]; moreover, blocking A_{2B} attenuated pulmonary hypertension in a murine model of emphysema and vascular remodeling [250].While adenosine signaling is closely linked to COPD pathogenesis and disease progression, the complex interplay between receptor subtype distribution, affinity, and downstream effects presents significant challenges in translating this knowledge into effective, targeted therapies.

In summary, cAMP-elevating agents hold significant promise for improving lung regeneration in COPD through their anti-inflammatory, bronchodilatory, and potentially pro-reparative effects. Collectively, these findings highlight the therapeutic potential and current limitations of cAMP-based interventions, emphasizing the need for more targeted and cell-specific approaches to enhance lung regeneration in COPD, especially in patients with advanced epithelial injury (Figure 2).

2.1.2 Glucocorticosteroids

Glucocorticosteroids represent one of the most common classes of drugs prescribed for chronic inflammatory respiratory illnesses, although the perspective on their clinical use in COPD has changed over the past decades. Initially one of the mainstay drugs for COPD management, it is now increasingly clear that corticosteroids do not sufficiently counteract inflammation in all patients with COPD, whereas they do increase the risk for side effects such as pneumonia [251-253]. Meta-analyses indicate that dual

bronchodilation with a long-acting anticholinergic and a long-acting β_2-adrenoceptor agonist is superior in terms of FEV_1 outcomes and prevention of exacerbations in comparison with the combination of a corticosteroid and a long-acting β_2-adrenoceptor agonist [198]. On the other hand, the risk of hospitalization due to pneumonia increases with dose and duration of corticosteroid treatment in patients with COPD [251-253]. A notable exception are patients with eosinophilic inflammation in whom post-bronchodilator FEV_1 improves with corticosteroid use and more so than in patients without eosinophilic inflammation, but this is not typical of emphysema [254]. Accordingly, corticosteroid use is no longer the recommended initial treatment for stable COPD patients, and is only considered for COPD patients with a high exacerbation risk if blood eosinophil numbers exceed 300/µl [255].

Corticosteroids are mostly used as inhaled corticosteroids, though systemic treatment with corticosteroids is also used in some patients, preferably for shorter time windows during acute exacerbation management to avoid side effects [255]. Clinically used inhaled corticosteroids include budesonide, fluticasone, ciclesonide and beclomethasone, whereas beclomethasone, dexamethasone, prednisone, prednisolone, methylprednisolone, hydrocortisone, and triamcinolone may all be used as systemic treatments for the management of COPD exacerbations [256]. The mode of action of glucocorticosteroids involves binding to the glucocorticoid receptor (GR), which in its inactive state is cytosolic and bound to heat shock protein 90. Dimerization of ligand-bound GR and nuclear translocation allows for the binding to glucocorticosteroid responsive elements (GREs) within the genome, resulting in the activation of anti-inflammatory genes such as lipocortin-1 and the repression of pro-inflammatory genes such as genes encoding for cytokines and cyclo-oxygenase-2. In addition, monomeric ligand-bound GR can bind to transcriptional regulators involved in pro-inflammatory gene expression such as AP-1 and NF-κB [257]. Moreover, non-genomic effects of glucocorticosteroids have been reported, including smooth muscle relaxation and immunosuppression, which are possibly dependent on a membrane bound GR [258, 259].

Glucocorticosteroids do not appear to have major beneficial direct effects on epithelial regeneration. In an elastase rabbit model of emphysema, intratracheal instillation of porcine pancreatic elastase induced changes in lung function and in airspace size, but these changes were not counteracted by

dexamethasone [260]. Similarly, in a mouse model of CS-exposure, treatment with budesonide failed to improve the regenerative capacity of AT2 cells [236]. In fact, *in vitro* exposure to budesonide even reduced the formation of alveolar epithelial organoids [236]. Studies using human airway epithelial cells report similar findings and show that the corticosteroid dexamethasone increases apoptosis and slows down wound closure [261]. Possibly, this effect is related to the activation of differentiation programs by corticosteroids, limiting the progenitor capacity of epithelial cells, particularly when corticosteroids are applied prior to the injury[261, 262]. Glucocorticosteroids also reduce the expression of HGF in fibroblast cultures, providing an additional explanation for the negative effects on progenitor function [263].

Despite these limited direct effects on progenitor cell function, glucocorticosteroids may contribute beneficially by inhibiting the inflamed lung microenvironment. Although indirect, and of limited effect size, beneficial effects of glucocorticosteroids on the progression of lung function decline, and on the progression of emphysema development assessed by computed tomography (CT) imaging, have been reported [264-266]. In addition, in at least a proportion of patients with COPD characterized by eosinophilia, glucocorticosteroids help to reduce the risk of exacerbations, disease events known to contribute to accelerated lung function decline [267, 268]. These indirect beneficial effects may be explained by changes in eosinophilic airway inflammation, by changes in matrix composition in the airways, or by changes in airway epithelium gene expression associated with cell cycle and oxidative phosphorylation [269, 270].

Moreover, corticosteroids inhibit mucus production by airway epithelial cells [271] which may have both direct and indirect effects on lung function decline. In summary, beneficial effects of glucocorticosteroids on disease progression may exist in at least a subgroup of patients with COPD, characterized by eosinophilic inflammation. These effects are unlikely to be the result of any direct beneficial effects of glucocorticosteroids on alveolar or airway repair, but instead related to suppression of inflammation in susceptible individuals. These findings highlight the unmet need for regenerative therapeutics in COPD, particularly for individuals with emphysema.

2.1.3 Mucolytic agents

Mucus plugging appears to play a crucial role in COPD as those patients without notable mucus plugs or those with resolvable mucus plugs have similar rates of lung function decline, whereas patients with persistent presence of mucus plugs have substantially accelerated decline of lung function [19]. Not only the presence but also the composition of mucus is altered in COPD. The presence of MUC5B is higher in COPD and so is the expression of the insoluble MUC2. Moreover, goblet cell metaplasia and an increased ratio of mucus cells to serous cells in the submucosal glands contributes to COPD [272]. Accordingly, mucolytic drugs are used in the treatment of COPD, which include N-acetylcysteine, carbocysteine, erdosteine, l-methylcysteine and fudosteine, all of which break up the cysteine bridges present in mucin proteins leading to less viscous mucus, and bromohexine, which targets glycosylation of mucin proteins, leading to less viscous mucus as well. Moreover, ambroxol is sometime used, which is an expectorant drug that drives fluid secretion[273]. The available clinical data indicate that mucolytics significantly reduce the rates of exacerbation, shortened the duration of antibiotic use and exacerbations, prolonged the time to first exacerbation, and had a tendency to reduce the occurrence of two or more exacerbations in patients with stable COPD compared to placebo [273]. Mucolytics did not improve lung function, mortality and quality of life. There is no direct evidence that mucolytics can be regenerative, but indirect effects driven by the beneficial effects on exacerbation management may lead to reductions in lung tissue injury.

2.1.4 PPARγ ligands

Peroxisome proliferator-activated receptor gamma (PPARγ) is, like the glucocorticosteroid receptor, a nuclear receptor that plays a critical role in regulating diverse cellular responses including glucose metabolism, lipid homeostasis, and adipocyte differentiation [274]. Unlike the glucocorticosteroid receptor, which resides in the cytoplasm when inactive and requires ligand-induced nuclear translocation, PPARγ is constitutively located in the nucleus and is activated through ligand-induced conformational changes. PPARγ is activated by endogenous ligands such as fatty acids and eicosanoids (such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2)) as well as synthetic ligands like thiazolidinediones [274]. Upon ligand binding, PPARγ undergoes a conformational change that facilitates

its heterodimerization with the retinoid X receptor (RXR). This PPARγ-RXR complex then binds to specific DNA sequences known as peroxisome proliferator response elements (PPREs) located in the promoter regions of target genes [275]. The activation of PPARγ leads to the recruitment of coactivators such as PGC-1α and the displacement of corepressors, ultimately resulting in the transcriptional regulation of genes [275].

15d-PGJ2 exerts anti-inflammatory effects by suppressing pro-inflammatory cytokines in part via PPARγ signaling and by inhibiting NF-κB signaling [276]. In addition, 15d-PGJ2 activates Nrf2 signaling to balance oxidant defense mechanisms [277]. Thiazolidinediones including rosiglitazone and pioglitazone represent selective and more stable PPARγ ligands, used for the management of type 2 diabetes, as they restore insulin sensitivity in peripheral organs such as the liver and fat tissues [256]. But PPARγ receptors are far from specific to fat and liver tissues, and are also widely expressed in structural and circulating cells present in the lung. Retrospective analysis of pioglitazone use in patients with COPD and type 2 diabetes hints to potential protective effects on COPD, but these would need to be confirmed in prospective studies [278]. Similar protective effects of thiazolidinedione use in patients with COPD has been associated with reductions in exacerbation risk [279].

Nonetheless, *in vitro* and *in vivo* evidence does support a beneficial role for thiazolidinediones in COPD. PPARγ ligands reduce the production of pro-inflammatory cytokines in alveolar macrophages obtained from patients with COPD, and enhanced gene expression associated with the alternative activation pathway [280]. PPARγ ligands also enhance efferocytosis and inhibit NF-κB signaling [281]. In line with a protective, anti-inflammatory function, the expression of PPARγ and of PGC-1α progressively decreases in the lungs of patients with moderate and severe COPD [282]. The expression of 15d-PGJ2 is also reduced in COPD, whereas that of the oxidative stress indicators HO-1 and NOX4 is increased [283]. Furthermore, PPARγ supports the expression of GPx3, which protects against oxidative stress in COPD [284]. Accordingly, treatment with thiazolidinediones has beneficial effects on the development of airway remodeling and emphysema development in mice and rats exposed to CS [285, 286]. These effects may in part be indirect, by reducing lung damage, as PPARγ ligands have anti-inflammatory effects on macrophages and restore the protease/antiprotease balance [285-288]. On the other hand, the experimental

PPARγ ligand LJ-529, which also acts as an adenosine A_3 receptor agonist, prevented emphysema development in a mouse model of elastase induced lung injury, suggestive of direct beneficial effects on epithelial repair as well [289]. A recent publication supports this contention and shows that rosiglitazone promotes lung organoid growth of both control and IPF-derived epithelial cells, whereas the PPARγ inverse agonist GW9662 reduces lung organoid growth [290]. The relevance of this effect for COPD remains to be established, but does warrant further investigation in view of the large number of patients with COPD who also have type 2 diabetes, in whom leveraging such a dual beneficial role for pioglitazone would be an attractive therapeutic strategy.

2.1.5 Retinoids

Retinoic acid (RA) signaling has potential to modulate population health at scale in part because active ligands are derived from dietary vitamin A, obtained from meat and plants as retinyl esters and carotenoids. Ingested retinoids are distributed to target tissues either postprandially in chylomicrons, or via hydrolysis into retinol and transport in blood while bound to retinol-binding protein, entering cells via the cell-surface receptor stimulated by retinoic acid 6 (STRA6) [291]. Intracellular retinol is metabolized into retinaldehyde by retinol dehydrogenases, then into transcriptionally active all-trans-RA (ATRA) by retinaldehyde dehydrogenases (RALDH1, 2, and 3) [292]. Alternatively, intracellular retinol can be esterified by lecithin:retinol acetyltransferase (LRAT) to be stored as lipid droplets, creating a reservoir for future RA synthesis [292]. Retinoids are unusual among vitamins for being stored at high levels within tissues to provide a buffer against periods of dietary vitamin A deficiency; these stores can be mobilized locally to respond to tissue damage, including in the lung[293]. Inappropriate RA signalling is limited in part through tight regulation of local RA concentration by CRABP1, which transports RA to cytoplasmic cytochrome P450 26 enzymes (CYP26A1, B1, and C1) for degradation [294].

Intracellular RA undergoes nuclear import by cellular retinoic acid binding protein 2 (CRABP2), whereupon RA interacts with cognate receptors of the nuclear receptor family, retinoic acid receptors (RAR-α -β, and -γ) to drive transcription [292]. RARs reside at RA response elements in regulatory regions

of target genes as heterodimers with RXR-α, -β, and -γ. Unliganded RAR:RXR heterodimers repress transcription through interactions with nuclear receptor corepressors (NCOR1 and NCOR2), which together with histone deacetylases (HDAC) and Polycomb proteins mediate chromatin compaction and gene silencing [292]. ATRA binding causes displacement of co-repressors by nuclear coactivators (NCOA1, 2, and 3), which recruit histone acetyltransferases and Trithorax proteins (MLL family) to mediate chromatin relaxation and activation of a diverse set of genes [292].

2.1.5.1 RA signaling control of lung cellular function

In vitro and *in vivo* studies have revealed that RA can exert powerful and cell-type specific effects on the major cell types of the distal lung. Cultured interstitial fibroblasts isolated from human or rat lungs increased synthesis of elastin, a key component of alveolar septa, in response to ATRA treatment [294, 295]. RA has long been known to promote differentiation of tracheal airway epithelial cells in air-liquid interface cultures [296]. A recent study found using organoids derived from adult distal lung tissue that ATRA promoted differentiation of distal lung epithelial progenitors including alveolar progenitors, whereas RA pathway inhibition blocked differentiation and promoted epithelial expansion [297]. The arrested differentiation in expanded epithelial organoids following RA inhibition was partially rescued by subsequent treatment with ATRA combined with HDAC inhibitors, suggesting agents that modulate chromatin accessibility at RA target genes could synergize with RA to improve regenerative outcomes in the lung [297]. Another study found that RA signaling, potentially through RAR-α, promoted angiogenesis in isolated human lung microvascular ECs [298]. Induction of angiogenesis is sufficient to induce regeneration in lung disease models [299]; together, this suggests that RA-driven angiogenesis could offer a potential strategy to induce lung regeneration. Accordingly, during lung development in mice, administration of vascular endothelial growth factor receptor 2 (VEGFR2) inhibitors which block angiogenesis caused alveolarization defects that were rescued with exogenous ATRA[300].

The lung is rich in retinoid esters, which were long thought to be stored in lipid-laden interstitial fibroblasts [301]. A recent study found that many additional lung cell types including the microvascular endothelium and AT 2 cells possess retinoid-containing lipid droplets [293]. Importantly, using a model of LPS-induced acute lung injury, this study found that mobilization of local retinoid stores is an

immediate response to tissue damage that is critical for successful resolution and survival [293]. It would be of interest to further investigate signals that trigger retinoid store mobilization following lung tissue damage.

2.1.5.2 RA in lung development, adult tissue maintenance and chronic lung disease

A key role for RA in lung development was revealed by mouse studies where targeted mutations in genes encoding RAR-α, -β or -γ caused impaired lung development including alveolarization defects [302-304]. In humans, vitamin A deficiency led to reduced lung function in offspring that was alleviated by maternal vitamin A supplementation [305]. Moreover, genetic studies in humans have identified associations between variants in numerous RA pathway genes and adult lung function including RARA, RARB and NCOR2 [306-308], which could reflect the role of RA signaling in lung development, but could also reflect a requirement for RA signaling in maintaining adult lung tissue integrity. A recent human study found that carotenoid intake and serum carotenoid levels in adults positively correlated with lung function, suggesting a protective role for RA signaling in adult lung maintenance [308]. Accordingly, low serum carotenoids were associated with increased risk of COPD [309]. It has long been recognized that vitamin A deficiency in adult rats can lead to parenchymal defects including emphysematous changes [310]. Thus, dysregulated RA signaling may be causal in the development of chronic lung disease. In support of this, emphysematous lung tissue showed increased expression of CYP26A1, which could increase local RA catabolism [298]. In addition, fibroblasts isolated from emphysematous lung had reduced levels of cellular retinoic acid binding protein 2 and failed to upregulate elastin in response to ATRA treatment [294]. Importantly, these changes may need to be overcome for the diseased lung to respond to exogenous RA.

2.1.5.3 Pharmacological treatment with RA

Among the first to explore regenerative pharmacology in the lung were studies from the 1990s and 2000s in which RA was administered in preclinical rodent models of chronic lung disease. ATRA administered in a rat model of elastase-induced emphysema induced lung regeneration, increasing alveolar numbers and restoring tissue architecture [311]. Subsequent studies in adult rodent models of emphysema supported these findings [312, 313]. Other studies failed to find an effect [314, 315], perhaps due to

differing sensitivities of different animal strains to retinoids [316, 317]. Nonetheless, this initial excitement led rapidly to human clinical trials for RA in chronic lung disease. Two studies investigated orally administered ATRA for patients with advanced emphysema, but failed to find an effect on CT, lung function, or quality of life scores [318, 319]. The reasons for the failures remain unclear but could be attributed to the advanced disease stage of the participants, where severe structural damage, depletion of progenitor cells, or a hostile local tissue microenvironment may have provided a barrier to therapeutic efficacy [33]. Moreover, it is unclear whether orally administered ATRA can reach the alveolar niche in sufficient quantities to drive repair.

It is possible that genes activated by RA during lung development are silenced in the ageing lung, and exogenous ATRA alone might be unable to overcome this. Further studies to characterize epigenetic changes in the ageing lung, and investigations into combining ATRA with approaches that modulate chromatin accessibility, may be warranted [297]. Encouragingly, a recent study showed that while lung regeneration following partial pneumonectomy was strongly impaired in aged mice, lung cells of aged mice remained responsive to exogenously administered ATRA, which indirectly activated PDGFRA signaling within resident PDGFRα+ alveolar fibroblasts, thereby augmenting alveolar regeneration [320].

2.1.5.4 Synthetic retinoids

Although ATRA has been in use therapeutically since the 1980s, known issues are off-target side effects and instability in solution [292]. Novel, synthetic retinoid derivatives that are stable and modulate discrete points in the RA pathway thus hold appeal for regenerative pharmacology. For example, the synthetic RAR-γ-selective agonist Palovarotene is primarily degraded by CYP3A4 enzymes and thus likely unaffected by increased CYP26A1 found in emphysematous lung [298]. Palovarotene was investigated in a parallel-group, placebo-controlled trial in patients with emphysema due to alpha 1-antitrypsin deficiency. Palovarotene appeared to cause small improvements in lung density and lung function relative to placebo, which although it failed to reach statistical significance, may indicate biological activity [321]. Other synthetic retinoids have been developed that specifically modulate the activity of RARs, CRABP1 and 2, LRAT, and CYP26 enzymes [322-324]. Proof-of-concept studies in lung cells *in*

vitro could probe the efficacy of such compounds to help shed light on their potential to promote regeneration in chronic lung disease.

2.1.6 WNT pathway modifiers

WNTs are a family of secreted glycoproteins that act as ligands for receptors and play crucial roles in cell-to-cell communication, especially during development, tissue regeneration, and stem cell maintenance. WNTs (19 distinct members in humans) bind and activate cell surface receptors called Frizzled receptors (FZD_1 through FZD_{10}) [325]. The binding of individual WNT ligands to specific Frizzled receptors, in conjunction with co-receptors, can elicit the activation of distinct signal transduction pathways. The WNT signaling pathways are mainly categorized as being β-catenin-dependent (classically referred to as canonical WNT signaling) or β-catenin-independent (i.e. non-canonical WNT signaling) [325]. In the absence of an extracellular WNT signal, cytosolic β-catenin is targeted for degradation by the so-called β-catenin destruction complex. Glycogen synthase kinase-3β (GSK-3β) plays a central role in the β-catenin destruction complex, serving as the main kinase that phosphorylates β-catenin, thereby targeting it for ubiquitination and proteasomal degradation. The destruction complex also includes several core components: AXIN, a scaffold protein and the rate-limiting factor of the complex; Adenomatous Polyposis Coli (APC), a tumor suppressor protein; and Casein kinase 1 alpha (CK1α), which initiates β-catenin phosphorylation, priming it for further phosphorylation by GSK-3β. Specific WNTs (e.g. WNT-3A) bind to specific FZD receptors and the co-receptors low-density lipoprotein-related receptors 5 and 6 (LRP5/6), resulting in inactivation of the destruction complex. Consequently, β-catenin degradation is reduced, it accumulates in the cytosol and subsequently translocates to the nucleus [326]. Nuclear β-catenin associates with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to regulate gene expression (Figure 3).

WNT signaling plays a crucial role in the development and maintenance of lung progenitor cells, particularly AT2 cells. It is essential for the proliferation and self-renewal capacity of AT2 cells, which are key for alveolar homeostasis and regeneration following lung injury [327]. AXIN2 functions as a negative feedback regulator, mediating β-catenin degradation and promoting commitment of AT2 cells

to differentiation towards AT1 cells [327, 328]. Importantly, AXIN2 expression peaks within WNT-active progenitor niches, establishing spatial gradients that define zones of self-renewal versus differentiation [327]. AT2 cells adjacent to WNT-secreting fibroblasts (e.g., WNT-3A and WNT-5A) maintain progenitor features, while AT2 cell positioned distally tend to differentiate into mature AT1 cells [50, 67, 329]. Disruption of WNT signalling gradients in COPD may lead to depletion or senescence of AT2 progenitor cells, thereby impairing their ability to self-renew and progress towards an AT1-like fate [330]. Restoring balanced WNT signalling is beneficial for lung tissue regeneration and, consequently, may offer therapeutic potential for the treatment of COPD {Kneidinger, 2011 #94}. However, excessive or prolonged β-catenin activation disrupts normal alveolar epithelial maturation dynamics in vitro [329] and the consequences of such overactivation on alveolar epithelial cell lineage progression in vivo remains incompletely defined.

2.1.6.1 Glycogen synthase kinase-3 inhibitors

GSK-3β is the primary kinase responsible for the phosphorylation of the WNT effector protein β-catenin. Upon phosphorylation, β-catenin is ubiquitinated and targeted for proteasomal degradation [325]. In COPD, β-Catenin expression is downregulated, particularly in the alveolar epithelium [330]. Pharmacological inhibition of GSK-3β leads to stabilization of β-catenin and activation of β-catenin-mediated gene transcription in various lung cells, with beneficial effects observed in multiple preclinical models of COPD. For example, lithium chloride (LiCl), an FDA approved drug used for the treatment of bipolar disorder, activates β-catenin signaling and has been shown to reduce elastase-induced emphysema in mice [330]. This therapeutic effect was recapitulated in three-dimensional (3D) *ex vivo* lung tissue cultures derived from patients with COPD [330]. Similarly, the structurally unrelated GSK-3β inhibitor CHIR/CT99021, also known as Laduviglusib, activated β-catenin signaling in lung tissue cultures of patients with COPD. Therapeutic application of CHIR/CT99021 also reduced CS-induced emphysema in mice [331]. Notably, pharmacological activation of β-catenin signaling by CHIR/CT99021 can partially restore the impaired function of distal lung progenitor cells, including AT2 cells, in experimental emphysema models [332]. In addition, SB216763, another GSK-3β inhibitor, has

demonstrated protective effects in a guinea pig model of lipopolysaccharide-induced pulmonary inflammation. In this model, which mimics aspects of COPD, treatment with SB216763 improved both lung pathology and skeletal atrophy [333, 334]. In addition to β-catenin activation, GSK-3β inhibitors also suppress NF-κB signaling, a key driver of COPD-related inflammation [335]. This might also be relevant for COPD pathogenesis as particulate matter (PM2.5) and CS-induced inflammatory responses *in vitro* were suppressed by SB216763 via suppression of NF-κB signaling [336, 337]. Together, these findings support the therapeutic potential of GSK-3β inhibitors in COPD. GSK -3β is required for proper proliferation and maturation of lung epithelial progenitors both, and the timing of its inhibition appears essential for achieving regenerative benefit [338, 339]. In a murine model of inflammatory lung injury, transient GSK-3β inhibition alleviates LPS-induced damage and promotes epithelial repair [338]. GSK-3 inhibition produced distinct effects on alveolar epithelial cell proliferation and differentiation depending on whether GSK-3 was blocked during the acute inflammatory phase or during the post-acute inflammatory phase following LPS-induced injury [338]. However, prolonged or sustained GSK-3β inhibition can impair the terminal differentiation of lung progenitors in vitro [339]. Collectively, these findings indicate that both dosage and timing of GSK-3 inhibition are critical to harness regenerative benefit. Alongside the aforementioned GSK-3 inhibitors, there are many more small molecules that inhibit GSK-3β and their therapeutic potential is being investigated for various disease conditions, but without any available data for COPD [340].

2.1.6.2 Alternative mechanisms of β-catenin activation

In addition to classical GSK-3β inhibition, several drugs have been identified that activate β-catenin signaling through alternative mechanisms. For instance, the FDA approved anti-inflammatory drug amlexanox and the pain reliever phenazopyridine hydrochloride both promote organoid formation in a β-catenin-dependent manner. Importantly, amlexanox has shown therapeutic efficacy *in vivo* by significantly reducing elastase-induced emphysema in a mouse model of COPD [341]. However, the activation of β-catenin by these drugs is most likely not related to direct inhibition of GSK-3β.

In addition to specific WNTs that activate β-catenin-dependent signaling, other WNTs can activate alternative (non-canonical) signaling pathways. In this context, WNT-5A is of particular interest, as its effect on β-catenin signaling depends on the receptor environment at the cell membrane. In the presence of the FZD_4 receptor, WNT-5A activates β-catenin-dependent signaling. FZD_4 is involved in β-catenin–driven alveolar lung repair and is significantly downregulated in human and experimental COPD [342]. In contrast, WNT-5A expression itself is upregulated in COPD and is associated with reduced β-catenin activity in alveolar epithelial cells [343].Therapeutic targeting of this pathway has yielded promising results. Inhibition of WNT-5A using either a neutralizing antibody or BOX5, a WNT-5A-derived, N-terminally butyloxycarbonyl-(Boc) protected hexa-peptide, attenuated lung tissue destruction, improved lung function, and restored expression of β-catenin-driven target genes and alveolar epithelial cell markers. These effects have been demonstrated in both elastase- and CS-induced models of COPD [343]. Collectively, these findings suggest that restoring FZD4 expression or inhibiting WNT-5A may provide therapeutic benefit in emphysema by reactivating β-catenin signaling. In addition, WNT-5A has profibrotic actions by enhancing fibroblast-to-myofibroblast differentiation and activation of the profibrotic growth factor latent TGF-β [342, 344]. While GSK-3 inhibition primarily enhances canonical WNT/β-catenin signalling and WNT-5A predominantly engages non-canonical pathways, crosstalk exists whereby canonical WNT activation can mitigate certain profibrotic effects of WNT-5A signalling, and vice versa.

2.1.6.3 WNT ligand and Frizzled receptor modulators:

Direct activation of canonical WNT signaling using ligand mimetics is emerging as a promising regenerative strategy in chronic lung diseases. One such approach involves the antibody R2M3-26, which has been engineered to simultaneously engage multiple Frizzled receptors (FZD_1, FZD_2, FZD_5, FZD_7, FZD_8) along with the LRP6 co-receptor. This multivalent targeting functionally mimics natural WNT ligands and activates β-catenin signaling across diverse target cells. A recent study demonstrated that R2M3-26 significantly enhanced alveolar organoid expansion *in vitro* using both mouse and human-derived AT2 cells [345]. *In vivo*, R2M3-26 treatment in mice with bleomycin-induced pulmonary

fibrosis reduced inflammation and collagen deposition, improved lung mechanics (increased lung compliance and decreased elastance), and upregulated Axin2 in epithelial, mesenchymal, and endothelial compartments, indicating widespread WNT pathway activation and tissue repair [345].

Building on this strategy, receptor-specific agonists targeting individual FZD subtypes have also shown efficacy in modulating epithelial regeneration. FZD_5- and FZD_6-specific agonist antibodies were recently shown to potently activate canonical WNT/β-catenin signaling in AT2 cells, enhancing their stem cell activity [346]. In this study, FZD_5 was identified as essential for AT2 self-renewal and epithelial regeneration following injury. Interestingly, FZD_6, which is traditionally associated with non-canonical signaling, was also found to activate β-catenin–dependent transcription in AT2 cells. Systemic administration of FZD_5- or FZD_6-specific agonists *in vivo* promoted AT2 proliferation and improved survival in bleomycin-treated mice [346]. In a murine emphysema model, systemic delivery of WNT-3A loaded extracellular vesicles enhanced AT2 cell proliferation, reduced alveolar space enlargement, and improved lung function [65]. Notably, this approach also led to activation of regenerative gene programs across epithelial, mesenchymal, and endothelial compartments. Taken together, these findings support the feasibility of ligand-based WNT activation as a regenerative strategy in COPD. This approach carries important context-dependent considerations. GSK-3 inhibition may act synergistically with FZD-targeted agonists to enhance β-catenin activation and thereby promote epithelial regeneration. A key caveat is that GSK-3 intersects with multiple WNT pathways: depending on the cellular context, GSK-3 (inhibition) can also modulate non-canonical WNT5A-driven signalling, which has been linked to pro-survival and profibrotic responses in fibroblasts [347]. Consequently, combining GSK-3 inhibition with FZD receptor agonism carries the potential for both pro-regenerative and profibrotic outcomes, depending on pathway bias and cellular context [348].

2.1.6.4 CK1α inhibitors

CK1α is a regulatory kinase involved in the phosphorylation of several components within the β-catenin destruction complex. Inhibition of CK1α can stabilize β-catenin and thereby enhance WNT signaling. While specific studies in COPD models are limited, the modulation of CK1α presents a potential

strategy for restoring epithelial regeneration in chronic lung diseases. One example is SJ7095, a recently developed molecular glue degrader of CK1α. This compound induces a specific interaction between an E3 ubiquitin ligase and the target protein, leading to its targeted degradation [349]. Whereas SJ7095 has shown promise in modulating WNT signaling through CK1α degradation, it remains to be tested in COPD models. Another compound, MU1742, has also been identified as a CK1α inhibitor with potential WNT-activating properties [350]. Despite their promise, targeting CK1α carries important considerations. Similar to GSK-3β, CK1α is involved in a diverse array of cellular processes, including circadian rhythm regulation, DNA repair, and apoptosis [351]. Additionally, CK1α also contributes to NF-κB activation. Its inhibition may therefore suppress inflammatory pathways, potentially dampening immune responses and impairing host defense mechanisms. This is an important consideration given the heightened susceptibility to infections in patients with COPD [352]. Taken together, while CK1α inhibitors represent a mechanistically compelling route to restore WNT activity and promote epithelial regeneration, their pleiotropic effects warrant careful evaluation in the context of COPD.

2.1.6.5 Dishevelled (DVL) activators/stabilizers

Dishevelled proteins (DVL1, 2, and 3) are central scaffolds in the WNT signaling cascade. They transmit signals from FZD receptors to downstream effectors, including β-catenin, and participated in both β-catenin-dependent and -independent pathways through their DIX, PDZ, and DEP domains [325]. Within the canonical WNT pathway, DVL contributes to β-catenin stabilization by inhibiting the β-catenin destruction complex, thereby enabling transcription of WNT target genes.

Pharmacological activation or stabilization of DVL has been proposed as a strategy to reinforce canonical WNT signaling and promote alveolar epithelial repair. One known negative regulator of DVL is CXXC5, which binds to the PDZ domain of DVL and attenuates β-catenin signaling [353]. Small molecule inhibitors such as KY-02061 and KY-02327 block this interaction. By preventing the binding of CXXC5 to DVL, these compounds relieve negative feedback inhibition and enhance WNT pathway activation [354]. These findings suggest that stabilizing DVL activity through targeted disruption of

inhibitory protein interactions may represent a promising therapeutic approach to promote epithelial regeneration in chronic lung diseases such as COPD.

2.1.6.6 sFRPs

Secreted frizzled-related proteins (sFRPs) are extracellular antagonists of WNT signaling that function by binding and sequestering WNT ligands, thereby preventing their interaction with FZD receptors [325]. Among the sFRP family members, sFRP1 and sFRP2 have been implicated in the pathogenesis of COPD by contributing to impaired epithelial repair. sFRP1 is elevated in emphysematous lung tissue and correlates with increased expression of matrix metalloproteinases (MMP) MMP-1 and MMP-9, implicating a role in ECM degradation and alveolar destruction [355]. Similarly, sFRP2 expression is increased in the small airway epithelium of smokers and patients with COPD. This protein suppresses β-catenin signaling and may thereby hinder epithelial regeneration by interfering with canonical WNT pathway activity [356]. Given this inhibitory role, neutralising sFRPs has been explored as a strategy to restore WNT signalling. Antibody-mediated blockade of SFRP1 has been shown to relieve extracellular WNT inhibition: in stressed epithelial and fibroblast systems, anti-SFRP1 antibodies attenuated SFRP1-dependent senescence and restored downstream β-catenin signalling [357]. Furthermore, small-molecule approaches have also been developed to target sFRPs. Bodine et al. identified sFRP-1 inhibitors (e.g., WAY-316606) through high-throughput screening, demonstrating that direct pharmacological inhibition of sFRP-1 selectively increases β-catenin activity in functional reporter assays [358]. Although this data originates from skeletal biology, they provide proof-of-concept that pharmacologically releasing extracellular WNT brakes is feasible. In addition, intracellular DVL activators (e.g., KY-02061, KY-02327) can bypass extracellular ligand sequestration entirely by relieving CXXC5-mediated inhibition of DVL, restoring downstream signalling even under conditions of elevated sFRP expression.

2.1.6.7 R-spondin proteins and RSPO agonists

R-spondins (RSPO1 to RSPO4) are secreted glycoproteins that enhance β-catenin dependent WNT signaling. They function by binding to leucine-rich repeat containing GPCRs (LGR4/5/6) and inhibiting

the E3 ubiquitin ligases ZNRF3 and RNF43. This interaction prevents internalization and degradation of WNT receptors, thereby stabilizing Frizzled and LRP5/6 on the cell surface and amplifying WNT signal transduction [359]. In the lung, RSPO2 is involved in epithelial patterning and branching morphogenesis during development, suggesting a potential role in progenitor cell regulation [360]. In a murine model of bleomycin-induced lung injury, RSPO2 administration enhanced WNT target gene expression and accelerated epithelial repair, further supporting its regenerative potential [361]. Despite these promising findings, the clinical application of recombinant RSPO proteins is limited due to issues with protein stability and delivery. To address these limitations, recent research has focused on developing small molecule agonists that mimic RSPO activity by targeting LGR4/5/6 receptors. Unfortunately, the development of small-molecule LGR4 agonists as RSPO mimetics has thus far not replicated RSPO's effect on β-catenin dependent WNT signaling. In one study, a β-arrestin-biased LGR4 agonist (referred to as compound 1) failed to enhance TCF and β-catenin reporter activity and instead slightly antagonized RSPO1-mediated signaling [362]. These findings suggest that current LGR4-targeting small molecules act via β-catenin-independent pathways, limiting their applicability for regenerative strategies aiming to restore alveolar β-catenin activity in COPD.

2.2 Small molecules that interfere with COPD-specific regenerative defects

2.2.1 Senotherapeutics

The incidence of COPD increases with age and is closely linked to the hallmarks of aging [6, 363]. Although genetic alterations in aging pathways are not well established in COPD, hallmarks of aging likely emerge from disease progression or environmental exposures such as CS [364]. Individuals with early-life lung impairment are particularly susceptible to accelerated aging [363, 365, 366]. Patients with COPD consistently show elevated markers of aging, with cellular senescence being among the most prominent. Senescence is a stress-induced, irreversible cell-cycle arrest state associated with senescence-associated secretory phenotype (SASP) secretion, which drives chronic inflammation and induces senescence in neighboring cells [367, 368]. Senescent cells have been identified within several lung compartments, including AT2 cells [60, 369], airway epithelium [370], endothelium [371, 372], smooth muscle cells [372, 373], and

fibroblasts [162, 188]. Their SASP, in part mediated by EVs, promotes paracrine inflammation, tissue remodeling, and immune dysregulation in COPD.

CS-induced oxidative stress accelerates senescence via telomere shortening, DNA and mitochondrial damage, and activation of the ATM-p53-p21 and p16-Rb pathways [374]. Accumulated senescent cells impair lung repair and sustain inflammation. Targeting senescence (senotherapy) is a promising therapeutic avenue in age-related diseases, including COPD [367, 375], with two main strategies under investigation: elimination of senescent cells (senolytics) or functional reprogramming (senomorphics).

2.2.1.1 Senolytics

Senolytics are compounds that target and eliminate senescent cells by disrupting their resistance to apoptosis. These cells rely on anti-apoptotic pathways to survive; senolytics reactivate programmed cell death specifically in these cells, enabling their clearance by the immune system. The idea of removing senescent cells to promote healthy aging originated from studies using the INK-ATTAC mouse model, where genetic ablation of p16(INK4a)-positive cells led to increased lifespan and reduced cancer incidence [376]. This foundational work inspired the development of drug screening platforms to identify senescence-targeting therapies. First-generation senolytics primarily targeted survival pathways, while newer agents focus on senescence-specific surface markers and phenotypes to enhance selectivity (Figure 4). The concept of depleting senescent cells is a promising concept since this would not require constant medication of the patient but a hit and run approach where the drugs can be administered intermittently.

The combination of dasatinib and quercetin (D+Q) remains one of the most extensively studied senolytic regiments [377]. Dasatinib, a tyrosine kinase inhibitor with selectivity for Abl and Src, and quercetin, a polyphenol, selectively eliminate senescent cells *in vitro*. D+Q are under clinical investigation for aging-related diseases, including IPF, with promising feasibility and safety data [378, 379].

Although no clinical data yet exist for senolytics in COPD, preclinical evidence is accumulating. Quercetin alone reduced inflammation and disease progression in elastase/LP-induced emphysema models, though its senolytic effect was not assessed [380]. Recent preclinical studies show that D+Q

reduces CS–induced senescence, inflammatory cell infiltration, and cytokine levels in COPD mouse models [381]. Similarly, D+Q reduced senescence and inflammation in air-liquid interface cultures from patients with COPD [381]. A randomized trial confirmed quercetin safety in patients with COPD, though without assessing cellular senescence markers [382].

A prominent target of senolytic strategies is the anti-apoptotic BCL-2 family, which is often upregulated in senescent cells (Figure 4). Compounds such as navitoclax (ABT-263), venetoclax (ABT-199), and ABT-737 mimic BH3 proteins by binding and inhibiting pro-survival proteins like BCL-2, BCL-XL, and BCL-w. These agents show differential efficacy across senescent fibroblasts, partially restoring ECM regulation *in vitro* [383]. Navitoclax also effectively eliminated senescent AT2 cells from patients with COPD [384]. Cardiac glycosides, including ouabain and digoxin, have been identified as effective senolytics, acting in part through induction of the pro-apoptotic BCL-2 family member NOXA and by disrupting the intracellular sodium–potassium gradient [385, 386]. While their senolytic efficacy has been demonstrated in animal models of fibrosis, no data are currently available regarding their use in models of COPD. Newer, potentially safer BCL-2 inhibitors such as UBX1325 have demonstrated senolytic activity, leading to improved retinal function and macular thickness in diabetic macular edema [387].

Additionally, PROTAC-based approaches that target BCL-XL for degradation via E3 ligases show promise in reducing senescence and inflammation while promoting proliferation in COPD small airway epithelial cells [388]. Natural compounds like fisetin, a flavonoid with anti-inflammatory properties [389], have also shown preliminary senolytic effects in COPD epithelial cells [390].

Of note, tight regulation of pro- and anti-apoptotic signaling determines cell fate during tissue remodeling and repair [391]. Apoptosis of epithelial cells has been described as a pathogenic mechanism in COPD [392, 393], whereas upregulation of anti-apoptotic pathways can exert protective effects on epithelial cells. This underscores that first-generation senolytics targeting pro-apoptotic pathways must be applied with caution to preserve a finely tuned apoptotic balance that supports tissue regeneration.

To enhance specificity, second-generation senolytics exploit unique features of senescent cells. One strategy targets increased lysosomal content and senescence-associated β-galactosidase (SA-β-gal) activity using galacto-oligosaccharide-coated nanoparticles or β-gal-activated prodrugs [394, 395]. Another

approach leverages iron dysregulation, a hallmark of senescent cells, which contributes to fibrosis and inflammation [396]. Iron-activated prodrugs like TRX-CBI selectively eliminate iron-overloaded senescent cells [397], a relevant strategy given the elevated iron levels in COPD and senescent airway cells [375, 398]. FOXO4, a longevity-associated transcription factor, binds p53 in senescent cells to block apoptosis. Disrupting this interaction with FOXO4-DRI induces senescent cell death and reduced fibrosis in an experimental fibrosis model [399]. In CS-induced senescent lung fibroblasts, DNA nanoparticles targeting Foxo4 displayed senolytic activity [400].

Senescent cells also show mitochondrial dysfunction and increased glutaminolysis. Elevated glutaminase-1 (GLS-1) breaks down glutamine into glutamate and ammonium, supporting survival. Inhibiting GLS-1 with BPTES induces senolysis and improves age-related organ function in mice [401]. Preliminary data suggests that it displays senolytic activity on senescent airway epithelial cells [402].

Alternative strategies to reduce senescent cells and their impact on chronic diseases include enhancing immune clearance or reactivating aging immune responses (Figure 4). CAR T cell therapies, initially developed for cancer, have been adapted to target senescent cells using surface markers such as uPAR and NKG2D ligands, showing efficacy in preclinical models—though uPAR is unsuitable for the lung due to its broad expression on non-senescent cell types including immune cells, endothelial and epithelial cells [403, 404]. In COPD and obesity, senescent T cells contribute to chronic inflammation, and targeted elimination, by vaccination against $CD153^+$ T cells, has improved tissue function in models [405], suggesting immune modulation may offer therapeutic potential in chronic lung diseases.

Notably, senescent cells play essential roles in normal embryonic development, tumor suppression, and wound healing [406]. In the adult murine lung, senescent fibroblasts have been shown to be required for epithelial regeneration [407]. Conversely, indiscriminate targeting of senescent cells may have adverse effects on wound healing and tissue homeostasis, emphasizing the need for strategies that specifically eliminate pathologically senescent cells while preserving their physiological functions.

2.2.1.2 Senomorphics

Senomorphics, which modulate senescence-associated pathways without directly eliminating senescent cells, represent a promising therapeutic strategy by attenuating chronic inflammation and tissue remodeling driven by the SASP. Key agents include mTOR inhibitors, Sirtuin activators, and JAK/STAT inhibitors. mTOR signaling regulates metabolism, proliferation, and senescence and is implicated in longevity. Its pharmacological inhibition has extended lifespan in model organisms [408]. In emphysema models, rapamycin reduced senescence markers [371]. Metformin, a widely used antidiabetic drug, activates AMPK and inhibits mTOR signaling, thereby reducing oxidative stress and SASP-driven inflammation in airway epithelial cells and ECs [409, 410], and protected mice from CS-induced injury in lung, kidney, and muscle [409]. Retrospective cohort analyses suggest clinical benefits of metformin in COPD [409, 411, 412]. JAK/STAT inhibition prevented senescence in emphysema models [372], with inhaled delivery improving tolerability and reducing SASP [413].

Sirtuins (SIRT1, SIRT3, SIRT6), NAD^+-dependent deacetylases, regulate inflammation, senescence, and mitochondrial function, playing critical roles in chronic lung disease progression [414-416]. Resveratrol, a sirtuin activator with lifespan-extending effects [417], has poor pharmacokinetic profile , prompting the development of more potent analogues as well as synthetic SIRT activators to reduce CS-induced lung inflammation. NAD^+ supplementation, e.g., via nicotinamide riboside, increased NAD^+ levels and reduced lung inflammation and senescence markers in patients with COPD in a recent clinical trial [418].

Modulating the SASP by targeting EVs, which are key SASP carriers, offers another novel therapeutic approach. Beyond targeting senescence, additional aging-related mechanisms in chronic lung disease include mitochondrial ROS inhibition (e.g., MitoQ, SkQ1), autophagy/mitophagy activation, and epigenetic modulation [419-421]. Modifying the gut-lung microbiome or using epigenetic clocks as biomarkers further expands options for non-invasive monitoring and intervention. Collectively, targeting the hallmarks of aging may not only slow COPD progression but also promote lung regeneration following injury.

2.2.2 ROCK inhibitors

Rho-associated coiled-coil kinase (ROCK) has a role in interfering with fibroblast function and differentiation towards myofibroblasts. Lung fibroblasts are required for alveolar epithelial regeneration by secreting growth factors, by producing ECM, and providing mechanical support. Cytokines such as TGF-β promote a myofibroblast phenotype, which is less able to support lung organoid formation [422, 423]. Although TGF-β is primarily associated with lung fibrosis, its levels are increased in COPD lung tissue as well [424]. The main mechanisms via which TGF-β pre-treatment of fibroblasts restricts the support function of mesenchymal cells includes modulation of Wnt pathway signaling as well as actin cytoskeletal remodeling. Thus, TGF-β–induced impairment of lung organoid formation can be mimicked by pre-treating lung fibroblasts with jasplakinolide, which enhances actin cytoskeletal stiffening [425].

Actin cytoskeletal remodeling depends on the conversion of globular actin to filamentous actin, which promotes the formation of stress fiber-like bundles of smooth muscle α-actin (sm-α-actin). This process also enhances sm-α-actin gene transcription by releasing G-actin–bound transcriptional regulators, such as MRTF-A, which translocate to the nucleus upon actin polymerization to activate target gene expression [426]. TGF-β and other actin factors that promote actin remodeling such as WNT-5A and WNT-11 utilize signaling via ROCK to enhance actin remodeling and subsequent MRTF-A dependent sm-α-actin expression [427, 428]. Accordingly, ROCK inhibitors are potential antagonists of actin cytoskeletal remodeling and its downstream effects. The most widely studied ROCK inhibitor is Y-27632, but other ROCK inhibitors are available, of which fasudil is even registered for clinical use in cerebral vasospasm [429].

Similar beneficial effects can be achieved for epithelial cell growth. In fact, ROCK inhibitors such as Y-27632 are often provided during lung organoid cultures to enhance progenitor cell activation. For example, ROCK inhibition using Y-27632 enhanced alveolar epithelial cell growth, Wnt pathway activation and expression of alveolar epithelial markers such as surfactant protein C (SFTPC) [430]. ROCK inhibitors are also able to reduce the negative effects of TGF-β on lung organoid formation, both if ROCK1/2 are simultaneously inhibited using compound A31 or when ROCK2 is inhibited selectively using compound A11 [425]. However, and in contrast to organoid number, the differentiation of lung

organoids towards $SFPTC^+$ alveolar epithelial cells was repressed by TGF-β and not reversed by ROCK inhibition [425]. The potential of ROCK inhibitors in reversing elastase-induced emphysema has not yet been reported, although CS-induced inflammation and vascular permeability have been shown to be ROCK-dependent [431]. In addition, because of the effects of ROCK inhibition on myofibroblast differentiation, indirect effects on MMP production may be envisaged. In conclusion, although ROCK inhibitors show therapeutic promise, current data are insufficient to draw firm conclusions about their role in lung regeneration in COPD.

2.2.3 Protease inhibitors

Lung emphysema is characterized by alveolar destruction, in part caused by an imbalance between proteases and anti-proteases in the lung [432]. This dysregulation leads to excessive proteolytic activity, resulting in ECM degradation and lung tissue damage. Extracellular proteases, particularly neutrophil elastase (NE), MMPs, and cathepsins, are enzymes responsible for the degradation of matrix components such as elastin and collagens [432]. As such, proteases play a physiological role in tissue remodeling, immune defense, and inflammatory responses. To prevent undesirable destruction of lung tissue, their activity must be tightly regulated by endogenous anti-proteases, including α_1-antitrypsin (A1AT), secretory leukocyte protease inhibitor (SLPI), and tissue inhibitors of metalloproteinases (TIMPs), which neutralize proteolytic enzymes and maintain lung structural integrity [432, 433].

In COPD and emphysema, the protease/anti-protease imbalance favoring proteolytic activity results in excessive degradation of alveolar walls, leading to loss of elastic recoil, and emphysema development. This may be caused by exposure to toxic chemicals, particles and gases such as CS, which trigger neutrophilic and macrophage-driven inflammation, leading to the release of NE and MMPs, which if persistent, contributes to lung tissue damage [434]. Moreover, oxidative stress generated by ROS inactivates anti-proteases such as A1AT, shifting the imbalance further. Genetic polymorphisms in the α_1-antitrypsin gene, leading to α_1-antitrypsin deficiency (AATD) is a well-established genetic cause of COPD leading to early-onset emphysema [435].

Targeting the protease-anti-protease imbalance for COPD and emphysema is an older concept, but with the exception of A1AT augmentation therapy for AATD patients, these approaches have not yet reached the clinic. Partly, this may be explained by the complexity of the protease / anti-protease network, rendering inhibition of individual proteases insufficient for clinical efficacy. Nonetheless, understanding this dynamic interplay between proteases and anti-proteases remains critical for developing novel interventions to halt or slow disease progression.

2.2.3.1 A1AT augmentation therapy

AATD is a genetically driven form of emphysema characterized by reduced serum levels (below 80 mg/dl) of functional A1AT, a serine protease inhibitor primarily responsible for protecting lung tissue from NE-mediated degradation [436]. The link between AATD and pulmonary emphysema led to the development of A1AT replacement as a potential therapeutic strategy. Initial efforts to develop augmentation therapy were reported in 1981, when Gadek and colleagues found that weekly A1AT supplementation was able to restore A1AT levels to normal in affected individuals [437]. Further developments led to the first FDA-approved plasma-derived intravenous A1AT therapy, Prolastin, which became available in 1987. Since then, additional formulations (e.g., Aralast, Zemaira, and Glassia) have been introduced, to restore circulating levels in deficient individuals [438]. A1AT augmentation therapy has demonstrated effects in reducing the progression of emphysema, by slowing the decline in lung density measured by CT imaging [439-441]. Current guidelines recommend augmentation therapy for individuals with severe AATD (PiZZ or PiSZ genotypes) and clinically significant emphysema [442].

Ongoing research is aimed at novel therapeutic strategies, including AAT replacement therapy using other administration routes and sources and gene therapy [443]. PEGylated AAT is under development as an inhaled formulation, which is not feasible using regular AAT because of the rapid clearance. INBRX-101 is a recombinant human AAT-Fc fusion protein that was found to be well-tolerated in AATD patients in a phase I trial, which increased the plasma AAT levels as well as AAT levels in epithelial lining fluid [444]. BEAM-302 is a lipid nanoparticle formulation containing base editing reagents designed

to correct the PiZ allele, which is now in phase 1/2 trials (NCT06389877). Clinical trials demonstrated safety with gene transfer of the *SERPINA1* gene that encodes for A1AT, though yet with limited efficacy [445]. Since then, several attempts have been made to package the gene in adenoviral transduction systems for replacement expression of the functional gene, with variable success [443]. Yet, the established safety of this approach is encouraging and suggest that adenoviral delivery holds promise for further optimization of effective gene therapy approaches.

2.2.3.2 MMP inhibitors

MMPs are secreted proteolytic enzymes that are mainly provided by inflammatory cells such as macrophages, neutrophils, and T cells. These enzymes degrade ECM proteins such as collagens and elastin [446]. In particular MMP-1, MMP-12, and MMP-28 have been demonstrated to contribute to emphysema development in mice [119, 447, 448], whereas MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-28 all have increased expression in COPD [447, 449-451].

Pharmacological intervention with dual inhibitors for MMP-9 and MMP-12 has been attempted, which led to successful inhibition of airway remodeling and emphysema development in CS-exposed guinea pigs using the inhibitor AZ11557272 [452]. The orally active dual MMP-9/MMP-12 inhibitor AZD1236 was evaluated in clinical trials in COPD, although not with emphysema progression or related outcomes as primary endpoints. The results showed no effects on inflammatory biomarkers such as differential cell counts and TNF-α levels in sputum, or in desmosine excretion in urine as a proxy for elastin breakdown [453]. Another interesting strategy to inhibit MMP activity is to target the delivery of pentagalloyl glucose to the lung using inhaled nanoparticles loaded with this drug. This resulted in suppression of MMP-12 activity and the preservation of elastin integrity in the lungs of elastase treated mice [454]. Collectively, although MMP inhibition appears effective in animal models, its clinical relevance remains to be established.

2.2.3.3 Cathepsin inhibitors (incl. DPP-1 inhibitors)

Cathepsins are lysosomal proteases that have been implicated in the pathogenesis of COPD through their role in ECM degradation, inflammation, and tissue remodeling. Their activity is inhibited by cystatins, and the ratio of cathepsin to cystatin expression was found increased in plasma of patients with COPD and was found to correlate to the degree of emphysema [455]. Cathepsin E expression is increased in COPD and its overexpression results in the activation of cell death and emphysema development in mice [456]. Cathepsin C is also known as dipeptidyl peptidase I (DPP-I), and contributes to lung tissue damage as well. Inhibitors of DPP-1 are under development mainly for bronchiectasis [457] but may be interesting to pursue as a therapeutic strategy for COPD too, given the protective effects of DPP-1 inhibition in animal models of COPD [458]. This is not only because of the direct effects on tissue damage, but also because DPP-1inhibitors reduce neutrophilic inflammation [459] which prevents extracellular matrix remodeling and potentially supports regeneration downstream. Indeed, the DPP-1 inhibitor brensocatib inhibits the activity not onbly of DPP-1 itself but of neutrophil elastase, proteinase 3 and cathepsin G as well, probably contributing to its broad mode of action [460].

2.2.3.4 Neutrophil elastase inhibitors

Given the central role of neutrophil elastase in elastolysis, neutrophil elastase inhibitors have long been considered for the inhibition of emphysema development. The inhibitor FR901277 prevents elastase induced emphysema development in rodents [461]. Furthermore, ONO-5046, another neutrophil elastase inhibitor, was shown to prevent CS-induced lung injury in mice [462]. Neutrophil elastase inhibitors are in clinical development for bronchiectasis. For example, the inhibitor BAY 85-8501 was found safe and showed target engagement in bronchiectasis patients [463]. Alvelestat (MPH966), an orally active neutrophil elastase inhibitor, is currently being evaluated for bronchiolitis obliterans syndrome [NCT02669251]. Whether neutrophil elastase inhibitors are suitable for long-term treatment and inhibition of emphysema progression is unclear at this moment.

2.2.4 Lymphotoxin-signaling inhibitors

The progression and severity of COPD are associated with increasing infiltration of the airways by both innate, predominantly neutrophils and macrophages, and adaptive immune cells (B and T lymphocytes). These form inducible bronchus-associated lymphoid tissue (iBALT), composed of B cells surrounded primarily by T cells [464-466]. The number of iBALT structures increases in the lung with disease severity [464, 467-469], and it was recently shown that they contribute to the pathogenesis of CS-induced COPD [331, 470, 471]. Furthermore, unbiased transcriptomics data obtained from the lungs of patients with COPD revealed activated adaptive immune cell signatures strongly associated with the development of emphysema [472-474], which is accompanied by a significant correlation between emphysema severity and lymphoid organ formation [469, 474].

Crucial to our understanding of COPD pathogenesis and subsequent treatment is to elucidate the molecular mechanisms underlying how iBALT contributes to both tissue injury (emphysema) and the dysregulated repair and regenerative pathways observed in COPD. Many of the pathways responsible for the development and maintenance of iBALTs in general, mirror those responsible for lymphoid organogenesis during ontogeny [475, 476]. Crucial is the interaction between the lymphotoxin-β receptor (LTβR) on stromal organizer cells and membrane bound lymphotoxin, heterotrimeric complexes of the TNF superfamily members LTα and LTβ (LTα1β2 or LTα2β1) [477], expressed on the surface of $CD45^{+}$-$CD3^{-}$-$CD4^{+}$-$ROR\gamma t^{+}$ lymphoid tissue inducer (LTi) cells [478-480]. In lymphoid tissue formation during chronic inflammation, lymphocytes are capable of fulfilling the role of LTi cells [481-484]. Lymphotoxin signaling triggers expression of downstream chemokines like CCL19, CCL21 and CXCL13 and cellular adhesion molecules such as VCAM1 and ICAM1, which attract and retain more hematopoietic cells [479]. LTβR signaling activates the non-canonical NF-κB pathway via NF-κB-inducing kinase (NIK), which phosphorylates and activates IKKα homodimers. Activated IKKα then phosphorylates the NF-κB precursor protein p100, leading to its partial proteasomal processing into p52. The resulting p52/RelB heterodimer translocates to the nucleus, where it drives transcription of target genes [485, 486]. Indeed, mice with a mutation in NIK (aly/aly mice), which lack non-canonical NF-κB signaling, have no lymph nodes and present disorganized thymic and splenic architecture with impaired T cell mediated immunity [487-489], a phenotype also observed in mice deficient in lymphotoxin [476]. Furthermore, blocking lymphotoxin

signaling using a LTβR-Ig fusion protein [490], impairs the development and maintenance of conventional lymphoid tissue [491, 492]. Indeed, LTβR-Ig treatment, used to block LT signaling both prophylactically and therapeutically in the presence of CS, significantly reduced iBALT formation and resulted in more dispersed immune cell localization [331]. Crucially, quantitative morphological analyses of lung tissue damage for airspace enlargement and alveolar surface density revealed that CS-induced emphysema was prevented by prophylactic LTβR-Ig treatment. Therapeutic treatment starting from four months, a time point at which airspace damage was already fully established in mice, led to full restoration of lung tissue, even in the continued presence of CS exposure [331].

Interestingly, LTβR-induced stabilization of NIK is crucial for TNFα-mediated cell death [493]. NIK is required for the activation of caspase-8 by promoting the assembly of the RIP1/FADD/caspase-8 death complex [493]. Consistent with this, increased AT2 cell death was observed in the lungs of both patients with COPD and mice chronically exposed to CS. *In vitro,* LTβR-signaling enhanced TNF induced AT2 cell death [331]. Single cell RNA-Sequencing clearly revealed that CS strongly induced a positive regulation of NIK-dependent signaling in AT2 cells, which was significantly reduced upon LTβR-Ig treatment. In line, high levels of *Ltbr* mRNA expression on AT2 cells were found indicating that NIK dependent signaling in AT2 cells can be triggered by LTβR-activation. This demonstrates the novel concept that therapeutic inhibition of LTβR-signaling restores lung architecture from smoking induced-emphysema by re-initiated endogenous Wnt/β-catenin-driven alveolar regeneration. Mechanistically, LTβR activation in progenitor AT2 cells suppresses Wnt/β-catenin signaling via the non-canonical NF-κB pathway, mediated by the NF-κB-inducing kinase NIK [331]. In primary AT2 cells and stable human and mouse cell lines treated with LTβR agonists, there was a clear downregulation of key WNT/β-catenin target genes *Axin2*, *Tcf4*, *Nkd1*, and *Lgr5*. Indeed, both *AXIN2* and *TCF4* expression were also suppressed in *ex vivo* human precision-cut lung slices stimulated with an LTβR agonist. Moreover, non-canonical NF-κB signaling induced by the alternative LTβR ligand TNFSF14 reduced β-catenin levels in a murine AT2 cell line [331]. Crucially, inhibition of GSK-3β ligand-independent β-catenin transcriptional reporter activity was prevented by LTβR activation, implying intracellular signal

modification downstream of the β-catenin destruction complex. Indeed, proteasome inhibition with bortezomib prevented LTβR driven β-catenin degradation [331].

Although direct LT-signaling inhibitors are lacking, small molecules targeting its downstream non-canonical NF-κB signaling pathway have been in development for a number of years [494-496]. Indeed, murine AT2 cells treated with the NIK inhibitor CMP137 prevented LTβR-signal induced degradation of β-catenin [331], suggesting NIK inhibition may be an alternative option for inducing lung regeneration in COPD. These series of experiments elegantly demonstrate that inhibition of LTβR-signaling in alveolar progenitors can both prevent epithelial cell death and activate WNT-induced regeneration promoted by β-catenin signaling.

2.2.5 Cytokine receptors and cytokine-targeted antibodies

A defining feature of the COPD lung microenvironment is the presence of persistent inflammation characterized by the presence of neutrophils, macrophages and lymphocytes, often present in iBALT structures as summarized in the previous section. These cells, and structural cells express several chemokines and cytokines to which the alveolar epithelial cell and its niche is continuously exposed [497]. Exacerbations offer periods of enhanced exposure to these inflammatory stimuli. A key question from both a medical biology and pharmacology perspective is what the effect is of this inflammatory microenvironment on lung tissue repair. As summarized in section 1.3.1, pro-inflammatory cytokines can have both detrimental effects and beneficial effects, dependent on the type of cytokine, and the duration of the exposure. It seems that acute inflammatory stimuli have beneficial effects on lung tissue repair, whereas chronic or persistent exposures have the reverse effect and counteract adequate lung tissue repair (Figure 5).

These findings suggest that targeting specific cytokines or their receptors would be beneficial for lung tissue repair, particularly when directed at the background of persistent inflammation. Indeed, persistent exposure to IL-1β was associated with elevated expression of a range of CXC chemokines including CXCL1, CXCL5, and CXCL8. In this setting, inhibition of the common receptor CXCR2 using the drug reparixin effectively reversed the detrimental effects of IL-1β exposure on lung organoid growth in the exposure model where mesenchymal cells were pre-exposed to IL-1β before inclusion in the lung

organoid assay [88]. Thus, IL-1β can both enhance alveolar epithelial cell proliferation and differentiation via the NF-κB pathway and, when dysregulated, impair mesenchymal support for epithelial growth.

Consistent with a detrimental role in emphysema development, mice deficient in IL1R1 or MyD88 develop reduced emphysema severity and ECM remodeling in response to elastase administration [498]. Critically, inflammation in response to elastase was also reduced in these animals, suggesting that inflammatory responses, in addition to direct elastase effects, partially contribute to disease pathology. Likewise, mice deficient in IL-6 have reduced inflammatory cell counts in bronchoalveolar lavage fluid (BALF), and attenuated emphysema development in response to elastase exposure [499]. The apparent contradiction with the previously mentioned protective roles for IL1R1 and IL-6 in lung repair [67, 79] is most likely explained by the difference in the model as these previously mentioned studies used influenza infection to model lung injury, which apparently produces different, even opposite, outcomes in comparison to the elastase model of emphysema.

Further support for targeted inhibition of pro-inflammatory cytokines comes from studies that used either genetically modified mice or function blocking antibodies against IL-17A. Mice deficient in IL-17A develop reduced levels of emphysema in response to elastase and have attenuated inflammation and cytokine levels in BALF. Levels of IL-17A were elevated already immediately (1 day) after elastase exposure in wild-type mice and remained elevated compared to nonexposed wild-type mice, suggesting that IL-17A may have contributed to the immediate development of inflammation in the elastase model [500]. On the other hand, in a disease model in which weekly low-dose elastase exposure for one month was followed up with LPS exposure and/or RSV infection, an antibody targeting IL-17 was protective against inflammation and emphysema development even when administered after the elastase exposures. This suggests that IL-17 contributes to the perpetuation of either lung damage or a reduced tissue repair response after elastase exposure [501]. This contention is further supported by the observation that antibodies targeting IL-23 or genetic ablation of IL-23 reduce the expression of Th17 cells and attenuate the development of emphysema in response to elastase in mice [502]. Whilst this does not indicate a direct role for IL-17 in tissue regeneration, there may be an indirect role because of inhibition of inflammation and subsequent epithelial injury.

Finally, the possibility of targeting type 2 inflammation is a strategy worth mentioning in the context of the recent developments on the protective effects of the IL-4R antibody dupilumab in patients with COPD [503]. In this context, the observation that IL-4 plays a role in the development of inflammation and emphysema in response to elastase is of interest [504]. Thus, interstitial macrophages produce MMP-12 in mice exposed to elastase and do so in an IL-4 dependent manner with basophils being the major source of IL-4 in the model. Mice deficient in IL-4 or mice with basophil-specific IL-4 deficiency fail to develop emphysema and have reduced expression of MMP-12 in response to elastase [503]. Furthermore, the cysteinyl leukotriene receptor antagonist montelukast attenuates emphysema development in response to elastase in mice, and inhibits the ovalbumin-aggravated response in a model of combined allergen and elastase exposure [505]. These findings raise the possibility that type 2 inflammation has an impact on emphysema development as well and that pharmacologically targeting this response may be beneficial.

2.2.6 Other anti-inflammatory strategies

2.2.6.1 Angiotensin pathway signaling

Angiotensin signaling is a complex biological pathway best known for the role of angiotensin II which contributes to hypertension mediated by the AT_1 receptor, and for which AT_1 receptor blockers such as losartan and ACE inhibitors such as captopril are used clinically [506]. However, in addition to its role in the cardiovascular system, angiotensin contributes to pulmonary physiology and pathophysiology as well, both by signaling via the AT_1 receptor and the AT_2 receptor. The AT_2 receptor, in contrast to AT_1, generally exerts protective effects. Moreover, angiotensin II can be converted by ACE2 into angiotensin(1-7), which not only binds to the AT_2 receptor but to the Mas receptor as well [506]. Intriguingly, compound 21, a selective peptide agonist for the AT_2 receptor, inhibits inflammation, p39 MAPK pathway activation, lung function changes, and emphysema development in response to CS in mice [507]. Moreover, an orally active formulation of angiotensin(1-7) was able to prevent emphysema development in response to elastase in mice, which was associated with repressed inflammation [508].

2.2.6.2 α7 Nicotinic receptor signaling

In contrast to the muscarinic receptor pathways, which are predominantly pro-inflammatory [509], α7 nicotinic receptor signaling has well-established anti-inflammatory effects [510]. α7 Nicotinic receptors are widely expressed on neurons and neuroendocrine cells, as well as on inflammatory cells such as macrophages [511]. The selective α7 nicotinic receptor agonist PNU-282987 strongly inhibited the development of emphysema in response to elastase in mice, both as a preventive strategy and as a therapeutic strategy. This was associated with an equally strong inhibition of inflammation in response to elastase [512]. PNU-282987 had similar protective effects on type 2 inflammation in animal models of allergen exposure [513].

2.2.6.3 Neuropeptide Y signaling

Neuropeptide Y (NPY) is a neuropeptide expressed by sympathetic neurons as well as by inflammatory cells and by epithelial cells, in particular neuroendocrine cells. Although there is limited literature available on NPY, reduced expression of the neuropeptide has been reported in the airways of patients with COPD [514]. In addition, an interaction between the presence of NPY and emphysema development was reported in NPY-/- mice. Although NPY-/- mice have no emphysematous abnormalities themselves, the absence of NPY aggravates the inflammatory response and emphysema development in response to elastase exposure [515]. It remains unknown whether NPY agonists could serve as therapeutic agents for emphysema.

2.2.6.4 RAGE signaling

Receptor for advanced glycation end products (RAGE) is highly expressed on AT1 cells and inflammatory cells, and its ligands (advanced glycation end products, AGEs) are increasingly expressed in patients with COPD [516]. AGEs are considered damage associated molecular patterns (DAMPs) with roles in linking tissue injury to inflammatory responses. In addition, they have negative effects on lung tissue repair as the antimicrobial protein LL-37 and HMGB1, both of which are endogenous RAGE

ligands, reduce lung organoid forming capacity [517]. In addition, they promote neutrophilic inflammation and emphysema development [517]. Accordingly, inhibition of RAGE signaling using the drug FPS-ZM1 prevented emphysema development, both in response to elastase and in response to LL-37 or HMGB1; it also reduced inflammatory cell infiltration, and suppresses DAMP-related signaling [517, 518].

2.3 Cell therapies

2.3.1 Mesenchymal stromal cell-based therapy

The most widely described stem cell population used for cell-based strategies in regenerative medicine is the mesenchymal stem/stromal cell (MSC). MSCs are multipotent stem cells that can be derived from various tissues, including bone marrow, adipose tissue, umbilical cord, and the lung [519, 520]. Due to the scarcity and limited numbers of adult human MSCs, human-induced pluripotent stem cells (iPSCs) are now increasingly used as a source of MSCs. iPSCs are derived by reprogramming of somatic cells from various tissues such as skin biopsies or urine samples, and can then be differentiated into iPSC-MSCs. Lung resident MSCs (LMSCs) are mesenchymal progenitors that replenish stromal cell populations, including lipofibroblasts, myofibroblasts and smooth muscle cells. They reside within the microenvironment of alveolar epithelial cells and ECs and support site-specific proliferative and differentiation responses, secreting trophic factors such as fibroblast growth factor (FGF)10, which is critical for embryonic lung development as well as adult lung homeostasis [521]. In mice, a subpopulation of FGF10-expressing cells has been reported to represent resident MSCs that are able to self-renew [522, 523]. MSCs as well as (lipo)fibroblasts are an important source of FGF10. Of note, MSCs and fibroblasts are difficult to distinguish on the basis of their secretory, surface molecule or gene expression profiles *in vitro*. Yet the regenerative potential of MSCs may be related to the higher proliferative capacity and lower susceptibility to undergo senescence upon expansion compared to fibroblasts [524]. As described above, increasing numbers of studies show that communication between mesenchymal cells and the alveolar epithelium is crucial for alveologenesis, normal lung homeostatic maintenance and alveolar epithelial repair upon lung injury. A wide variety of growth factors secreted by MSCs have been implicated in mesenchymal-epithelial crosstalk during alveolar epithelial developmental and repair

processes, including FGF10 and other FGFs, keratinocyte growth factor (KGF), WNT ligands, BMPs, and HGF. In addition, MSCs can secrete micro-RNAs and anti-inflammatory factors into the damaged micro-environment, suppressing allograft rejection and protecting against inflammation-induced injury. Rather than by their direct engraftment, MSCs are thought to exert their therapeutic effects mainly through their paracrine function. In addition to growth factors and (anti-)inflammatory mediators, their secretome consists of other soluble proteins and extracellular vesicles (EVs), including exosomes. In preclinical models of ALI and ARDS, administration of MSC's secretome has been shown to improve survival, restore lung architecture, decrease fibrin deposition, and attenuate inflammation [525]. The cell-free nature of secretome-based therapy offers advantages over live cell transplantation, including reduced risk of tumorigenicity and immune rejection [526]. BM-MSC-derived EVs were shown to be safe in a clinical trial on the treatment ARDS in patients with COVID-19 [527], yet secretome-based therapy offers new challenges such as limited duration of effects. Moreover, clinical translation is limited because of the heterogeneity in secretome composition, optimal dosing, delivery methods, and large-scale manufacturing challenges. EV treatment in COPD will be further discussed in section 2.4

Besides their paracrine effects, MSCs derived from adult tissues and iPSC-derived MSCs have been shown capable of mitochondrial transfer, reducing lung tissue damage upon smoking and oxidative stress and protecting against mitochondrial dysfunction in animal models [528-530]. Accordingly, various animal studies have shown that MSCs are beneficial in lung disease, with the ability to ameliorate emphysematous lesions when administered either prophylactically or therapeutically [531].

MSCs from adult tissues have been extensively and successfully used in clinical trials aiming at dampening immune reactions and enhancing tissue regeneration, such as for the treatment of Graft-versus-Host Disease [532], Crohn's disease [533], cardiac ischemia, and following solid organ transplantation [534]. However, the clinical application of MSCs in lung disease is still in its infancy and evidence for beneficial clinical effects of transplanted MSCs on lung function is limited. In the first clinical trial in COPD, bone marrow-derived MSCs (BM-MSCs) were delivered intravenously. The treatment was well-tolerated and resulted in a significant, early reduction in systemic C-reactive protein (CRP) levels, but without effect on lung function [535]. In a post-hoc analysis, the treatment significantly improved lung

function in those patients with high CRP levels, indicating clinical benefit through anti-inflammatory effects [536]. In another clinical trial in severe emphysema patients undergoing lung volume reduction, treatment with BM-MSCs confirmed that treatment was safe. Moreover, this study reported increased CD31 expression, suggesting responsiveness of microvascular ECs, yet again without a beneficial effect on lung function [537].

Because of the complex architecture of the lung and the extensive alveolar destruction in emphysema, the challenge of achieving lung tissue repair is considerable, and important questions on the optimal route, dosage, frequency of treatment and source of MSCs remain to be answered. Various animal studies have compared the use of MSCs from different sources. In an elastase-based mouse model, comparison of LMSCs to BM-MSCs showed higher retention of LMSCs in the lungs, which was accompanied by higher ICAM-1, integrin-α2, and PDGFRα expression, and may thus relate to higher ability of MSCs to adhere to ECs and migrate into the lung tissue [538]. LMSCs and BM-MSCs showed similar growth factor receptor and inflammatory mediator expression profiles, and both cell types reduced elastase-induced lung damage. In another study comparing the effects of intravenous and intratracheal installation of adipose derived MSCs (AD-MSCs), BM-MSCs, and LMSCs in a mouse model, cells from all sources reduced elastase-induced mean linear intercept, neutrophil infiltration and alveolar epithelial and EC damage, and increased elastic fiber content, independent of administration route. However, only BM-MSCs displayed beneficial systemic effects, while AD-MSCs and LMSCs showed a more significant reduction in the fractional area of alveolar collapse than BM-MSCs [539]. This may be linked to the immunomodulatory/anti-inflammatory profile of BM-MSCs [540], which translated into reduced systemic cytokines [541] and protection not only of the lung but also in extrapulmonary tissues in models of acute lung injury upon their administration in a pre-clinical model [542]. In contrast, the more localized effects of AD-MSCs and LMSCs may be attributed to their tissue-specific gene expression profiles. LMSCs, in particular, express higher levels of growth factors such as FGF10 and HGF [164].

For their application in the clinic, one of the hurdles that needs to be taken, irrespective of the cell source and route of delivery, is the short retention time of MSCs in the lung. Even though intravenously

administered MSCs initially become trapped in pulmonary capillaries, they are cleared within a few days [543]. While retention was initially higher in mice with elastase-induced emphysema [543], a hostile lung microenvironment in COPD, with high levels of oxidative stress, inflammation and loss of ECM may significantly impact on the attachment and survival of MSCs [544]. Furthermore, administered MSCs may be cleared rapidly by phagocytosing immune cells. This needs to be taken into account when considering the most appropriate dosing frequency and route of administration. A potential solution is the use of a delivery scaffold, such as microgel encapsulation to protect the cells and improve their retention time [545]. Insight into factors in the MSC secretome that are crucial for alveolar repair will further guide the design of such a delivery scaffold and/or pre-conditioning strategies of the cells. When the strategy is aimed at the use of autologous MSCs, it is important to consider abnormalities in gene expression profiles and pathways of MSCs derived from patients with COPD. COPD-derived LMSCs express lower levels of HGF and FGF10 [546]. Strikingly, even more differences in gene expression were observed between BM-MSCs and AD-MSCs from patients with COPD and controls [164]. One of the pathways that may be dysregulated in MSCs from COPD patients is the Hedgehog (Hh)-Glioma-associated oncogene 1 (GLI1) axis, which is regulated by COPD susceptibility gene *HHIP* [547], encoding Hh interacting protein. GLI1 was found higher expressed in fibroblasts, which share mesenchymal stem cell features with MSCs, from smokers and COPD patients (PMID: 25815884). In animal models, loss of HHIP expression resulted in activation of Hh signaling in fibroblasts, promoting emphysematous manifestations [548] and potentiating the release of IL-7 by Gli^+ fibroblasts [549]. Moreover, $Gli1^+$ MSCs were shown to contribute to abnormal alveolar differentiation upon injury [550]. Animal models provide evidence that the HHIP/Hh axis is a reachable target and pharmacological modulation of Hh pathways may thus represent an opportunity to enhance lung tissue repair [551].

Besides the dysregulation of regenerative pathways in COPD, another limitation of autologous MSC is *in vitro* expansion of MSCs, which can lead to the induction of replicative senescence, and the induction of senescence is accompanied by lower levels of FGF10 [524]. Of note, when compared to fibroblasts from lung tissue of the same donors, MSCs showed lower sensitivity towards both stress-induced and replicative senescence, indicating that MSCs are less likely to senesce upon *in vitro* expansion [524].

Additionally, the use of iPSC-derived MSCs may overcome this issue, but this comes with the risk of tumorigenesis. Here, microencapsulation to prevent proliferation may be an option to explore for future strategies. Together, with continued insight into the action of (iPSC-induced) MSCs and how to overcome their limitations, the application of these cells holds significant promise in regenerative medicine in the lung.

2.3.2 Organoid and iPSC induced epithelial cell therapy

In COPD the epithelial barrier is compromised due to disrupted tight and adherens junctions, leading to increased permeability and susceptibility to pathogens. In the upper airways, the local stem cells, called basal cells, exhibit dysregulated differentiation, contributing to ciliary dysfunction and mucus overproduction. Similarly in the distal lung, the alveolar progenitor cells (AT2) show impaired differentiation into AT1 cells, compromising gas exchange [552]. Aggravating risk factors like cigarette smoke and other pollutants are thought to impair regeneration by increasing oxidative stress, leading to senescence, sustained tissue damage and remodeling [553, 554]. However other mechanisms for failed regeneration likely also contribute and our understanding remains limited. Recent advances in regenerative medicine, particularly involving iPSCs, lung organoids, and adult stem cell transplantation, are opening new avenues for both disease modeling and therapeutic intervention. These innovative approaches aim at providing the damaged tissue with an alternative source of progenitor cells required for lung repair and integrity.

2.3.2.1 Transplantation of iPSC-derived lung cells

Several recent studies have demonstrated that when iPSC-derived basal cells are transplanted into injured airways of immunocompromised mice, they can engraft, self-renew, and contribute to the long-term regeneration of functional airway epithelium [555-557]. In addition to basal cells, other lung-resident epithelial stem and progenitor populations have been derived from iPSCs and tested in preclinical models. For example, lung tip progenitor cells, characterized by expression of transcription factors such as SOX9 and ID2, have been generated and successfully engrafted into the distal lung of mice following naphthalene-induced injury, supporting localized repair [558]. Similarly, studies have reported the

generation and engraftment of AT2 from patient-specific iPSCs, which are critical for surfactant production and alveolar homeostasis. These iPSC-derived AT2s have been shown to survive and integrate into the alveolar niche *in vivo*, contributing to alveolar regeneration after injury [559, 560]. These findings suggest that multiple kinds of lung progenitors could be employed to repair or replace damaged epithelial tissues in COPD patients, especially in scenarios where chronic inflammation and repeated injury disrupt epithelial integrity and repair capacity.

2.3.2.2 Transplant of lung primary cells and their derivatives.

Whereas iPSC-derived cell therapies offer a customizable and patient-specific approach, concerns about immune system avoidance, tumorigenic potential, and genomic instability remain significant barriers to clinical translation. As an alternative, transplantation of endogenous airway and alveolar progenitor cells is emerging as a promising and potentially safer strategy for long-term lung regeneration.

Basal cells have demonstrated stable engraftment and long-term regenerative capacity in preclinical models. In a key study [556], human basal cells were expanded in *ex vivo* culture and transplanted into the airways of immunocompromised mice, where they engrafted successfully, maintained basal cell identity, and differentiated into multiple airway epithelial lineages over time. Similar studies demonstrated efficient expansion of human airway basal cells and their successful engraftment into bleomycin, elastase, and LPS mouse lung injury models [561-563]. These findings suggest that isolated and expanded adult basal cells could support durable epithelial repair after transplantation.

Beyond the proximal airways, efforts have also focused on regenerating distal lung structures, particularly the alveoli. Lung epithelial organoids derived from adult alveolar progenitor cells—primarily AT2 cells—have shown significant regenerative potential in preclinical models. Organoid-derived AT2 cells have been transplanted into bleomycin injured immunocompromised mouse lungs. These cells were engrafted in the alveolar regions and contributed to epithelial repair. Importantly, recipient mice showed decreased fibrosis and immune infiltration compared to control mice, indicating the therapeutic potential of alveolar stem cell delivery [564, 565]. A similar study showed AT2 organoid transplant in flu-injured mice aids in oxygen saturation recovery and lung repair [552], and another

demonstrated whole lung cell transplantation can aid in the resolution of a pulmonary fibrosis bleomycin mouse model [566].

Together, these studies support the growing consensus that transplant of airway and alveolar progenitor cells represent a promising approach to restore the lungs regenerative capacity in chronic lung diseases impacting epithelial cells such as COPD.

2.3.2.3 Autologous transplantation in human: P63⁺ lung progenitor cell transplantation

A step toward clinical translation was recently reported with the first-in-human trial investigating the autologous transplantation of P63⁺ airway basal progenitor cells in COPD patients [567, 568] . These cells were isolated via bronchoscopic brushing and expanded ex vivo in a pharmaceutical grade culture system before patient transplantation via bronchoscopy. Patients who received transplanted cells showed improved diffusing capacity for carbon monoxide (DLCO), six-minute walk distance (6MWD), and patient-reported respiratory symptoms, whereas control patients had continued lung function decline. This on-going clinical trial underscores the potential of patient cell transplantation for therapeutic benefit and epithelial regeneration in COPD patients, although consideration must be taken for possible genomic instability in transplanted cells from ex vivo culture.

2.3.2.4 Challenges and future directions

Despite these promising developments, several challenges remain. Efficient differentiation of iPSCs into functional lung epithelial subtypes, the time required to derive iPSCs from each patient, and long-term engraftment stability are critical barriers to overcome. Perhaps one of the most significant challenges of iPSC-derived cells are their lack of maturation compared to cells from adult tissues; further development of culturing platforms may aide this deficiency in the future.

It is notable that the human lung is significantly larger than murine lungs which have been used for the proof-of-principle transplantation and engraftment studies; a major question that remains is what is the number of cells required for engraftment to have a therapeutic benefit for COPD patients? The number of cells needed may be proportional to the size difference, however it is possible that engraftment of a portion of the lungs will be sufficient, and overall likely is highly dependent on the severity of their disease. To mimic lung injury and to promote engraftment, all developed methods to date require

preconditioning of the recipient lung with damaging agents, akin to the ablation of the bone marrow that precedes transplantation of hematopoietic stem cells. However, it is not clear how to utilize these treatments in patients with lung disease. Furthermore, as we learn more about the lung microenvironment in repair and disease, new transplant approaches need to consider the role of endothelial and mesenchymal cells as both co-transplant adjuvant as well as in engraftment efficiency. Finally, immune barriers must be addressed in clinical settings to prevent rejection and allow for enough cell engraftment to effectively restore lung function.

iPSC and organoid technologies are rapidly advancing from experimental models to potential clinical interventions for COPD. As demonstrated by recent studies, regenerative epithelial cell therapies have the potential to restore epithelial integrity and regenerative capacity in COPD patients. Continued interdisciplinary research and carefully designed clinical trials will be essential to investigate therapeutic potential of these novel approaches.

2.3.3 Endothelial cell therapy in COPD

Recent investigations have uncovered the functional role of ECs in lung regeneration, suggesting targeting pulmonary ECs is an effective intervention to restore functional gas exchange in respiratory disease. Currently, corneal EC therapy is widely conducted in clinical trials [569-571], but limited information is available for respiratory diseases. Intravenous infusion of autologous endothelial progenitor cells (EPCs) is feasible and safe and may be beneficial to patients with idiopathic pulmonary arterial hypertension [572]. The delivery of EPCs overexpressing endothelial nitric oxide synthase is also tolerated hemodynamically in patients with pulmonary arterial hypertension [573] with a trend toward improvement in total pulmonary resistance during a short delivery period, although one severe adverse event occurred after discharge.

2.3.3.1 Therapeutic potential of endothelial progenitor cells therapy in COPD

COPD is significantly associated with endothelial dysfunction contributing to both airway remodeling and alveolar destruction [574-576]. Pulmonary ECs lining along the arteries, veins and capillaries mediate

the interactions between blood and lung tissue, which is vital for angiogenesis, regulation of blood flow, vascular permeability, wound healing, and inflammation [577, 578]. The primary function of the respiratory system is gas exchange where the functional compartment responsible for it is the alveolus that is comprised of multiple epithelial, endothelial, and mesenchymal cell subtypes.

Though there is currently no EC therapy trial conducted in patients with COPD, EC therapy has been beneficial in animal models of elastase-induced and CS-induced COPD [138, 579, 580]. Specifically, co-transplantation of tissue-resident AMs and EPCs improves the efficacy of EPCs therapy in hyperoxia-injured lungs [580]. To identify the regenerative potential of EC therapy in emphysema, GFP-labeled E4ORF1-transduced lung ECs were intravenously delivered at days 7 and 14 after elastase treatment. This intervention significantly reduced parenchymal destruction and decreased mean cord length [138]. Delivery of human induced pluripotent stem cell-derived distal ECs together with pneumocytes in an elastase-induced rat emphysema model via intratracheal injection led to about 15% engraftment in the host alveoli and these cells integrated to form vascularized alveoli together with host cells [579]. In animal models of COPD induced by cigarette smoke exposure, systemic administration of EPCs has been shown to alleviate multi-organ senescence and modulate disease-associated pathways, including the USP7/p300 axis, where USP7 stabilizes the coactivator p300 involved in gene regulation and cell differentiation. However, these interventions have not reversed established tissue morphological changes [581]. Taken together, these studies indicate that EPCs possess significant value for restoration of alveolar destruction associated with chronic lung diseases in different animal models, and their regenerative potential can be achieved both via intravenous and intratracheal delivery.

2.3.4 Platelet-rich plasma therapy for COPD

Platelet-rich plasma (PRP) therapy has been postulated as a potential adjunct therapy for COPD, with the notion that it could slow the progression of the disease and improve patient quality of life. PRP is an autologous product, derived from the patient's own blood; a number of methods have been developed to extract the desired components from whole blood in a short period of time (~15 min) for administration back to the patient [582]. Previous studies have demonstrated that positive therapeutic

outcomes following musculoskeletal injury can be achieved with doses around 3.5 billion platelets per administration, with cumulative doses reaching up to 10-12 billion platelets in multiple dosing strategies [583]. PRP contains an array of biological factors that have the potential to modulate inflammation and remodeling processes in disease tissues.

PRP has the capability to modulate chronic disease pathology via a number of mechanisms. PRP has been found to reduce tissue inflammation, a primary characteristic of the COPD lung. In the context of musculoskeletal injuries, PRP administration was able to reduce the levels of pro-inflammatory cytokines (IL-17A, IL-1β, TNF-α, IL-6, and IFN-γ), increase the expression of angiogenic factors (HGF, VEGF, PDGF, IGF-1, and TGF-β), and improve joint structure assessed by magnetic resonance imaging [584]. In the context of lung disease, PRP has been shown to reduce IL-1β levels in COVID-19 patients [585].

The administration of PRP to damaged tissue may also have regenerative potential. Both nebulized and non-nebulized PRP promoted fibroblast proliferation *in vitro* compared to controls [586]. In the lung, PRP has been found to increase lung vascularity and alveolar regeneration in mice following right lung pneumonectomy [587]. Mechanistically, this was found to occur through a WNT-dependent pathway involving LRP5 phosphorylation and activation of the Tie2 receptor in ECs. PRP treatment resulted in accelerated EC sprouting *in vitro* and improved lung tissue regeneration in mice following unilateral pneumonectomy. Platelet-derived factors, in particular CXCL12 (SDF-1), have also been shown to prime the pulmonary capillary vascular niche and promote alveolar regeneration. Following left lobe pneumonectomy in mice, platelet-derived CXCL12 stimulation of the CXCR4/7-Akt pathway in pulmonary capillary endothelial cells induced metalloproteinase MMP14 expression and caused the release of HB-EGF, thereby stimulating the proliferation of alveolar epithelial cells driving neo-alveolarization [588].

Recently, a clinical case series described the use of submucosal injections of autologous PRP in three patients with tracheobronchial fistulae; this treatment was successful in all patients with no treatment-related complications, suggesting the potential of PRP to promote localized tissue repair [589]. In COPD

patients, a number of small cohort studies have been performed to assess the ability of PRP to improve lung function and quality of life. While some of these studies have reported an increase in FEV_1 and symptom scores, due to the lack of data on patient COPD severity and comorbidities, as well as incomplete reporting of lung function data [590], it remains to be seen if PRP is a viable adjunct treatment strategy for COPD. Large scale double blinded and controlled studies are eagerly anticipated to explore this further.

Currently, it is thought that much of the regenerative effect of PRP therapy for tissue repair is mediated by extracellular vesicles (EVs) released from activated platelets [591]. While the mechanistic basis for the regenerative potential of PRP-ECs is an area of continuing investigation, recent studies focusing on the cargo of platelet-derived EVs have revealed that target cell pyroptosis could be inhibited via EV-delivered long non-coding RNAs and microRNAs interfering with the SIRT1 axis [592]. The advantages of EV-based treatments, including lower immunogenicity, improved tissue penetration, and the ability to deliver bioactive molecules directly to target cells certainly make EV-based therapies attractive for tissue regeneration, but this enthusiasm must be tempered by the current lack of standardization regarding the cell type of EV origin and EV dosing strategies.

2.4 Extracellular vesicles

As mentioned previously, MSCs have emerged as a promising option for regenerative therapies, particularly in the treatment of respiratory diseases such as COPD [521, 593, 594]. MSCs have shown great potential in preclinical studies, where they have demonstrated the ability to reduce inflammation, modulate immune responses, promote angiogenesis, and support tissue repair through their paracrine effects [521, 593, 594]. However, despite these promising preclinical results, clinical trials with MSCs have often yielded disappointing outcomes. One of the challenges has been the rapid clearance of MSCs by the body's immune system, particularly by macrophages, which limits their effectiveness in the targeted lung tissue. Additionally, the complexity and high costs associated with MSC-based therapies, including the need for careful formulation, delivery, and sometimes surgical procedures, have further complicated their clinical application [593-595]. An alternative approach is to induce or enhance lung repair

using biologically active factors from the secretome of mesenchymal cells, which could be applied at an early stage of the disease and on a larger scale [594]. MSCs and other cell types within the alveolar niche release EVs, which locally influence neighboring cells. Initially, EVs were thought to function primarily as a mechanism for cellular waste disposal [596]. However, subsequent research has revealed their critical roles in intercellular communication and regulation of various biological processes, offering promising applications for disease diagnosis and treatment.

EVs are heterogeneous, cell-secreted particles enclosed by a phospholipid bilayer membrane [597]. The two most studied EV subtypes, large vesicles (microvesicles) and small vesicles (exosomes), are classified based on their size and biogenesis. Microvesicles, which range from 0.1 to 1–2 micrometers in diameter, bud directly from the plasma membrane. In contrast, exosomes, typically 30–150 nanometers in diameter, originate from endosomal multivesicular bodies and are released when these structures fuse with the plasma membrane [598]. Due to their overlapping size, density, and protein markers, isolating pure vesicle populations remains challenging. In line with the Minimal Information for Studies of Extracellular Vesicles guidelines, the term EVs will be used generically in this review to describe all lipid-bilayer-delimited particles naturally released from cells that lack replication ability [599]. EVs carry various bioactive molecules, including proteins, lipids, and genetic material (mRNA and microRNA). Once released, they interact with target cells via ligand-receptor interactions or are internalized through phagocytosis, endocytosis, or direct membrane fusion [600]. The activation of specific membrane receptors on target cells triggers signaling cascades that modulate biological processes, influencing cell behavior and tissue homeostasis.

EVs have recently attracted significant attention as potential regenerative agents, with an increasing body of research exploring their therapeutic role in tissue repair and regeneration. In this context, EVs from a variety of cellular sources have been investigated as potential treatments for COPD, further underscoring their relevance in regenerative pharmacology. Among these, MSCs have been the most widely studied source, with EVs derived from bone marrow, umbilical cord, and adipose tissue being

evaluated in several preclinical models, such as mouse models of emphysema (see Table 1) [541, 601-604]. While bone marrow- and umbilical cord-derived MSC EVs have demonstrated anti-inflammatory effects and protection against emphysema progression, adipose-derived MSC EVs failed to improve lung function, highlighting the variability in MSC-derived EV efficacy depending on their cellular origin [541, 601-604]. Beyond MSCs, other cell types have also shown promise as EV sources. For instance, we demonstrated that alveolar lung fibroblast-derived EVs (MRC5) improved lung function and reduced elastase-induced lung injury, suggesting that resident lung cells may play an important role in alveolar repair [149]. Positioned in situ near alveolar progenitor cells, these fibroblasts may facilitate localized EV signaling, directly supporting progenitor cell survival and regeneration [149]. Similarly, platelet-derived EVs have shown protective effects in a CS-induced COPD model, while genetically modified HEK293T cell-derived EVs (WNT-3A-transfected) enhanced alveolar repair and lung function recovery in an elastase-induced emphysema model [65, 605]. These findings suggest that EVs derived from non-MSC sources may provide alternative strategies for lung regeneration, particularly if their cargo can be engineered or optimized for targeted therapeutic effects.

In addition to the wide range of cellular sources under investigation, the dosing strategies employed in EV-based therapies for COPD vary substantially across studies (see Table 1). Reported doses range from approximately $0.5x10^8$ - $5.0x10^{10}$ EVs per dose, with differences not only in the absolute quantity but also in the number of administrations used. Most preclinical studies investigated a single EV dose without assessing dose-response relationships, thereby limiting our understanding of the optimal therapeutic window. Furthermore, it is important to acknowledge that reported EV doses are typically quantified based on total particle number, which is an indirect measurement and potentially confounded by non-EV contaminants [598]. This lack of a biologically meaningful and standardized quantification method complicates dose comparison across studies and presents a significant challenge for therapeutic standardization. Several studies report EV yields relative to the number of cultured donor cells rather than an absolute EV quantity, which introduces significant variability due to differences in culture conditions and isolation protocols. Compounding this issue is the use of diverse isolation methods

across studies, which can affect the purity and biological composition of the final EV preparations [606]. Most notably, differential ultracentrifugation remains the most widely applied method, yet it is known to co-isolate soluble proteins and other non-EV components unless followed by additional purification steps [607, 608]. These impurities may contribute to biological effects that are erroneously attributed to EVs themselves. Indeed, recent evidence suggests that non-EV components of conditioned media can account for a substantial portion of the observed regenerative activity [608]. Collectively, these challenges underscore the urgent need for standardized EV quantification, purification, and reporting practices to improve reproducibility and facilitate meaningful comparisons across studies [599].

The route of administration is a critical factor in the therapeutic application of EVs for COPD. Most preclinical studies have used either intratracheal or intravenous delivery of EVs (Table 1). In many cases, both administration routes yielded therapeutic effects. However, direct comparisons have highlighted differences in efficacy. For instance, Huang and colleagues demonstrated that while intratracheal administration of EVs significantly reduced lung injury, the same EVs administered intravenously failed to show efficacy [603]. These findings suggest that local pulmonary delivery may be more effective than systemic approaches for targeting lung tissue. Despite the promising results of intratracheal administration in rodents, this method is not feasible in clinical practice. An alternative strategy is nebulization, which allows non-invasive aerosolized delivery of EVs directly to the lung. Several studies have demonstrated the feasibility and therapeutic benefit of nebulized EVs. In a recent study, nebulized EVs improved lung function and reduced inflammation, whereas intravenous administration had no observable effect [541]. Similarly, nebulized EV were found to reduce lung injury and enhance lung function in other models of lung damage [605, 609].

While nebulization enables the delivery of relatively high doses of active pharmaceutical ingredients directly to the lungs, it is also associated with several limitations. These include limited delivery efficiency, considerable inter-patient variability, prolonged administration times, and challenges in achieving consistent dosing [610, 611]. Furthermore, many biopharmaceuticals, including EVs, exhibit

instability in aqueous solution or suspension, which complicates long-term storage and distribution without appropriate formulation strategies [610, 611]. Dry powder inhalers (DPI) represent a promising alternative for pulmonary delivery of EVs, as dry powder formulations can significantly enhance the storage stability of sensitive biologics [610-612]. Two principal strategies have been explored for formulating EVs as dry powders: freeze drying and spray drying. Freeze drying is a well-established technique used to preserve biological products by removing water through sublimation [613-615]. Several studies have demonstrated that EVs retain their physical properties and biological activity after freeze drying [616-619]. Moreover, distribution studies in murine and non-human primate models have shown that inhaled, freeze-dried EVs carrying GFP mRNA successfully localize to both bronchioles and parenchyma, resulting in functional protein expression within lung cells [620]. These findings indicate that EVs can reach their cellular targets and deliver biologically active cargo via the inhaled route. Spray drying offers an alternative, single-step method for producing respirable dry powders [610, 614, 615]. This process typically yields spherical and homogeneously sized particles with favorable aerodynamic flow properties [611]. Although research in this area is still limited, our work shows that lung fibroblast-derived EVs can be successfully spray-dried using excipients such as inulin and leucine, as stabilizer and dispersibility enhancer, respectively [616]. The resulting powder retained their structural integrity and biological activity for at least 12 weeks and exhibited properties suitable for deep lung deposition using a DPI [616]. While these findings are currently based on *in vitro* data, they provide a promising foundation for future *in vivo* studies aimed at confirming the therapeutic efficacy of spray-dried EV formulations delivered via inhalation.

Although preclinical studies offer compelling evidence for the regenerative potential of EVs in COPD, their clinical translation is still in its infancy. To date, only a limited number of clinical studies have explored the therapeutic use of EVs in respiratory disease, and in the context of COPD, only one published study has been identified. In this study, patients received weekly inhalations of Exo-d-MAPPS, a formulation containing MSC-derived EVs supplemented with high concentrations of immunomodulatory factors [621]. Thirty patients with COPD were treated once per week for three weeks

with 0.5 mL of Exo-d-MAPPS via inhalation [621]. All treated patients exhibited improvements in pulmonary function and quality of life, as evidenced by increases in FEV_1, peak expiratory flow, six-minute walking distance, and reductions in Clinical COPD Questionnaire scores [621]. While it remains unclear which components of the formulation drove these effects, the treatment was well tolerated, demonstrating the feasibility and safety of inhaled EV-based therapies in a clinical COPD population. This study offers a valuable first benchmark for future trials, particularly regarding the use of inhalation as a delivery method.

However, several translational challenges must still be addressed. Scalable, GMP-compliant EV production systems are needed to ensure consistent yield and purity, yet current manufacturing practices often result in variable product composition and potential contamination [621-624]. Most efforts still rely on multilayered cell factories, though advances such as hollow-fiber bioreactors, microcarrier-based stirred tanks, and 3D spheroid cultures show promise for clinical upscaling [622, 625-627]. Beyond production, EV therapies demand rigorous quality control, including batch-to-batch consistency, validated potency assays, and comprehensive molecular characterization [625, 628]. Finally, dedicated regulatory frameworks for EV-based products have not yet been established, requiring concerted efforts from regulatory agencies, scientific societies, and industry stakeholders to guide safe and effective clinical implementation [598].

2.5 Cell-derived therapeutic proteins

In view of the well-established roles of the alveolar niche in guiding epithelial repair and regeneration, major research efforts have been directed at identifying the secreted factors derived from lung fibroblasts, endothelial cells and immune cells in an attempt to utilize these as drugs or as leads for drug development. This section will summarize the niche-derived proteins with potential therapeutic value (Figure 6).

2.5.1 Fibroblast growth factors

FGFs represent a large family of growth factors mainly expressed by MSCs, that signal to FGF receptors, for which four different receptor tyrosine kinase subtypes (FGFR1-4) exist. The ligand-receptor interactions between most FGFs (e.g., FGF10) and FGFR involve FGF binding to heparan sulphate, which maintains FGFs localized to the tissue of origin, ensuring a paracrine function [629]. On the other hand, endocrine FGFs that circulate in the bloodstream such as FGF23, require binding to α-Klotho to activate FGFR signaling [630]. The degree of heparan sulphate binding of paracrine FGFs is a key determinant of their biological action radius. For example, FGF10, which binds to heparan sulphate with higher affinity than FGF7 does, has a more restricted action radius than FGF7, which is key to the differential roles of FGF10 and FGF7 in epithelial gland budding (FGF10) versus branching (FGF7) during development [631]. Downstream of the FGF-FGFR interaction is the tyrosine kinase-dependent activation of proliferation and survival pathways such as PI3K, p42/p44 MAPK, and FAK [629].

In lung fibroblasts, the main FGFs expressed are FGF2, FGF7, and FGF10 and these FGF family members are also the best studied in the context of lung tissue repair and regeneration [82, 632]. On the other hand, FGFR1 and FGFR2 are the main receptors expressed, for which FGFR1 is mainly expressed by lung fibroblasts and FGFR2 mainly by epithelial cells [82, 632], underscoring the role of FGF/FGFR signaling in both autocrine fibroblast functions and in mesenchymal to epithelial cell signaling in the lung. The expression of FGF7 is upregulated in lung fibroblasts exposed to a cocktail of cytokines relevant to the COPD exacerbation [82], whereas TGF-β reduces the expression of FGF7 and FGF10, whilst increasing the expression of FGF2 [422]. The expression of FGF1, FGF2, and FGFR1 is increased in the airways of patients with COPD [633], whereas the expression of FGF7 and FGF10 by lung fibroblasts is decreased in COPD [634]. In addition, an interaction with SNPs in the FGF7 gene region and COPD susceptibility has been reported [635], although it is difficult to disentangle whether this is due to a direct role for FGF7 in COPD development or in lung development, predisposing to COPD later in life.

The biological roles of FGF7 (also known as KGF) and FGF10 in lung regeneration have been consistently reported to be supportive. Recombinant FGF7 and FGF10 promote survival and proliferation of epithelial cells in culture [422, 636, 637]. FGF7 appears to be a stronger stimulus than FGF10

in this respect [422]; in fact, FGF7 signaling is required for alveolar epithelial cell organoids formation in fibroblast-free culture conditions [638]. *In vivo*, FGF10 signaling is consistently reported to be required for both airway and alveolar epithelial repair. Thus, in the airways, FGF10 is expressed by bronchial smooth muscle and its conditional deletion impairs the epithelial repair response to naphthalene injury [639]. In the alveolar region, lipofibroblasts express FGF10 in abundance [523] and deletion or reduction in FGF10 expression is associated with hypomorphic lungs and impaired alveolar epithelial growth and differentiation during development [640]. FGF7 plays key roles in lung development as well, particularly in branching morphogenesis [641] and in alveolar epithelial cell survival and differentiation [642-644]. Interestingly, and in addition to direct effects on epithelial repair, FGF signaling has also been reported to control elastin turnover by lung fibroblasts. Deficiency in FGFR3 and FGFR4 leads to aberrant lung development, characterized by enlarged airspaces and defective regulation of genes involved in elastogenesis. Isolated lung fibroblasts obtained from these mice did produce elastin, indicating that FGF signaling controls alveolar development and elastogenesis in utero by supporting mesenchymal-epithelial interactions that govern these responses [645]. Because of these critical roles in both epithelial regeneration and matrix homeostasis, FGFs have been extensively studied as potential therapeutic agents for COPD.

2.5.1.1 FGF2

In spite of the subtle differences in FGF spatial regulation during homeostatic lung development and maintenance of lung tissue, exogenous administration of recombinant FGF proteins or modified versions hereof, tend to have quite similar and consistently protective effects in animal models. FGF2 deficiency fails to resolve the inflammation and epithelial repair response following either bleomycin or naphthalene injury [646]. Furthermore, recombinant FGF2 protein, administered via intraperitoneal injection, reduced the inflammatory response and alveolar capillary leak in response to LPS exposure [647]. Most relevant to the topic of this review, however, modified versions of FGF2, being either collagen-binding FGF2 or protein transduction domains conjugated FGF2, improved both airspace enlargement and inflammatory outcomes in mouse models of elastase-induced emphysema [648, 649]. Recombinant

FGF2 also improves inflammatory outcomes in an animal model of CS exposure [166], and appears to be safe as an inhaled drug, although short-term (during a period of two weeks) administration in patients with stable COPD did not improve lung function or Borg scale outcomes [166]. It will be of interest to evaluate the efficacy of this treatment in the context of COPD exacerbations as will be discussed in the section on clinical outcomes below.

2.5.1.2 FGF7/KGF

Recombinant FGF7 (KGF) is one of the most extensively studied of these three FGF proteins, and is protective *in vivo* as well. KGF pre-treatment improves lung repair outcomes in a mouse model of idiopathic pneumonia syndrome following bone marrow transplantation [650]. KGF administration *in vivo* in rats also enhances the subsequent growth and repair response of AT2 cells *in vitro* indicating a direct relationship with epithelial cell activation [651]. Similar protective effects were observed in a mouse model of oleic acid induced acute lung injury [652]. When applied as a MSC-therapy approach that overexpresses KGF, the treatment protected against LPS-induced acute lung injury as well [653]. Relevant to COPD, KGF administration in a preventive therapeutic regimen, attenuated the inflammatory response, and protected from elastase-induced emphysema development in a mouse model, but not when administered in a therapeutic regimen [654]. This is at odds with a study that reported therapeutic effects of recombinant KGF in a mouse model of elastase exposure, in which effects on AT2 cell proliferation and the activation of p42/p44 MAPK signaling were also reported [655]. The main differences between these two studies is the mode of administration, which was subcutaneous [654] and via oropharyngeal instillation [655]. On a speculative note, this may have impacted on the local bio-availability of KGF in the lung, hence explaining the better outcomes in [655]. Interestingly, recombinant KGF is available as a clinically approved formulation (Palifermin) for chemotherapy-induced oral mucositis and has been clinically evaluated for safety and preliminary efficacy outcomes in human volunteers exposed to inhaled LPS. The results of this trial indicate that the preparation is safe, and that early beneficial effects on surfactant protein D and IL-Ra were observed [656]. Moreover, BALF of volunteers treated with KGF, promoted alveolar epithelial repair and fibroblast proliferation *in vitro* [656]. Whilst these studies are promising, the

clinical trial mentioned is over 10 years old already indicating no immediate additional follow-up was pursued. Clinicaltrials.gov does mention a trial in asthma, but without publication of results.

2.5.1.3 FGF10

Similar protective effects are reported for recombinant FGF10. Intratracheal application of FGF10 inhibits oxidative stress and ferroptosis in response to particulate matter in mouse lungs [657]. This is associated with Gpx4 and Nrf2 pathway activation. A related study also reported protective effects on pyroptosis [658]. In line with these findings, recombinant HGF protects against emphysema development in response to either CS or elastase in mice [659]. Interestingly, in that study protective effects on pulmonary vascular changes of FGF10 were also observed, which is of interest in the context of altered EC function in emphysema. Further to this point, FGF10 restores the defective endothelial glycocalyx and prevents EC apoptosis in a mouse model of CS-induced COPD [660].

2.5.2 Hepatocyte growth factor

HGF is a secreted protein in the human lung that is among the best studied factors driving epithelial repair, both from a biological and from a pharmacological point of view. HGF signals via the receptor tyrosine kinase c-MET and plays critical roles in epithelial homeostasis and in lung cancer development. In that respect, any potential therapeutics targeting the HGF/c-MET pathway will have to balance the benefit of tissue repair with the risk of promoting lung cancer, in which HGF/c-MET inhibition is a desirable outcome [661]. FGF10 is mainly expressed by lung fibroblasts and myofibroblasts as well as pericytes and pulmonary neuroendocrine cells [662]. Its expression is also higher in LMSCs than in adipocyte or bone-marrow derived stromal cells [164]. Its receptor c-MET on the other hand is abundantly expressed in most epithelial cell types and in the endothelium [662], in line with a role in lung mesenchymal to epithelial communication. Downstream of the c-MET receptor are the typical RTK-induced signaling pathways such as PI3K signaling, p42/p44 MAPK signaling, and FAK signaling [663].

Consistent with a link between lung injury and repair, the expression of HGF increases in otherwise healthy patients with community acquired pneumonia, however this increase is not observed in patients with acute exacerbations of COPD [664]. Furthermore, COPD patient-derived LMSCs have reduced expression of HGF mRNA [164], and lung fibroblasts obtained from emphysema patients have reduced capacity for HGF production [165]. Furthermore, cytokines relevant to COPD pathology such as TGF-β reduce HGF expression in lung fibroblasts, contributing to the negative effects of TGF-β on epithelial repair [422]. Intriguingly, whereas one study reports reduced expression of HGF in the epithelial lining fluid of patients with COPD [665], other studies report no change or increased expression of HGF in BALF or in plasma [666, 667]. Thus, whereas the reduced capacity for mesenchymal HGF production in COPD is consistently reported, this is not necessarily reflected in other pulmonary compartments or in the systemic compartment. It will be of interest to investigate this apparent contradiction in further detail and to involve severity of COPD and the presence of emphysema versus airway disease in the analysis to understand this relationship more.

Consistent with a major role for HGF in emphysema development, genetic deletion of the c-Met receptor in mice is sufficient to induce airspace enlargement in mice [668]. HGF based therapeutics have been evaluated both for emphysema and for other respiratory conditions in need for lung tissue repair such as acute lung injury and lung fibrosis. The first published report on the potential use of HGF in emphysema dates from 20 years ago already and used *in vivo* gene transfection of HGF in rat lungs as a proof-of-concept approach. In this study, an HVJ packaged plasmid encoding for HGF was administered intravenously as a single dose, which provided sustained increases in HGF gene expression for one week in the rat lung tissues. This was accompanied by improvements in airspace enlargement associated with reduced apoptosis and improved proliferation of alveolar cells after elastase exposure [669]. Interestingly, the treatment also restored pulmonary microvascular changes and functional changes on lung function [669]. Similar beneficial effects of HGF were demonstrated for intranasal administration of recombinant HGF protein, which was effective even after one week of treatment duration already in an elastase model of emphysema in mice, without additional benefit from prolonged treatment for up to four weeks of duration [670]. Cell therapy with human MSCs is also HGF

dependent since both MSC therapy and treatment with conditioned media from these cells improved elastase induced emphysema, whereas MSCs deficient in HGF were nearly ineffective [671]. *In vitro* studies confirm that these effects are achieved by acting on alveolar epithelial cells, since HGF depletion inhibited the beneficial effects of bone marrow derived stem cells on the growth and differentiation of AT2 cells [672], whereas direct administration of recombinant HGF boosts growth of alveolar epithelial cells grown in lung organoids [422]. Antisense oligonucleotides against HGF also interfere with the growth and differentiation of fetal rat lung explants [673]. On the other hand, alveolar organoids can form in the absence of HGF [638], indicating that its presence is supportive, but not strictly required.

2.5.3 Bone morphogenetic proteins

BMPs are members of the TGF-β superfamily, and were initially discovered for their role in bone and cartilage formation [674-676]. Besides their classical functions, BMPs have since emerged as pleiotropic factors involved in iron homeostasis, immune modulation, angiogenesis, stem cell regulation, and tissue repair. BMP ligands bind to type I receptors (activin receptor-like kinase 2 (ALK2 (ActR-I)), ALK3 (BMPR-IA), and ALK6 (BMPR-IB), as well as type II receptors (activin receptor type IIA (ActR-IIA), ActR-IIB, and BMPR-II. This binding promotes phosphorylation of SMAD1/5/8 proteins. These combine with cytoplasmic SMAD4 as an active transcriptional complex and translocate to the nucleus, resulting in the activation of gene transcription [674].

The best studied BMP ligands in the context of alveolar regeneration are BMP2, BMP4, and BMP6, which have the highest expression in whole lung tissue [139]. The BMP signaling pathway plays a crucial role in AT2 cell growth and differentiation, though in ligand and context specific manners. For example, recombinant BMP4 appears to reduce alveolar epithelial organoid growth, whereas antagonists of BMP signaling such as follistatin and noggin promote AT2 self-renewal [677]. In sharp contrast, cocultures of bronchioalveolar stem cells (BASCs) with endothelial cells require BMP4 for alveolar lineage specification, indicating the context-dependent effect of BMPs [135]. In line with this contention, BMP6 promotes alveolar epithelial cell growth [139], whereas BMP2 promotes AT2 cell differentiation into AT1

cells [678]. Moreover, *Bmpr1a* ablation disrupted club cell regeneration in mice [679]. However, recombinant BMP6 was unable to restore elastase induced lung injury in precision cut lung slices [139].

BMP-Smad1/5/8 signaling is important for maintaining lung homeostasis and lung function. Interestingly, Smad1/5/8 signaling is downregulated in emphysema and mice that express the BMP antagonist Noggin selectively in AT2 cells develop emphysema spontaneously [680]. In COPD, BMP6 expression is decreased in the lungs, an effect that is also observed in smokers and in mice exposed to CS [681]. An association between BMP6 and lung function has been described in mice as well, leading to reduced total lung capacity and increased dynamic elasticity and tissue damping in *Bmp6-/-* mice [681]. This is further supported by genome-wide association studies reporting associations between variants in the *BMP6* gene and forced vital capacity [682]. Moreover, *BMP6* mRNA levels are downregulated during acute exacerbations compared to stable COPD [683]. Similarly, BMP6 was upregulated in COPD rats treated with high-intensity electroacupuncture, which correlated with increased lung function and reduced inflammation [684].

Mechanistically, *Bmp6*-deficient mice showed iron accumulation in multiple organs and loss of iron regulatory feedback mechanisms [685]. Iron overload may further harm the surrounding tissues by promoting oxidative stress and cell death. Indeed, BMP6 is pro-angiogenic in both *in vitro* and *in vivo* [686, 687]. This angiogenic activity is important to COPD, as emphysema is associated with microvascular dysfunction and remodeling resulting from a reduction in capillary length and density [136]. BMP6 works in both canonical and non-canonical pathways (SMAD-dependent and independent). Specifically, it triggers cell migration via p38-HSP27 signaling axis in tip cells, while inducing the activation of SMAD1/5 signaling in stalk cells [686]. Consequently, increased migration in tip cells and proliferation in stalk cells occurs, leading to enhanced angiogenesis. BMP6 appears to be preferentially expressed by pulmonary microvascular ECs, but with functional effects on alveolar epithelial organoid growth as well, which is mechanistically explained by reduced oxidative stress signaling and enhanced WNT signaling [139]. These findings highlight BMPs as a key regulator of alveolar and vascular repair, which is impaired in COPD. Given its dual role in epithelial regeneration and angiogenesis, further research into BMP-based therapies is warranted.

2.5.4 Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a key regulator with important pro-angiogenic activity. Its role as a family of signaling proteins for vascular development and angiogenesis is well established [[688]. The VEGF family of growth factors consists of several subtypes, being VEGF-A, -B, -C, and -D, as well as placental growth factor (PIGF). VEGF-A (often called "VEGF") primarily functions as the main mediator of new blood vessel formation, while VEGF-C and -D are key regulators of lymphatic vessel formation. VEGF-B and PIGF, along with VEGF receptor-1 (VEGFR-1), have more restricted roles, with a less clear contribution to angiogenesis [689].

VEGFs specifically interact with one or more type V receptor tyrosine kinases (RTKs), VEGFR-1, -2, and -3 and with distinct co-receptors such as neuropilins and heparan sulfate proteoglycans [690]. VEGFR1 plays a regulatory role by negatively modulating VEGFR2 activity and promoting the migration of monocytic cells. VEGFR2 serves as the driver of angiogenesis, orchestrating the differentiation of blood vascular EPCs, EC migration, proliferation, and survival. It also regulates sprouting angiogenesis, flow sensing, and vascular permeability [691]. In contrast, VEGFR3 is predominantly involved in lymphangiogenesis, supporting the migration of lymphatic EPCs, lymphatic vessel expansion, and, to a lesser extent, contributing to blood vascular sprouting angiogenesis [691].

Decreased VEGF levels have been reported in sputum from patients with emphysema compared to healthy individuals [692]. In emphysema, reduced VEGF may contribute to or result from alveolar capillary loss and tissue destruction, while in chronic bronchitis, elevated VEGF may reflect ongoing angiogenesis, vascular remodeling in inflamed small airways [693, 694]. This indicates VEGF can be both beneficial and harmful. It may act as a protective feature to prevent emphysema while potentially detrimental by exacerbating inflammation in bronchial disease. Additionally, VEGF is emerging as a biomarker that might help to differentiate COPD phenotypes and possibly indicate underlying emphysema development [695-697].

VEGF signaling may intersect with multiple pathogenic processes in COPD. Inhibition VEGF receptor signaling disrupts maintenance of alveolar structure, promotes oxidative stress and cell apoptosis,

thereby contributing to pathogenesis of emphysema [698]. Conversely, VEGF agonism supports cell survival by preventing increased oxidative stress, apoptosis, and modulates inflammation by affecting immune cell trafficking and survival [693, 699]. Both *in silico* and *in vivo* modeling showed that prominin-1-derived peptide (PR1P), a novel short peptide that increases VEGF binding to ECs, prevents proteolytic degradation by enzymes such as elastase and plasmin, and reduced lung injury in 4- and 21-day elastase induced murine emphysema models [699, 700]. Unlike direct treatment with VEGF protein which could have off-target effects, stabilizing VEGF via PR1P may be considered safer. PR1P is currently in the preclinical stage, but still represents a promising therapeutic avenue for emphysema treatment. Since no such treatment is currently available to clinically address COPD, recent studies provide a hopeful foundation suggesting that further investigation of the VEGF pathway as a therapeutic target may benefit patients in the future.

2.5.5 Osteoglycin and its active fragments

Osteoglycin (OGN), also known as mimecan, has recently emerged as a promising candidate in regenerative pharmacology for COPD. Initially referred to as osteoinductive factor due to its role in bone formation, OGN was later found to be ubiquitously expressed. It is an endogenous small leucine-rich proteoglycan involved in various biological processes, including tissue development, ECM organization, and fibrosis regulation [701]. The mature protein (~37 kDa) comprises seven tandem leucine-rich repeats and a C-terminal tail, and contains multiple glycosylation sites [701, 702]. Our recent proteomics-guided drug discovery approach identified OGN as a key factor secreted by lung fibroblasts that promotes alveolar epithelial repair [703]. This aligns with the growing recognition of the pivotal role lung fibroblasts play in orchestrating epithelial regeneration within the alveolar niche.

Using murine and COPD patient-derived lung epithelial organoids, OGN was shown to significantly increase the colony-forming efficiency of alveolar epithelial progenitors without affecting organoid size, suggesting a specific effect on progenitor cell activation rather than a broad proliferative response [703]. Notably, OGN enhanced alveolar ($SFTPC^+$) organoid differentiation even under injurious

conditions such as CS extract exposure, indicating its potential to support epithelial maturation under COPD-relevant stressors [703]. Interestingly, a smaller C-terminal fragment (~15 kDa), comprising leucine-rich repeats 4 through 7 (MC002), was equally effective in supporting organoid formation and differentiation. Both OGN and MC002 also reduced elastase-induced lung injury in murine precision-cut lung slices, and high doses of MC002 significantly improved lung function parameters in an elastase-induced lung injury mouse model [703].

Although initially identified for its role in bone formation, subsequent studies revealed that OGN is widely expressed across tissues, including the lung [701, 702]. In non-disease human lung samples, OGN expression was positively correlated with age [704]. In contrast, OGN expression appears dysregulated in the lungs of individuals with COPD. Lin and colleagues reported reduced OGN mRNA expression in lung biopsies of patients with severe COPD [705]. Similarly, immunostaining for OGN on human lung tissue has shown proportionally lower expression in current smokers compared to non-smokers, with ex-smokers displaying intermediate levels, suggesting that CS may exert a lasting suppressive effect on OGN expression. A trend toward lower OGN levels has also been observed in lung tissue from patients with severe early-onset COPD [703]. These findings suggest that age-related upregulation of OGN in the lung may be disrupted by smoking, potentially contributing to impaired alveolar repair capacity. Given that reduced OGN levels are also present in otherwise healthy smokers, this dysregulation could represent an early molecular event that predisposes individuals to COPD, possibly requiring additional environmental or genetic insults to drive disease progression.

While OGN expression appears to correlate with disease severity and smoking history, the mechanism of action of OGN and its active fragment is not fully understood yet. Transcriptomic analyses of OGN-treated lung epithelial cells revealed upregulation of protective epithelial pathways, including those involved in oxidative stress detoxification and iron homeostasis, processes increasingly implicated in COPD pathogenesis [703]. Although these pathway-level changes provide initial mechanistic insight, the specific binding partners or downstream signaling cascades mediating OGN's regenerative effects are currently unknown. However, emerging evidence suggests that OGN may modulate several key tissue repair pathways. For instance, studies in pulmonary fibrosis models demonstrated that OGN

downregulation by microRNA-140 was associated with activation of the Wnt/β-catenin signaling pathway [706]. OGN has also been shown to modulate TGF-β signaling, a central pathway in alveolar remodeling. In cardiac fibrosis, OGN suppressed fibroblast proliferation and migration through inhibition of LPAR3/MMP2/EGFR signaling, reducing ECM deposition [707]. In cancer, OGN overexpression inhibited epithelial-to-mesenchymal transition and reduced cell proliferation via downregulation of the PI3K/Akt/mTOR pathway [708]. Beyond the lung, OGN also appears to play a role in systemic metabolic regulation, as knockout models revealed increased bone formation and altered insulin sensitivity [709].

Taken together, these findings highlight OGN as a promising therapeutic candidate for COPD with demonstrated regenerative potential. However, further studies are required to elucidate its precise molecular binding partners and mechanisms of action, as well as to guide future clinical development.

2.5.6 Other extracellular matrix-based strategies

In addition to OGN, several other matrix proteins or fragments hereof have been proposed as therapeutic options in preclinical models of emphysema. The protein Hyaluronan and proteoglycan link protein 1 (HAPLN1) was shown to increase expression of sirtuins and reduce markers of cellular senescence in AT2 cells. Recombinant HAPLN1 protein also reduced emphysema development in an elastase-induced mouse model of emphysema [710].

Similar protective effects were reported for keratan sulfate. In this study, a disaccharide repeating unit of keratan sulfate was shown to reduce emphysema development in response to elastase in the mouse, and to attenuate inflammation in both the elastase model and in an exacerbation model in which CS exposure is combined with LPS [711].

3. CLINICAL OUTCOMES AND FEASIBILITY

In recent years, significant progress has been made in discovering therapeutic strategies aimed at tissue repair and regeneration in COPD. Numerous preclinical studies have demonstrated promising regenerative effects in experimental models, sparking optimism about the potential of these therapies

to modify disease progression. However, despite scientific progress, none of these candidates have successfully advanced to clinical approval. The translation of regenerative therapies from bench to bedside remains challenging due to the complexity of COPD and the difficulty of demonstrating disease modification in clinical trials. Here, we will discuss the clinical endpoints typically used to evaluate therapeutic efficacy in COPD trials, the feasibility of applying these endpoints to regenerative interventions, and how emerging biomarkers and alternative trial designs may help to overcome current translational barriers.

Safety issues may form a significant barrier to clinical introduction. COPD patients suffer from many comorbidities. among which lung cancer is most prominent in patients with a history of smoking, showing a two- to six-times higher risk compared to the general population [712]. This should be kept in mind when evaluating the safety of regenerative therapies, since they intrinsically may further increase this risk. Cell based therapies (MSC- or iPSC-based) carry a tumorigenic risk, therefore, these therapies should be evaluated already in early development stages on their tumorigenicity [713]. On the other hand, stem cell-based therapies used in COPD were proven to be relatively safe so far [714, 715]. However long-term data are scarce.

Similar to cell-based therapies also EVs or exosomes may contain growth factors that may favor tumor progression. From this perspective, regenerative therapies that consist of only a single therapeutic compound should be preferred since the safety of such a single entity is much easier to establish than of the complex mixtures that occur in cells or cell derived EVs or other mixtures.

3.1 Clinical Endpoints

While preclinical research into regenerative therapies for COPD has shown promising results, translating these findings into effective clinical treatments remains a major hurdle. According to the European Medicines Agency (EMA), demonstrating disease modification or slowing of disease progression requires long-term clinical trials that convincingly show a change in the trajectory of lung function decline [716, 717]. This is typically assessed through periodic measurements of FEV_1, the volume of air a person can forcibly exhale in the first second [718]. In clinical trials, the trough or pre-

bronchodilator FEV_1 is the most commonly used parameter [719]. A minimal clinically important difference (MCID) of 100 milliliters is generally accepted to represent a meaningful improvement [258, 718-720]. However, FEV_1 alone is now considered insufficient to fully capture the therapeutic benefit of an intervention [719]. When FEV_1 is used as a primary endpoint, regulatory guidelines require it to be supported by a co-primary endpoint that reflects symptoms and patient-reported outcomes [716, 719]. Among the most frequently used additional clinical endpoints is the Transition Dyspnea Index, which evaluates changes in the severity of dyspnea across three domains: functional impairment, magnitude of task, and magnitude of effort. Each is scored from minus three to plus three, resulting in a total score ranging from minus nine to plus nine [721, 722]. A change of at least one point is considered clinically meaningful [717-719, 721, 722]. Another widely accepted endpoint is the St. George's Respiratory Questionnaire, a self-administered instrument that assesses health status across symptoms, activity limitations, and psychosocial impacts [719]. Total scores range from zero to one hundred, with a four-point change considered the MCID [717-719, 721, 722]. Additional outcomes commonly used in COPD trials include exacerbation frequency, exercise capacity (e.g., six-minute walk distance), rescue medication use, and imaging-based endpoints such as quantitative computed tomography to assess emphysema progression or airway remodeling. [716, 719]. Recent updates in regulatory and academic consensus now emphasize integrated and multidimensional endpoints, including composite measures such as clinically important deterioration (CID) or clinically important improvement (CII), to better capture the complexity of COPD and the potential for disease modification [722]. Moreover, the use of biomarkers, functional respiratory imaging, and digital monitoring tools is gaining importance in evaluating treatment response, particularly for precision-medicine approaches and regenerative interventions aiming to restore lung structure and function [722].

To detect changes in disease trajectory, particularly in the context of regenerative therapies aimed at modifying disease progression, clinical trials require large patient cohorts and extended follow-up periods of at least three to five years [723, 724]. This duration is necessary to generate statistically and clinically meaningful data, particularly when assessing the slope of FEV_1 decline over time between

treatment groups. However, the long timelines, substantial costs, and logistical complexity of such trials present a significant barrier to the clinical translation of regenerative approaches in COPD. These challenges highlight the urgent need for alternative trial designs or early biomarkers that can serve as surrogates for long-term disease progression, thereby facilitating the development of disease-modifying therapies in this space.

To date, fibrinogen remains the only prognostic biomarker formally recognized by both the U.S. Food and Drug Administration (FDA) and the EMA for use in COPD drug development [725]. As a blood clotting factor and acute-phase reactant, fibrinogen plays a key role in the body's response to inflammation. In the context of COPD, the lung epithelium has been shown to produce fibrinogen in response to inflammatory stimuli. Elevated plasma fibrinogen levels (>3.5 g/L) have been associated with an increased risk of acute exacerbations and higher overall mortality among patients with COPD [726-730]. As such, fibrinogen serves as an important biomarker for disease prognosis and identifying high-risk patient populations in clinical trials. In addition to fibrinogen, promising analytical techniques such as proteomics, metabolomics, single-cell and single-nucleus RNA sequencing, mass cytometry, and advanced imaging are increasingly being used to identify novel biomarkers in COPD [731-734]. For instance, soluble RAGE is under consideration for COPD to help identify subjects at risk for emphysema progression [725, 735]. Another proposed biomarkers is the peptide mid-range proadrenomedullin as a predictor for mortality in COPD [730, 736, 737]. Additionally, elevated levels of serum surfactant protein D, a multimeric glycoprotein involved in pulmonary innate immunity, correlated with progressive lung function decline and worsening of the health status of severe patients with COPD [738-741]. Many types of COPD biomarkers have been identified, including blood protein biomarkers, cellular markers, and protease enzymes, which have been collected from diverse biological sources such as peripheral blood, sputum, bronchoalveolar lavage fluid, exhaled air, and genetic material [742, 743]. Moreover, an emerging area of interest is the use of composite biomarker panels, which combine multiple markers to better predict disease severity, progression, and mortality [744-746]. These multi-analyte approaches may ultimately improve patient stratification and the development of personalized treatment strategies in COPD [747]. Taken together, integrating biomarkers with traditional

clinical measures could significantly enhance the monitoring of COPD progression during clinical trials, potentially reducing trial duration and enabling the rapid identification and discontinuation of ineffective compounds ("fast fail" strategies).

3.2 Disease heterogeneity

In addition to clinical readouts, the lack of progress in COPD drug development may largely be attributed to the heterogeneity of the disease. The traditional phenotypic distinctions of 'pink puffers' and 'blue bloaters' offer a simplistic view of the heterogeneous conditions encompassed by COPD. Studies have identified several phenotypic subpopulations within COPD, including eosinophilic, emphysema, and respiratory failure phenotypes [748]. Furthermore, COPD exacerbations are categorized as eosinophil-driven, bacteria-driven, or frequent exacerbators [748]. Patients with COPD can also be classified according to significant risk factors, including genetics, early-life events, infections, smoking and environmental tobacco smoke, and environmental exposure [363, 749]. Understanding the various trajectories and taxonomy of COPD, along with recent advances in COPD pathophysiology, has expanded its definition from a single disease to a syndrome with diverse manifestations and underlying mechanisms [3, 363, 745, 749]. To improve clinical trial outcomes, it is essential to address this complexity by designing trials that reflect the complexity of COPD. Advances in high-throughput methods offer new opportunities for personalizing treatments and drug discovery. By leveraging these methods, clinical trials can be designed to better match the diverse manifestations of COPD. For example, patient biopsies could be subjected to transcriptomic, proteomic, or metabolic profiling to identify specific molecular phenotypes [750, 751]. Single-cell sequencing of lung biopsies can reveal progenitor cell populations that regenerative therapies may target [33, 752]. Alternatively, creating lung organoids from patient-derived cells can serve as a platform for high-throughput compound screening, facilitating the discovery of personalized therapeutics [32].

An emerging framework to address COPD heterogeneity is the treatable traits approach, which focuses on identifying and targeting specific, measurable characteristics that contribute to an individual patient's disease burden, regardless of traditional diagnostic labels [753, 754]. These traits may include airway inflammation type (eosinophilic vs. neutrophilic), bacterial colonization, exacerbation

susceptibility, comorbidities, or impaired tissue repair capacity [753, 754]. By focusing on what mechanistically goes wrong in each patient, rather than the broad disease category, this approach enables more personalized interventions. However, applying a treatable traits framework to repair or regeneration therapy remains challenging, as the loss of lung function in COPD results from multifactorial processes, such as chronic inflammation, infection, protease imbalance, and cellular senescence. Nevertheless, defining traits related to regenerative potential, such as progenitor cell exhaustion or matrix remodeling capacity, could help identify subgroups most likely to benefit from regenerative or reparative interventions in future clinical trials [755]. This approach will ensure that clinical trials are tailored to these well-defined patient cohorts, improving the likelihood of translating preclinical successes into clinical practice. By adopting these strategies and embracing a broader, more nuanced understanding of COPD, clinical trials can more effectively address the complexity of the disease and enhance therapeutic outcomes [749, 756].

3.3 The exacerbation period as a key window of opportunity.

While patient heterogeneity is a major barrier to therapeutic success in COPD, another challenge lies in trial design, specifically the long timelines required to demonstrate clinical efficacy. An alternative approach may lie in reconsidering the timing of therapeutic intervention. Instead of focusing solely on long-term decline, the exacerbation period may represent a critical and underutilized window of opportunity for the prevention of excessive lung function decline. These exacerbations are a hallmark of COPD, particularly in patients with more advanced disease and many of which are triggered by viral and/or bacterial infections [757, 758]. Prior history of exacerbations, older age, the presence of comorbidities, COPD severity and the presence of eosinophilic inflammation are the most significant predictors for the occurrence of exacerbations, underscoring their multifactorial nature [759]. Clinically, an exacerbation is defined as a worsening of the patient's baseline dyspnea, cough, and/or sputum that is acute in onset and necessitates a change in regular medication [14]. Beyond their immediate impact on symptoms and quality of life, exacerbations are increasingly recognized as key drivers of disease progression. It is a relatively new understanding that progressive lung function decline in COPD is not linear, but rather episodic in nature as a result of incomplete recovery from loss of function during an

exacerbation (Figure 7). In fact, exacerbations are estimated to account for the majority of the accelerated lung function loss throughout the life of a patient with COPD [760]. Therefore, treatments aimed at reducing exacerbation risk may have an important preventive effect in the management of lung function decline.

In the context of regenerative pharmacology, the exacerbation period offers an additional and compelling therapeutic opportunity. In the healthy lung, bacterial and viral infections trigger robust activation of alveolar epithelial progenitors to facilitate repair after injury [761, 762]. While this repair response is also observed in COPD, it is often incomplete, leading to a net decline in lung function following each exacerbation [760] (Figure 7). Thus, the exacerbation period is characterized by both heightened disease burden during the exacerbation phase and the concurrent activation of endogenous repair pathways in the period following an exacerbation. This makes the exacerbation window itself an opportunity for therapeutic intervention, particularly for therapies that antagonize the detrimental effects of the inflammatory microenvironment on lung repair, whilst the recovery phase following an exacerbation may be suitable for therapeutics that directly promote repair. Targeting the relatively well-defined time window of (the aftermath of) an exacerbation offers several advantages. Lung function recovery post-exacerbation can be assessed over a matter of weeks, rather than years, and within a relatively controlled clinical setting using the patient as their own control. This could substantially reduce the duration and complexity of clinical trials for regenerative therapies. Moreover, this temporally confined setting may allow for the use of therapeutics that are unsuitable for long-term maintenance, thereby reducing cumulative risk exposure. Taken together, the exacerbation period may represent a biologically and clinically optimal window to evaluate and deliver regenerative interventions aimed at restoring lung function in COPD.

3.4 Route of administration and formulation

Drugs and advanced therapy medicinal products (ATMPs) under development for regenerative lung therapies in COPD do not differ from any other medicinal product in the sense that their route of administration and formulation are mainly determined by the combination of their physicochemical

properties, their structure, their site of action and their intended therapeutic objectives. The formulation scientist must evaluate and balance these four interrelated technical and biopharmaceutical factors, each presenting specific opportunities and constraints, when making decisions during the design and development of the dosage form. What may make the formulation and administration of products for lung regeneration special is that so far, the described therapies, cover the full range of possible drug substance options varying form small organic molecules, therapeutic proteins, nanosized vesicle like structures to advanced cell therapies as summarized in the sections above [32, 749]. In addition, inhalation of the medicine offers a unique option for targeted drug administration to the lungs, which may substantially increase the therapeutic effect that can be obtained with certain therapies through increased exposure of the lungs to the drug [763, 764]. However, at the same time this route of administration is not suitable for all medicines, since mucosal and epithelial barriers may prevent the drug from reaching the target, especially when this target is not at the luminal side of the epithelium.

Oral administration is most convenient for the patient, however, its application for administering drugs for lung regeneration is limited to those drugs that have sufficient oral bioavailability. The oral route is especially suitable for drugs that show the tendency to accumulate in the lungs to concentrations surpassing blood levels, irrespective of the route of administration. The anti-tuberculosis drug bedaquiline is an example of such a drug [765].

In contrast to oral administration the inhalation route, applying liquid or solid aerosols, offers a more targeted approach for therapies acting locally in the lungs. This route of administration enables high drug concentrations at the site of action while avoiding hepatic first-pass metabolism. As such inhalation is an attractive option for a wide range of drug substances, from small molecules to large biopharmaceuticals. Basically, an inhaled drug has to overcome two barriers before it may exert any therapeutic activity. First, penetration and deposition of the aerosol into the airways is required. This, physical barrier can be overcome by generating aerosols with a size range between 1 and 5 µm. Larger particles will not sufficiently penetrate the airways, whereas particle in the nanometer range will not show deposition and merely be exhaled again. Secondly, the drug must reach its site of action which may often require the passage of the epithelial barrier of the airways or alveoli. The airway and alveolar

epithelium are highly permeable to orally inhaled small molecules, which allows these molecules to reach also targets beyond the luminal side of the lung epithelium. Over the past decade a several excellent reviews and books have been published on the development and use of inhalation systems and the formulations used for small molecules and will therefore not be further discussed here [766, 767].

Many of the drugs and ATMPs currently under investigation for regenerative therapies in COPD are biopharmaceuticals [749]. In addition, sparked by the development of inhaled insulin [766], there is today a plethora of information on the formulation and administration of peptide- and protein-based drugs. In general, the protein's instability is a major issue in formulating them. Approaches to tackle their instability includes the application of stabilizing excipients such as, buffers, (poly)saccharides, polyols, surfactants and specific salts and drying of the formulation by lyophilization or spray-drying [768-770]. Next to the formulation, the inhalation device is relevant to the success of the therapy. Since, dried formulations, produced through lyophilization or spray-drying are more stable than liquid formulation, dry powder inhalers may be more suitable for the administration of proteins than the liquid-based nebulizers. Furthermore, ultrasonic nebulizers may affect the structural integrity of the protein [771, 772]. Proteins were among the first biopharmaceuticals explored for the treatment of COPD. Whether the pulmonary route is suitable for a protein is determined by the protein's molecular weight (size) and the location of the target. The molecular mass of proteins that exert their action in the lumen of the airway or alveoli, is not relevant. However, for proteins with a site of action beyond the epithelial lining of the lungs, the molecular weight is important. Proteins with a molecular mass over 1.0 to 1.3 kDa do not pass the airway epithelium, whereas the proteins with a molecular mass over 22 kDa are not absorbed via the alveolar lining. Higher molecular weight proteins may therefore be unable to reach their site of action after inhalation [771, 773].

Currently, the field of biopharmaceutics has developed beyond peptides and proteins, and today also includes nucleic acid-based therapies (mRNA, siRNA and antisense oligonucleotides) and extracellular vesicles (EVs), like the proteins, these therapies may also be suitable for pulmonary administration. Nucleic acid-based therapies are often formulated into lipid nanoparticles (LNPs). These particles protect the genetic material from enzymatic breakdown and enhance the cellular uptake by endocytosis

or pinocytosis. LNPs consist of ionizable cationic lipids, pegylated lipids, phospholipids and cholesterol, and microfluidic technologies have been used to encapsulate the oligonucleotide (e.g. mRNA) in these particles [774]. There is evidence that after inhalation, LNPs can transfect lung cells [775, 776]. Recently, it was demonstrated that pulmonary endothelial-targeted LNPs were capable to deliver mRNA to enhance vascular repair [777]. It is important to realize that LNPs are too large to pass the airway epithelium. Instead, they are internalized by cells located in the superficial layer of the airway epithelium, where the cargo delivered by the LNPs can subsequently exert its therapeutic effect. As an alternative to inhalation, intravenous administration of self-assembling, one-component ionizable Janus dendrimer-based LNPs has been proposed for lung-targeted gene delivery, demonstrating effective mRNA delivery and potential for lung regeneration [778].

As summarized in 2.4, EVs have recently emerged as a potential therapeutic for regenerative treatments [594, 598, 749]. EVs are characterized by their poor stability, which makes them unsuitable as regular therapeutics, certainly when they are dispersed in a liquid where they require storage at -80°C. However, recently it has been found that EVs incorporated in an inulin matrix in the glassy state by freeze drying, stabilized the fragile vesicle structure even at temperatures up to 20°C for 12 weeks at 43 %RH, while maintaining the biophysical properties and regenerative capacity. A similar spray dried powder formulation which next to the inulin also contained 4 % leucine was suitable for inhalation via a dry powder inhaler [616]. Recently, various techniques to produce powders for inhalation containing different biopharmaceuticals, including EVs were reviewed [779].

Finally, bone marrow mononuclear cells (BMMCs) such as mesenchymal BM-MSCs or iPSCs are used for regenerative therapies [32, 714, 749]. Since autologous stem cell therapies are fully personalized therapies, the sourcing, isolation growing and formulation of the cells can only be done in the hospital or in highly specialized nearby the hospital. Cells are usually kept in culture media such as Dulbecco's Modified Eagle Medium (DMEM). For storage the cells can be frozen in liquid nitrogen when the DMEM is supplemented with 10% dimethyl sulfoxide. For infusion the cells are generally formulated in saline or phosphate buffered saline which may be supplemented with human serum albumin. Cells are unsuitable for administration via the inhalation route. Having sizes significantly over 5 µm (15-20 µm) implies

that upon inhalation lung deposition would not reach beyond the first two bifurcations and most of the cells would end up in the throat. When the size of the cells would be reduced to less than 5 to 7 the cellular structure would be destroyed, and the cells would lose their functionality.

4. CONCLUSIONS

The field of regenerative medicine in COPD is advancing rapidly, propelled by new insights into epithelial progenitor biology, inflammatory signaling, and the molecular pathways that govern alveolar repair. This review has highlighted how COPD represents not merely a disease of progressive tissue destruction, but a failure of endogenous repair systems, a concept that reshapes both our understanding of pathogenesis and our therapeutic ambitions.

The alveolar epithelial niche is shaped by a dynamic interplay between epithelial cells, immune cells, fibroblasts, ECs, and the ECM. Key signaling pathways such as WNT/β-catenin, FGF, BMP, and HGF have emerged as central regulators of epithelial proliferation, differentiation, and survival. However, these pathways are frequently disrupted in COPD through inflammatory cytokines, cellular senescence, oxidative stress, and altered mesenchymal-epithelial crosstalk. Strategies that restore balance in these signaling networks, whether through direct activation, suppression of antagonistic signals, or modulation of the niche environment, have shown encouraging results in preclinical models.

At the same time, this review has underscored the multifaceted challenges that remain. COPD is a disease with heterogenous endotypes and stages, and regenerative approaches must contend not only with damaged epithelium but also with persistent inflammation, matrix remodeling, vascular dysfunction, and cellular senescence. As such, future therapies are unlikely to succeed through single-target strategies. Instead, multimodal interventions, combining regenerative, anti-inflammatory, and senolytic components, may be needed to overcome the entrenched pathological milieu.

The preclinical advances summarized here provide a robust foundation, but translation to patients will require rigorous clinical validation, optimized delivery systems (e.g., inhaled biologics, vesicle-mediated delivery), and improved patient stratification tools. Biomarkers that predict regenerative potential or senescence burden could enhance trial design and treatment outcomes. Moreover, the timing of intervention, early versus late-stage disease, will likely determine therapeutic success. In this context, exacerbations may offer a clinically actionable window of opportunity: a transient phase of epithelial injury and heightened niche activity that could be leveraged to support regeneration. Designing clinical trials around these episodes may therefore improve both biological efficacy and measurable outcomes.

In conclusion, the field stands at a pivotal moment. A growing arsenal of regenerative candidates, ranging from small molecules and cell therapies to EV-loaded ligands and cell-derived proteins, is entering a phase of translational readiness. The next decade may witness a paradigm shift in COPD care: from symptom management and damage control, to interventions that restore lung architecture and function.

Acknowledgements

Some of the Figures created with BioRender.com. This work was supported in part by research grants from Boehringer Ingelheim (RG, BNM), Lung Foundation Netherlands (10.1.23.021) and is part of the project RecovAir with file number P21-14 of the research programme Perspectief 2021-2022 2022 TTW which is (partly) financed by the Dutch Research Council (NWO) under the grant 2022/TTW/01358852 (IH, RG). A Ph.D. scholarship from the Molecular Life and Health Program of the University of Groningen supported LvdK. LvdK and HWF are employees and stock owners of MimeCure. DN was supported by an Indonesian Education Scholarship (BPI)Scholarship from The Agency for the Assessment and Application of Technology (BPPT) Indonesia and the Indonesia Endowment Fund for Education Agency (LPDP). BNM reports financial support was provided by the Northern CARA Foundation and Netherlands Organisation for Health Research and Development (ZonMW). ML acknowledges support from the German Center for Lung Research (DZL), the Deutsche

Forschungsgemeinschaft (DFG, German Research Foundation) – 512453064 and the von Behring Röntgen Foundation (71_0011). AÖY was supported by the German Center for Lung Research (DZL). Jill R. Johnson has received grant support from the UK Medical Research Council, the UK Biotechnology and Biological Sciences Research Council, and Lung Foundation Netherlands. RG, AFMD and CFK are members of the LONGFONDS Accelerate Project BREATH. CFK received support from the Cystic Fibrosis Foundation Awards KIM19P0 and KIM21G0, R35HL150876, P01HL158505, Gilda and Alfred Slifka, Gail and Adam Slifka, the Cystic Fibrosis/Multiple Sclerosis Fund Foundation Inc., and the Harvard Stem Cell Institute. CFK reports financial support was further provided by the National Heart Lung Blood Institute R35HL150876. CFK is a founder of Cellforma. TMC, AOY, ML, IH, CFK, RG are members of the COPD-iNET consortium (www.copd-inet.com) [780]. The work represented in this paper was part of discussions leading up to and held at the ERS Research Seminar: Regenerating the lung – a multidisciplinary approach to combat COPD (Prague, CZ, November 2025). AFMD has received funding from the European Respiratory Society (ERS) and the European Union's H2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 847462. The authors are solely responsible for its content; it does not represent the opinion of ERS and the European Commission and ERS and the EU Commission are not responsible for any use that might be made of data appearing therein.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to remove English language and grammar mistakes. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

1. Boers E, Barrett M, Su JG, Benjafield AV, Sinha S, Kaye L, et al. Global Burden of Chronic Obstructive Pulmonary Disease Through 2050. *JAMA Netw Open*. 2023;6(12):e2346598.
2. WHO. 2025 [Available from: https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd).
3. Celli B, Fabbri L, Criner G, Martinez FJ, Mannino D, Vogelmeier C, et al. Definition and Nomenclature of Chronic Obstructive Pulmonary Disease: Time for Its Revision. *Am J Respir Crit Care Med*. 2022;206(11):1317–25.
4. Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. *Annu Rev Pathol*. 2009;4:435–59.
5. Rivas M, Gupta G, Costanzo L, Ahmed H, Wyman AE, Geraghty P. Senescence: Pathogenic Driver in Chronic Obstructive Pulmonary Disease. *Medicina (Kaunas)*. 2022;58(6).
6. MacNee W. Is Chronic Obstructive Pulmonary Disease an Accelerated Aging Disease? *Ann Am Thorac Soc*. 2016;13 Suppl 5:S429–S37.
7. Barnes PJ. Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev*. 2004;56(4):515–48.
8. Agusti A, Hogg JC. Update on the Pathogenesis of Chronic Obstructive Pulmonary Disease. *N Engl J Med*. 2019;381(13):1248–56.
9. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet*. 2018;392(10159):2052–90.
10. Kim V, Criner GJ. Chronic bronchitis and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2013;187(3):228–37.
11. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med*. 2011;365(17):1567–75.
12. Higham A, Quinn AM, Cancado JED, Singh D. The pathology of small airways disease in COPD: historical aspects and future directions. *Respir Res*. 2019;20(1):49.
13. Hogg JC, McDonough JE, Suzuki M. Small airway obstruction in COPD: new insights based on micro-CT imaging and MRI imaging. *Chest*. 2013;143(5):1436–43.
14. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur Respir J*. 2017;49(3).
15. Wedzicha JA, Seemungal TA. COPD exacerbations: defining their cause and prevention. *Lancet*. 2007;370(9589):786–96.
16. Hillas G, Perlikos F, Tzanakis N. Acute exacerbation of COPD: is it the "stroke of the lungs"? *Int J Chron Obstruct Pulmon Dis*. 2016;11:1579–86.
17. Viniol C, Vogelmeier CF. Exacerbations of COPD. *Eur Respir Rev*. 2018;27(147).
18. Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2000;161(5):1608–13.
19. Mettler SK, Nardelli P, Campo MI, San Jose Estepar R, Manapragada PP, Abozeed M, et al. Longitudinal Changes in Airway Mucus Plugs and FEV(1) in COPD. *N Engl J Med*. 2025;392(19):1973–5.
20. Hahn EJ, Rayens MK, Adkins S, Simpson N, Frazier S, Mannino DM. Fewer hospitalizations for chronic obstructive pulmonary disease in communities with smoke-free public policies. *Am J Public Health*. 2014;104(6):1059–65.
21. Patel N. An update on COPD prevention, diagnosis, and management: The 2024 GOLD Report. *Nurse Pract*. 2024;49(6):29–36.
22. Duffy SP, Criner GJ. Chronic Obstructive Pulmonary Disease: Evaluation and Management. *Med Clin North Am*. 2019;103(3):453–61.

23. Bhatt SP, Rabe KF, Hanania NA, Vogelmeier CF, Bafadhel M, Christenson SA, et al. Dupilumab for chronic obstructive pulmonary disease with type 2 inflammation: a pooled analysis of two phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med*. 2025;13(3):234–43.
24. Abraham S, Symons G. Non-pharmacological management of chronic obstructive pulmonary disease. *S Afr Med J*. 2015;105(9):789.
25. Mulhall P, Criner G. Non-pharmacological treatments for COPD. *Respirology*. 2016;21(5):791–809.
26. Patel N, DeCamp M, Criner GJ. Lung transplantation and lung volume reduction surgery versus transplantation in chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2008;5(4):447–53.
27. van Agteren JE, Carson KV, Tiong LU, Smith BJ. Lung volume reduction surgery for diffuse emphysema. *Cochrane Database Syst Rev*. 2016;10(10):CD001001.
28. Sakaguchi T, Slebos DJ. Single-Use Flexible Bronchoscope in Facilitating Endobronchial Valve Treatment in Severe Emphysema. *Int J Chron Obstruct Pulmon Dis*. 2025;20:313–7.
29. Sakaguchi T, Hartman JE, Slebos DJ. An update on endobronchial valve therapy for severe emphysema: real world data and special indications. *Expert Rev Respir Med*. 2024;18(12):1003–11.
30. Yamamoto S, Horita N, Imai R, Niitsu T. Surgical and Bronchoscopic Lung Volume Reduction for Severe Emphysema: A Systematic Review and Network Meta-analysis. *Lung*. 2025;203(1):22.
31. Wang C, Zhou J, Wang J, Li S, Fukunaga A, Yodoi J, et al. Progress in the mechanism and targeted drug therapy for COPD. *Signal Transduct Target Ther*. 2020;5(1):248.
32. Khedoe P, Wu X, Gosens R, Hiemstra PS. Repairing damaged lungs using regenerative therapy. *Curr Opin Pharmacol*. 2021;59:85–94.
33. Ng-Blichfeldt JP, Gosens R, Dean C, Griffiths M, Hind M. Regenerative pharmacology for COPD: breathing new life into old lungs. *Thorax*. 2019;74(9):890–7.
34. Brouwer KM, Hoogenkamp HR, Daamen WF, van Kuppevelt TH. Regenerative medicine for the respiratory system: distant future or tomorrow's treatment? *Am J Respir Crit Care Med*. 2013;187(5):468–75.
35. Butler JP, Loring SH, Patz S, Tsuda A, Yablonskiy DA, Mentzer SJ. Evidence for adult lung growth in humans. *N Engl J Med*. 2012;367(3):244–7.
36. Laros CD, Westermann CJ. Dilatation, compensatory growth, or both after pneumonectomy during childhood and adolescence. A thirty-year follow-up study. *J Thorac Cardiovasc Surg*. 1987;93(4):570–6.
37. Basil MC, Katzen J, Engler AE, Guo M, Herriges MJ, Kathiriya JJ, et al. The Cellular and Physiological Basis for Lung Repair and Regeneration: Past, Present, and Future. *Cell Stem Cell*. 2020;26(4):482–502.
38. Hogan BL, Barkauskas CE, Chapman HA, Epstein JA, Jain R, Hsia CC, et al. Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell*. 2014;15(2):123–38.
39. Rock J, Konigshoff M. Endogenous lung regeneration: potential and limitations. *Am J Respir Crit Care Med*. 2012;186(12):1213–9.
40. Martins LR, Sieverling L, Michelhans M, Schiller C, Erkut C, Grunewald TGP, et al. Single-cell division tracing and transcriptomics reveal cell types and differentiation paths in the regenerating lung. *Nat Commun*. 2024;15(1):2246.
41. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci U S A*. 2009;106(31):12771–5.
42. Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am J Physiol Lung Cell Mol Physiol*. 2004;286(4):L643–9.
43. Plasschaert LW, Zilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature*. 2018;560(7718):377–81.
44. Wu M, Zhang X, Lin Y, Zeng Y. Roles of airway basal stem cells in lung homeostasis and regenerative medicine. *Respir Res*. 2022;23(1):122.

45. Basil MC, Cardenas-Diaz FL, Kathiriya JJ, Morley MP, Carl J, Brumwell AN, et al. Human distal airways contain a multipotent secretory cell that can regenerate alveoli. *Nature*. 2022;604(7904):120–6.
46. Liu Q, Liu K, Cui G, Huang X, Yao S, Guo W, et al. Lung regeneration by multipotent stem cells residing at the bronchioalveolar-duct junction. *Nat Genet*. 2019;51(4):728–38.
47. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell*. 2005;121(6):823–35.
48. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, et al. Type 2 alveolar cells are stem cells in adult lung. *J Clin Invest*. 2013;123(7):3025–36.
49. Yang J, Hernandez BJ, Martinez Alanis D, Narvaez del Pilar O, Vila-Ellis L, Akiyama H, et al. The development and plasticity of alveolar type 1 cells. *Development*. 2016;143(1):54–65.
50. Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA, Desai TJ. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science*. 2018;359(6380):1118–23.
51. Chapman HA, Li X, Alexander JP, Brumwell A, Lorizio W, Tan K, et al. Integrin alpha6beta4 identifies an adult distal lung epithelial population with regenerative potential in mice. *J Clin Invest*. 2011;121(7):2855–62.
52. Ahmadvand N, Khosravi F, Lingampally A, Wasnick R, Vazquez-Armendariz AI, Carraro G, et al. Identification of a novel subset of alveolar type 2 cells enriched in PD-L1 and expanded following pneumonectomy. *Eur Respir J*. 2021;58(5).
53. Zheng D, Soh BS, Yin L, Hu G, Chen Q, Choi H, et al. Differentiation of Club Cells to Alveolar Epithelial Cells In Vitro. *Sci Rep*. 2017;7:41661.
54. Kathiriya JJ, Brumwell AN, Jackson JR, Tang X, Chapman HA. Distinct Airway Epithelial Stem Cells Hide among Club Cells but Mobilize to Promote Alveolar Regeneration. *Cell Stem Cell*. 2020;26(3):346–58 e4.
55. Lv Z, Liu Z, Liu K, Lin X, Pu W, Li Y, et al. Alveolar regeneration by airway secretory-cell-derived p63(+) progenitors. *Cell Stem Cell*. 2024;31(11):1685–700 e6.
56. Brandsma CA, de Vries M, Costa R, Woldhuis RR, Konigshoff M, Timens W. Lung ageing and COPD: is there a role for ageing in abnormal tissue repair? *Eur Respir Rev*. 2017;26(146).
57. Melo-Narvaez MC, Stegmayr J, Wagner DE, Lehmann M. Lung regeneration: implications of the diseased niche and ageing. *Eur Respir Rev*. 2020;29(157).
58. Birch J, Barnes PJ, Passos JF. Mitochondria, telomeres and cell senescence: Implications for lung ageing and disease. *Pharmacol Ther*. 2018;183:34–49.
59. Cordoba-Lanus E, Cazorla-Rivero S, Espinoza-Jimenez A, de-Torres JP, Pajares MJ, Aguirre-Jaime A, et al. Telomere shortening and accelerated aging in COPD: findings from the BODE cohort. *Respir Res*. 2017;18(1):59.
60. Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med*. 2006;174(8):886–93.
61. Yu H, Lin Y, Zhong Y, Guo X, Lin Y, Yang S, et al. Impaired AT2 to AT1 cell transition in PM2.5-induced mouse model of chronic obstructive pulmonary disease. *Respir Res*. 2022;23(1):70.
62. Liberti DC, Kremp MM, Liberti WA, 3rd, Penkala IJ, Li S, Zhou S, et al. Alveolar epithelial cell fate is maintained in a spatially restricted manner to promote lung regeneration after acute injury. *Cell Rep*. 2021;35(6):109092.
63. Abdelwahab EMM, Rapp J, Feller D, Csongei V, Pal S, Bartis D, et al. Wnt signaling regulates trans-differentiation of stem cell like type 2 alveolar epithelial cells to type 1 epithelial cells. *Respir Res*. 2019;20(1):204.
64. Lin CR, Bahmed K, Kosmider B. Impaired Alveolar Re-Epithelialization in Pulmonary Emphysema. *Cells*. 2022;11(13).
65. Gao L, Sun Y, Zhang X, Ma D, Xie A, Wang E, et al. Wnt3a-Loaded Extracellular Vesicles Promote Alveolar Epithelial Regeneration after Lung Injury. *Adv Sci (Weinh)*. 2023;10(18):e2206606.
66. Zhou R, Wang H, Zhang Y, Mai J, Yang L. Small airway disease as a key factor in COPD: new perspectives and insights. *Front Med (Lausanne)*. 2025;12:1648612.
67. Zepp JA, Zacharias WJ, Frank DB, Cavanaugh CA, Zhou S, Morley MP, et al. Distinct Mesenchymal Lineages and Niches Promote Epithelial Self-Renewal and Myofibrogenesis in the Lung. *Cell*. 2017;170(6):1134–48 e10.

68. Liao D, Li H. Dissecting the Niche for Alveolar Type II Cells With Alveolar Organoids. *Front Cell Dev Biol*. 2020;8:419.
69. Wagner DE, Bonenfant NR, Parsons CS, Sokocevic D, Brooks EM, Borg ZD, et al. Comparative decellularization and recellularization of normal versus emphysematous human lungs. *Biomaterials*. 2014;35(10):3281–97.
70. Sauler M, McDonough JE, Adams TS, Kothapalli N, Barnthaler T, Werder RB, et al. Characterization of the COPD alveolar niche using single-cell RNA sequencing. *Nat Commun*. 2022;13(1):494.
71. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2016;138(1):16–27.
72. Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax*. 2000;55(2):114–20.
73. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med*. 1996;153(2):530–4.
74. Sapey E, Ahmad A, Bayley D, Newbold P, Snell N, Rugman P, et al. Imbalances between interleukin-1 and tumor necrosis factor agonists and antagonists in stable COPD. *J Clin Immunol*. 2009;29(4):508–16.
75. Grumelli S, Corry DB, Song LZ, Song L, Green L, Huh J, et al. An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. *PLoS Med*. 2004;1(1):e8.
76. Eickmeier O, Huebner M, Herrmann E, Zissler U, Rosewich M, Baer PC, et al. Sputum biomarker profiles in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) and association between pulmonary function. *Cytokine*. 2010;50(2):152–7.
77. Zhang L, Cheng Z, Liu W, Wu K. Expression of interleukin (IL)-10, IL-17A and IL-22 in serum and sputum of stable chronic obstructive pulmonary disease patients. *COPD*. 2013;10(4):459–65.
78. Sapey E, Stockley RA. COPD exacerbations . 2: aetiology. *Thorax*. 2006;61(3):250–8.
79. Katsura H, Kobayashi Y, Tata PR, Hogan BLM. IL-1 and TNFalpha Contribute to the Inflammatory Niche to Enhance Alveolar Regeneration. *Stem Cell Reports*. 2019;12(4):657–66.
80. Kida H, Yoshida M, Hoshino S, Inoue K, Yano Y, Yanagita M, et al. Protective effect of IL-6 on alveolar epithelial cell death induced by hydrogen peroxide. *Am J Physiol Lung Cell Mol Physiol*. 2005;288(2):L342–9.
81. Zhang X, Ali M, Pantuck MA, Yang X, Lin CR, Bahmed K, et al. CD8 T cell response and its released cytokine IFN-gamma are necessary for lung alveolar epithelial repair during bacterial pneumonia. *Front Immunol*. 2023;14:1268078.
82. Kortekaas RK, Geillinger-Kastle KE, Fuentes-Mateos R, van Orsoy R, Al-Alyan N, Burgess JK, et al. The disruptive effects of COPD exacerbation-associated factors on epithelial repair responses. *Front Immunol*. 2024;15:1346491.
83. Patel BV, Wilson MR, O'Dea KP, Takata M. TNF-induced death signaling triggers alveolar epithelial dysfunction in acute lung injury. *J Immunol*. 2013;190(8):4274–82.
84. Wang Z, Zheng T, Zhu Z, Homer RJ, Riese RJ, Chapman HA, Jr., et al. Interferon gamma induction of pulmonary emphysema in the adult murine lung. *J Exp Med*. 2000;192(11):1587–600.
85. Ma B, Kang MJ, Lee CG, Chapoval S, Liu W, Chen Q, et al. Role of CCR5 in IFN-gamma-induced and cigarette smoke-induced emphysema. *J Clin Invest*. 2005;115(12):3460–72.
86. Katsura H, Sontake V, Tata A, Kobayashi Y, Edwards CE, Heaton BE, et al. Human Lung Stem Cell-Based Alveolospheres Provide Insights into SARS-CoV-2-Mediated Interferon Responses and Pneumocyte Dysfunction. *Cell Stem Cell*. 2020;27(6):890–904 e8.
87. Dost AFM, Balážová K, Casellas CP, van Rooijen LM, Epskamp W, van Son GJF, et al. A human organoid model of alveolar regeneration reveals distinct epithelial responses to interferon-gamma. *bioRxiv*. 2025:2025.01.30.635624.
88. Ciminieri C, Woest ME, Reynaert NL, Heijink IH, Wardenaar R, Spierings DCJ, et al. IL-1beta Induces a Proinflammatory Fibroblast Microenvironment that Impairs Lung Progenitors' Function. *Am J Respir Cell Mol Biol*. 2023;68(4):444–55.

89. Choi J, Park JE, Tsagkogeorga G, Yanagita M, Koo BK, Han N, et al. Inflammatory Signals Induce AT2 Cell-Derived Damage-Associated Transient Progenitors that Mediate Alveolar Regeneration. *Cell Stem Cell*. 2020;27(3):366–82 e7.
90. Kadur Lakshminarasimha Murthy P, Sontake V, Tata A, Kobayashi Y, Macadlo L, Okuda K, et al. Human distal lung maps and lineage hierarchies reveal a bipotent progenitor. *Nature*. 2022;604(7904):111–9.
91. Shaykhiev R, Krause A, Salit J, Strulovici-Barel Y, Harvey BG, O'Connor TP, et al. Smoking-dependent reprogramming of alveolar macrophage polarization: implication for pathogenesis of chronic obstructive pulmonary disease. *J Immunol*. 2009;183(4):2867–83.
92. Takiguchi H, Yang CX, Yang CWT, Sahin B, Whalen BA, Milne S, et al. Macrophages with reduced expressions of classical M1 and M2 surface markers in human bronchoalveolar lavage fluid exhibit pro-inflammatory gene signatures. *Sci Rep*. 2021;11(1):8282.
93. Eapen MS, Hansbro PM, McAlinden K, Kim RY, Ward C, Hackett TL, et al. Abnormal M1/M2 macrophage phenotype profiles in the small airway wall and lumen in smokers and chronic obstructive pulmonary disease (COPD). *Sci Rep*. 2017;7(1):13392.
94. Bazzan E, Turato G, Tine M, Radu CM, Balestro E, Rigobello C, et al. Dual polarization of human alveolar macrophages progressively increases with smoking and COPD severity. *Respir Res*. 2017;18(1):40.
95. Kunz LI, Lapperre TS, Snoeck-Stroband JB, Budulac SE, Timens W, van Wijngaarden S, et al. Smoking status and anti-inflammatory macrophages in bronchoalveolar lavage and induced sputum in COPD. *Respir Res*. 2011;12(1):34.
96. Hu Y, Shao X, Xing L, Li X, Nonis GM, Koelwyn GJ, et al. Single-Cell Sequencing of Lung Macrophages and Monocytes Reveals Novel Therapeutic Targets in COPD. *Cells*. 2023;12(24).
97. Donnelly LE, Barnes PJ. Defective phagocytosis in airways disease. *Chest*. 2012;141(4):1055–62.
98. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*. 2014;41(1):14–20.
99. Aegerter H, Lambrecht BN, Jakubzick CV. Biology of lung macrophages in health and disease. *Immunity*. 2022;55(9):1564–80.
100. Evren E, Ringqvist E, Willinger T. Origin and ontogeny of lung macrophages: from mice to humans. *Immunology*. 2020;160(2):126–38.
101. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity*. 2013;38(1):79–91.
102. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med*. 2013;210(10):1977–92.
103. van de Laar L, Saelens W, De Prijck S, Martens L, Scott CL, Van Isterdael G, et al. Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity*. 2016;44(4):755–68.
104. Mould KJ, Barthel L, Mohning MP, Thomas SM, McCubbrey AL, Danhorn T, et al. Cell Origin Dictates Programming of Resident versus Recruited Macrophages during Acute Lung Injury. *Am J Respir Cell Mol Biol*. 2017;57(3):294–306.
105. Wohnhaas CT, Bassler K, Watson CK, Shen Y, Leparc GG, Tilp C, et al. Monocyte-derived alveolar macrophages are key drivers of smoke-induced lung inflammation and tissue remodeling. *Front Immunol*. 2024;15:1325090.
106. Cornwell WD, Kim V, Fan X, Vega ME, Ramsey FV, Criner GJ, et al. Activation and polarization of circulating monocytes in severe chronic obstructive pulmonary disease. *BMC Pulm Med*. 2018;18(1):101.
107. Gibbings SL, Thomas SM, Atif SM, McCubbrey AL, Desch AN, Danhorn T, et al. Three Unique Interstitial Macrophages in the Murine Lung at Steady State. *Am J Respir Cell Mol Biol*. 2017;57(1):66–76.
108. Tan SY, Krasnow MA. Developmental origin of lung macrophage diversity. *Development*. 2016;143(8):1318–27.
109. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, et al. Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. *Science*. 2019;363(6432).

110. Schyns J, Bai Q, Ruscitti C, Radermecker C, De Schepper S, Chakarov S, et al. Non-classical tissue monocytes and two functionally distinct populations of interstitial macrophages populate the mouse lung. *Nat Commun*. 2019;10(1):3964.
111. Dewhurst JA, Lea S, Hardaker E, Dungwa JV, Ravi AK, Singh D. Characterisation of lung macrophage subpopulations in COPD patients and controls. *Sci Rep*. 2017;7(1):7143.
112. Mitsune A, Yamada M, Fujino N, Numakura T, Ichikawa T, Suzuki A, et al. Upregulation of leukocyte immunoglobulin-like receptor B4 on interstitial macrophages in COPD; their possible protective role against emphysema formation. *Respir Res*. 2021;22(1):232.
113. Kapellos TS, Bassler K, Aschenbrenner AC, Fujii W, Schultze JL. Dysregulated Functions of Lung Macrophage Populations in COPD. *J Immunol Res*. 2018;2018:2349045.
114. Demedts IK, Bracke KR, Van Pottelberge G, Testelmans D, Verleden GM, Vermassen FE, et al. Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2007;175(10):998–1005.
115. Decramer M, Janssens W, Miravitlles M. Chronic obstructive pulmonary disease. *Lancet*. 2012;379(9823):1341–51.
116. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. *Lancet*. 2011;378(9795):1015–26.
117. Finkelstein R, Fraser RS, Ghezzo H, Cosio MG. Alveolar inflammation and its relation to emphysema in smokers. *Am J Respir Crit Care Med*. 1995;152(5 Pt 1):1666–72.
118. Ofulue AF, Ko M. Effects of depletion of neutrophils or macrophages on development of cigarette smoke-induced emphysema. *Am J Physiol*. 1999;277(1):L97–105.
119. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science*. 1997;277(5334):2002–4.
120. Le Quement C, Guenon I, Gillon JY, Valenca S, Cayron-Elizondo V, Lagente V, et al. The selective MMP-12 inhibitor, AS111793 reduces airway inflammation in mice exposed to cigarette smoke. *Br J Pharmacol*. 2008;154(6):1206–15.
121. Pervizaj-Oruqaj L, Ferrero MR, Matt U, Herold S. The guardians of pulmonary harmony: alveolar macrophages orchestrating the symphony of lung inflammation and tissue homeostasis. *Eur Respir Rev*. 2024;33(172).
122. Wei X, Qian W, Narasimhan H, Chan T, Liu X, Arish M, et al. Macrophage peroxisomes guide alveolar regeneration and limit SARS-CoV-2 tissue sequelae. *Science*. 2025;387(6738):eadq2509.
123. van Riet S, van Schadewijk A, de Vos S, Vandeghinste N, Rottier RJ, Stolk J, et al. Modulation of Airway Epithelial Innate Immunity and Wound Repair by M(GM-CSF) and M(M-CSF) Macrophages. *J Innate Immun*. 2020;12(5):410–21.
124. Boorsma CE, Draijer C, Melgert BN. Macrophage heterogeneity in respiratory diseases. *Mediators Inflamm*. 2013;2013:769214.
125. Byrne AJ, Mathie SA, Gregory LG, Lloyd CM. Pulmonary macrophages: key players in the innate defence of the airways. *Thorax*. 2015;70(12):1189–96.
126. Boe DM, Hulsebus HJ, Najarro KM, Mullen JE, Kim H, Tan AC, et al. Advanced age is associated with changes in alveolar macrophages and their responses to the stress of traumatic injury. *J Leukoc Biol*. 2022;112(6):1371–86.
127. Rymut N, Heinz J, Sadhu S, Hosseini Z, Riley CO, Marinello M, et al. Resolvin D1 promotes efferocytosis in aging by limiting senescent cell-induced MerTK cleavage. *FASEB J*. 2020;34(1):597–609.
128. Shaykhiev R, Crystal RG. Innate immunity and chronic obstructive pulmonary disease: a mini-review. *Gerontology*. 2013;59(6):481–9.
129. Taylor AE, Finney-Hayward TK, Quint JK, Thomas CM, Tudhope SJ, Wedzicha JA, et al. Defective macrophage phagocytosis of bacteria in COPD. *Eur Respir J*. 2010;35(5):1039–47.
130. Rochford I, Joshi JC, Rayees S, Anwar M, Akhter MZ, Yalagala L, et al. Evidence for reprogramming of monocytes into reparative alveolar macrophages in vivo by targeting PDE4b. *Am J Physiol Lung Cell Mol Physiol*. 2021;321(4):L686–L702.
131. Mammoto A, Mammoto T. Vascular Niche in Lung Alveolar Development, Homeostasis, and Regeneration. *Front Bioeng Biotechnol*. 2019;7:318.
132. Ding BS, Nolan DJ, Guo P, Babazadeh AO, Cao Z, Rosenwaks Z, et al. Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. *Cell*. 2011;147(3):539–53.

133. Leiby KL, Yuan Y, Ng R, Raredon MSB, Adams TS, Baevova P, et al. Rational engineering of lung alveolar epithelium. *NPJ Regen Med*. 2023;8(1):22.
134. Niethamer TK, Stabler CT, Leach JP, Zepp JA, Morley MP, Babu A, et al. Defining the role of pulmonary endothelial cell heterogeneity in the response to acute lung injury. *Elife*. 2020;9.
135. Lee JH, Bhang DH, Beede A, Huang TL, Stripp BR, Bloch KD, et al. Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. *Cell*. 2014;156(3):440–55.
136. Ackermann M, Werlein C, Plucinski E, Leypold S, Kuhnel MP, Verleden SE, et al. The role of vasculature and angiogenesis in respiratory diseases. *Angiogenesis*. 2024;27(3):293–310.
137. Ravi A, Jia L, Wu X, van der Meer AD, Gosens R, Stolk J, et al. Loss of Pulmonary Endothelial Cells in Emphysema Impairs Support of Human Alveolar Epithelial Cell Growth. *Am J Respir Cell Mol Biol*. 2024;70(2):149–52.
138. Hisata S, Racanelli AC, Kermani P, Schreiner R, Houghton S, Palikuqi B, et al. Reversal of emphysema by restoration of pulmonary endothelial cells. *J Exp Med*. 2021;218(8).
139. van der Koog L, Wu X, Nugraha DF, Ravi A, Wolters JC, Verhamme FM, et al. Pulmonary Microvascular Endothelial Cells Support Alveolar Epithelial Growth via Bone Morphogenetic Protein 6 Secretion. *Am J Respir Cell Mol Biol*. 2025.
140. Yamamoto H, Yun EJ, Gerber HP, Ferrara N, Whitsett JA, Vu TH. Epithelial-vascular cross talk mediated by VEGF-A and HGF signaling directs primary septae formation during distal lung morphogenesis. *Dev Biol*. 2007;308(1):44–53.
141. Peinado VI, Barbera JA, Ramirez J, Gomez FP, Roca J, Jover L, et al. Endothelial dysfunction in pulmonary arteries of patients with mild COPD. *Am J Physiol*. 1998;274(6):L908–13.
142. Niethamer TK, Levin LI, Morley MP, Babu A, Zhou S, Morrisey EE. Atf3 defines a population of pulmonary endothelial cells essential for lung regeneration. *Elife*. 2023;12.
143. Vila Ellis L, Cain MP, Hutchison V, Flodby P, Crandall ED, Borok Z, et al. Epithelial Vegfa Specifies a Distinct Endothelial Population in the Mouse Lung. *Dev Cell*. 2020;52(5):617–30 e6.
144. Doyle MF, Tracy RP, Parikh MA, Hoffman EA, Shimbo D, Austin JH, et al. Endothelial progenitor cells in chronic obstructive pulmonary disease and emphysema. *PLoS One*. 2017;12(3):e0173446.
145. Ushakumary MG, Riccetti M, Perl AT. Resident interstitial lung fibroblasts and their role in alveolar stem cell niche development, homeostasis, injury, and regeneration. *Stem Cells Transl Med*. 2021;10(7):1021–32.
146. Osei ET, Noordhoek JA, Hackett TL, Spanjer AI, Postma DS, Timens W, et al. Interleukin-1alpha drives the dysfunctional cross-talk of the airway epithelium and lung fibroblasts in COPD. *Eur Respir J*. 2016;48(2):359–69.
147. Hallgren O, Nihlberg K, Dahlback M, Bjermer L, Eriksson LT, Erjefalt JS, et al. Altered fibroblast proteoglycan production in COPD. *Respir Res*. 2010;11(1):55.
148. Larsson-Callerfelt AK, Hallgren O, Andersson-Sjoland A, Thiman L, Bjorklund J, Kron J, et al. Defective alterations in the collagen network to prostacyclin in COPD lung fibroblasts. *Respir Res*. 2013;14(1):21.
149. van der Koog L, Boerrigter MJ, Gorter IC, Gosens R, Nagelkerke A. Lung fibroblast-derived extracellular vesicles and soluble factors alleviate elastase-induced lung injury. *Eur J Pharmacol*. 2024;974:176612.
150. Mason RJ. Biology of alveolar type II cells. *Respirology*. 2006;11 Suppl:S12–5.
151. Worrell JC, MacLeod MKL. Stromal-immune cell crosstalk fundamentally alters the lung microenvironment following tissue insult. *Immunology*. 2021;163(3):239–49.
152. Behzad AR, McDonough JE, Seyednejad N, Hogg JC, Walker DC. The disruption of the epithelial mesenchymal trophic unit in COPD. *COPD*. 2009;6(6):421–31.
153. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. *Mol Med*. 2011;17(3-4):293–307.
154. Weibel ER. On the tricks alveolar epithelial cells play to make a good lung. *Am J Respir Crit Care Med*. 2015;191(5):504–13.
155. Bhatt SP, Soler X, Wang X, Murray S, Anzueto AR, Beaty TH, et al. Association between Functional Small Airway Disease and FEV1 Decline in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2016;194(2):178–84.

156. Lin YC, Sung YK, Jiang X, Peters-Golden M, Nicolls MR. Simultaneously Targeting Myofibroblast Contractility and Extracellular Matrix Cross-Linking as a Therapeutic Concept in Airway Fibrosis. *Am J Transplant*. 2017;17(5):1229–41.
157. Barnes PJ. Small airway fibrosis in COPD. *Int J Biochem Cell Biol*. 2019;116:105598.
158. Bekker NJ, van Pijkeren A, Wolters JC, Sanchez Brotons A, Guryev V, Woldhuis RR, et al. A proteomics approach to identify COPD-related changes in lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol*. 2023;324(4):L521–L35.
159. Melo-Narvaez MC, Bramey N, See F, Heinzelmann K, Ballester B, Steinchen C, et al. Stimuli-Specific Senescence of Primary Human Lung Fibroblasts Modulates Alveolar Stem Cell Function. *Cells*. 2024;13(13).
160. Pouwels SD, Heijink IH, ten Hacken NH, Vandenabeele P, Krysko DV, Nawijn MC, et al. DAMPs activating innate and adaptive immune responses in COPD. *Mucosal Immunol*. 2014;7(2):215–26.
161. Togo S, Holz O, Liu X, Sugiura H, Kamio K, Wang X, et al. Lung fibroblast repair functions in patients with chronic obstructive pulmonary disease are altered by multiple mechanisms. *Am J Respir Crit Care Med*. 2008;178(3):248–60.
162. Wrench CL, Baker JR, Monkley S, Fenwick PS, Murray L, Donnelly LE, et al. Small airway fibroblasts from patients with chronic obstructive pulmonary disease exhibit cellular senescence. *Am J Physiol Lung Cell Mol Physiol*. 2024;326(3):L266–L79.
163. Krimmer DI, Burgess JK, Wooi TK, Black JL, Oliver BG. Matrix proteins from smoke-exposed fibroblasts are pro-proliferative. *Am J Respir Cell Mol Biol*. 2012;46(1):34–9.
164. Kruk D, Yeung ACY, Faiz A, Ten Hacken NHT, Timens W, van Kuppevelt TH, et al. Gene expression profiles in mesenchymal stromal cells from bone marrow, adipose tissue and lung tissue of COPD patients and controls. *Respir Res*. 2023;24(1):22.
165. Plantier L, Marchand-Adam S, Marchal-Somme J, Leseche G, Fournier M, Dehoux M, et al. Defect of hepatocyte growth factor production by fibroblasts in human pulmonary emphysema. *Am J Physiol Lung Cell Mol Physiol*. 2005;288(4):L641–7.
166. Kim YS, Hong G, Kim DH, Kim YM, Kim YK, Oh YM, et al. The role of FGF-2 in smoke-induced emphysema and the therapeutic potential of recombinant FGF-2 in patients with COPD. *Exp Mol Med*. 2018;50(11):1–10.
167. Zandvoort A, Postma DS, Jonker MR, Noordhoek JA, Vos JT, Timens W. Smad gene expression in pulmonary fibroblasts: indications for defective ECM repair in COPD. *Respir Res*. 2008;9(1):83.
168. Elowsson Rendin L, Lofdahl A, Kadefors M, Soderlund Z, Tykesson E, Rolandsson Enes S, et al. Harnessing the ECM Microenvironment to Ameliorate Mesenchymal Stromal Cell-Based Therapy in Chronic Lung Diseases. *Front Pharmacol*. 2021;12:645558.
169. Hussell T, Lui S, Jagger C, Morgan D, Brand O. The consequence of matrix dysfunction on lung immunity and the microbiome in COPD. *Eur Respir Rev*. 2018;27(148).
170. Sirianni FE, Chu FS, Walker DC. Human alveolar wall fibroblasts directly link epithelial type 2 cells to capillary endothelium. *Am J Respir Crit Care Med*. 2003;168(12):1532–7.
171. Sohal SS, Reid D, Soltani A, Ward C, Weston S, Muller HK, et al. Reticular basement membrane fragmentation and potential epithelial mesenchymal transition is exaggerated in the airways of smokers with chronic obstructive pulmonary disease. *Respirology*. 2010;15(6):930–8.
172. Burgess JK, Mauad T, Tjin G, Karlsson JC, Westergren-Thorsson G. The extracellular matrix - the under-recognized element in lung disease? *J Pathol*. 2016;240(4):397–409.
173. Zhou Y, Horowitz JC, Naba A, Ambalavanan N, Atabai K, Balestrini J, et al. Extracellular matrix in lung development, homeostasis and disease. *Matrix Biol*. 2018;73:77–104.
174. Wight TN, Frevert CW, Debley JS, Reeves SR, Parks WC, Ziegler SF. Interplay of extracellular matrix and leukocytes in lung inflammation. *Cell Immunol*. 2017;312:1–14.
175. Korpos E, Wu C, Song J, Hallmann R, Sorokin L. Role of the extracellular matrix in lymphocyte migration. *Cell Tissue Res*. 2010;339(1):47–57.
176. Joglekar MM, Nizamoglu M, Fan Y, Nemani SSP, Weckmann M, Pouwels SD, et al. Highway to heal: Influence of altered extracellular matrix on infiltrating immune cells during acute and chronic lung diseases. *Front Pharmacol*. 2022;13:995051.

177. Gray AL, Pun N, Ridley AJL, Dyer DP. Role of extracellular matrix proteoglycans in immune cell recruitment. *Int J Exp Pathol*. 2022;103(2):34–43.
178. Annoni R, Lancas T, Yukimatsu Tanigawa R, de Medeiros Matsushita M, de Morais Fernezlian S, Bruno A, et al. Extracellular matrix composition in COPD. *Eur Respir J*. 2012;40(6):1362–73.
179. Karakioulaki M, Papakonstantinou E, Stolz D. Extracellular matrix remodelling in COPD. *Eur Respir Rev*. 2020;29(158).
180. Liu G, Philp AM, Corte T, Travis MA, Schilter H, Hansbro NG, et al. Therapeutic targets in lung tissue remodelling and fibrosis. *Pharmacol Ther*. 2021;225:107839.
181. Pelosi P, Rocco PR, Negrini D, Passi A. The extracellular matrix of the lung and its role in edema formation. *An Acad Bras Cienc*. 2007;79(2):285–97.
182. Cantor J. The Role of the Extracellular Matrix in the Pathogenesis and Treatment of Pulmonary Emphysema. *Int J Mol Sci*. 2024;25(19).
183. Knudsen L, Ochs M. The micromechanics of lung alveoli: structure and function of surfactant and tissue components. *Histochem Cell Biol*. 2018;150(6):661–76.
184. Joglekar MM, Bekker NJ, Koloko Ngassie ML, Vonk JM, Borghuis T, Reinders-Luinge M, et al. The lung extracellular matrix protein landscape in severe early-onset and moderate chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol*. 2024;327(3):L304–L18.
185. Burgess JK, Harmsen MC. Chronic lung diseases: entangled in extracellular matrix. *Eur Respir Rev*. 2022;31(163).
186. Suki B, Bates JH. Lung tissue mechanics as an emergent phenomenon. *J Appl Physiol (1985)*. 2011;110(4):1111–8.
187. Ito JT, Lourenco JD, Righetti RF, Tiberio I, Prado CM, Lopes F. Extracellular Matrix Component Remodeling in Respiratory Diseases: What Has Been Found in Clinical and Experimental Studies? *Cells*. 2019;8(4).
188. Woldhuis RR, Heijink IH, van den Berge M, Timens W, Oliver BGG, de Vries M, et al. COPD-derived fibroblasts secrete higher levels of senescence-associated secretory phenotype proteins. *Thorax*. 2021;76(5):508–11.
189. Noordhoek JA, Postma DS, Chong LL, Menkema L, Kauffman HF, Timens W, et al. Different modulation of decorin production by lung fibroblasts from patients with mild and severe emphysema. *COPD*. 2005;2(1):17–25.
190. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Nolte IM, Boezen HM. Decorin and TGF-beta1 polymorphisms and development of COPD in a general population. *Respir Res*. 2006;7(1):89.
191. Kelly MP, Nikolaev VO, Gobejishvili L, Lugnier C, Hesslinger C, Nickolaus P, et al. Cyclic nucleotide phosphodiesterases as drug targets. *Pharmacol Rev*. 2025;77(3):100042.
192. Yang A, Yu G, Wu Y, Wang H. Role of beta2-adrenergic receptors in chronic obstructive pulmonary disease. *Life Sci*. 2021;265:118864.
193. Billington CK, Ojo OO, Penn RB, Ito S. cAMP regulation of airway smooth muscle function. *Pulm Pharmacol Ther*. 2013;26(1):112–20.
194. Schmidt M, Cattani-Cavalieri I, Nunez FJ, Ostrom RS. Phosphodiesterase isoforms and cAMP compartments in the development of new therapies for obstructive pulmonary diseases. *Curr Opin Pharmacol*. 2020;51:34–42.
195. Metaxas EI, Balis E. The safety of indacaterol for the treatment of COPD. *Expert Opin Drug Saf*. 2018;17(6):637–42.
196. Blair HA. Tiotropium/Olodaterol: A Review in COPD. *Drugs*. 2019;79(9):997–1008.
197. Vestbo J, Leather D, Diar Bakerly N, New J, Gibson JM, McCorkindale S, et al. Effectiveness of Fluticasone Furoate-Vilanterol for COPD in Clinical Practice. *N Engl J Med*. 2016;375(13):1253–60.
198. Horita N, Goto A, Shibata Y, Ota E, Nakashima K, Nagai K, et al. Long-acting muscarinic antagonist (LAMA) plus long-acting beta-agonist (LABA) versus LABA plus inhaled corticosteroid (ICS) for stable chronic obstructive pulmonary disease (COPD). *Cochrane Database Syst Rev*. 2017;2(2):CD012066.
199. Oba Y, Pathak M, Maduke T, Fakhouri EW, Dias S. Dual combination therapy versus long-acting bronchodilators alone for chronic obstructive pulmonary disease (COPD): a systematic review and network meta-analysis. *Cochrane Database Syst Rev*. 2025;4(4):CD015997.

200. Barnes PJ. Biochemical basis of asthma therapy. *J Biol Chem*. 2011;286(38):32899–905.
201. Perkins GD, Gao F, Thickett DR. In vivo and in vitro effects of salbutamol on alveolar epithelial repair in acute lung injury. *Thorax*. 2008;63(3):215–20.
202. Spurzem JR, Gupta J, Veys T, Kneifl KR, Rennard SI, Wyatt TA. Activation of protein kinase A accelerates bovine bronchial epithelial cell migration. *Am J Physiol Lung Cell Mol Physiol*. 2002;282(5):L1108–16.
203. Sriram K, Insel MB, Insel PA. Inhaled beta2 Adrenergic Agonists and Other cAMP-Elevating Agents: Therapeutics for Alveolar Injury and Acute Respiratory Disease Syndrome? *Pharmacol Rev*. 2021;73(4):488–526.
204. Peitzman ER, Zaidman NA, Maniak PJ, O'Grady SM. Agonist binding to beta-adrenergic receptors on human airway epithelial cells inhibits migration and wound repair. *Am J Physiol Cell Physiol*. 2015;309(12):C847–55.
205. Plosa EJ, Benjamin JT, Sucre JM, Gulleman PM, Gleaves LA, Han W, et al. beta1 Integrin regulates adult lung alveolar epithelial cell inflammation. *JCI Insight*. 2020;5(2).
206. Steenhuis P, Huntley RE, Gurenko Z, Yin L, Dale BA, Fazel N, et al. Adrenergic signaling in human oral keratinocytes and wound repair. *J Dent Res*. 2011;90(2):186–92.
207. Pullar CE, Manabat-Hidalgo CG, Bolaji RS, Isseroff RR. beta-Adrenergic receptor modulation of wound repair. *Pharmacol Res*. 2008;58(2):158–64.
208. Ghoghawala SY, Mannis MJ, Pullar CE, Rosenblatt MI, Isseroff RR. Beta2-adrenergic receptor signaling mediates corneal epithelial wound repair. *Invest Ophthalmol Vis Sci*. 2008;49(5):1857–63.
209. Bennett WD. Effect of beta-adrenergic agonists on mucociliary clearance. *J Allergy Clin Immunol*. 2002;110(6 Suppl):S291–7.
210. Meyer T, Reitmeir P, Brand P, Herpich C, Sommerer K, Schulze A, et al. Effects of formoterol and tiotropium bromide on mucus clearance in patients with COPD. *Respir Med*. 2011;105(6):900–6.
211. Celli BR, Thomas NE, Anderson JA, Ferguson GT, Jenkins CR, Jones PW, et al. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med*. 2008;178(4):332–8.
212. Fan Chung K. Phosphodiesterase inhibitors in airways disease. *Eur J Pharmacol*. 2006;533(1-3):110–7.
213. Jones NA, Leport M, Holand T, Vos T, Morgan M, Fink M, et al. Phosphodiesterase (PDE) 7 in inflammatory cells from patients with asthma and COPD. *Pulm Pharmacol Ther*. 2007;20(1):60–8.
214. Nourian YH, Salimian J, Ahmadi A, Salehi Z, Karimi M, Emamvirdizadeh A, et al. cAMP-PDE signaling in COPD: Review of cellular, molecular and clinical features. *Biochem Biophys Rep*. 2023;34:101438.
215. Martinez FJ, Rabe KF, Calverley PMA, Fabbri LM, Sethi S, Pizzichini E, et al. Determinants of Response to Roflumilast in Severe Chronic Obstructive Pulmonary Disease. Pooled Analysis of Two Randomized Trials. *Am J Respir Crit Care Med*. 2018;198(10):1268–78.
216. Wedzicha JA, Calverley PM, Rabe KF. Roflumilast: a review of its use in the treatment of COPD. *Int J Chron Obstruct Pulmon Dis*. 2016;11:81–90.
217. Calverley PM, Rabe KF, Goehring UM, Kristiansen S, Fabbri LM, Martinez FJ, et al. Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised clinical trials. *Lancet*. 2009;374(9691):685–94.
218. Anzueto A, Barjaktarevic IZ, Siler TM, Rheault T, Bengtsson T, Rickard K, et al. Ensifentrine, a Novel Phosphodiesterase 3 and 4 Inhibitor for the Treatment of Chronic Obstructive Pulmonary Disease: Randomized, Double-Blind, Placebo-controlled, Multicenter Phase III Trials (the ENHANCE Trials). *Am J Respir Crit Care Med*. 2023;208(4):406–16.
219. Al Matni MY, Meliton L, Dudek SM, Letsiou E. Dual Inhibition of Phosphodiesterase 3 and 4 Enzymes by Ensifentrine Protects against MRSA-Induced Lung Endothelial and Epithelial Dysfunction. *Cells*. 2024;13(21).
220. Hubert S, Kok-Carriere A, De Ceuninck F. Ensifentrine (Ohtuvayre) for chronic obstructive pulmonary disease. *Trends Pharmacol Sci*. 2024;45(10):941–2.
221. Zarogoulidis P, Kosmidis C, Kougkas N, Lallas A, Petridis D, Hohenforst-Schmidt W, et al. Modification of Apremilast from Pills to Aerosol a Future Concept. *Int J Environ Res Public Health*. 2021;18(21).

222. Al-Harbi NO, Imam F, Al-Harbi MM, Qamar W, Aljerian K, Khalid Anwer M, et al. Effect of Apremilast on LPS-induced immunomodulation and inflammation via activation of Nrf2/HO-1 pathways in rat lungs. *Saudi Pharm J*. 2023;31(7):1327–38.
223. Facchinetti F, Civelli M, Singh D, Papi A, Emirova A, Govoni M. Tanimilast, A Novel Inhaled Pde4 Inhibitor for the Treatment of Asthma and Chronic Obstructive Pulmonary Disease. *Front Pharmacol*. 2021;12:740803.
224. Lea S, Metryka A, Li J, Higham A, Bridgewood C, Villetti G, et al. The modulatory effects of the PDE4 inhibitors CHF6001 and roflumilast in alveolar macrophages and lung tissue from COPD patients. *Cytokine*. 2019;123:154739.
225. Kistemaker LEM, Oenema TA, Baarsma HA, Bos IST, Schmidt M, Facchinetti F, et al. The PDE4 inhibitor CHF-6001 and LAMAs inhibit bronchoconstriction-induced remodeling in lung slices. *Am J Physiol Lung Cell Mol Physiol*. 2017;313(3):L507–L15.
226. Nguyen HO, Salvi V, Tiberio L, Facchinetti F, Govoni M, Villetti G, et al. The PDE4 inhibitor tanimilast shows distinct immunomodulatory properties associated with a type 2 endotype and CD141 upregulation. *J Transl Med*. 2022;20(1):203.
227. Moretto N, Caruso P, Bosco R, Marchini G, Pastore F, Armani E, et al. CHF6001 I: a novel highly potent and selective phosphodiesterase 4 inhibitor with robust anti-inflammatory activity and suitable for topical pulmonary administration. *J Pharmacol Exp Ther*. 2015;352(3):559–67.
228. Stellari FF, Sala A, Ruscitti F, Buccellati C, Allen A, Rise P, et al. CHF6001 Inhibits NF-kappaB Activation and Neutrophilic Recruitment in LPS-Induced Lung Inflammation in Mice. *Front Pharmacol*. 2019;10:1337.
229. Villetti G, Carnini C, Battipaglia L, Preynat L, Bolzoni PT, Bassani F, et al. CHF6001 II: a novel phosphodiesterase 4 inhibitor, suitable for topical pulmonary administration--in vivo preclinical pharmacology profile defines a potent anti-inflammatory compound with a wide therapeutic window. *J Pharmacol Exp Ther*. 2015;352(3):568–78.
230. Zaslona Z, Peters-Golden M. Prostanoids in Asthma and COPD: Actions, Dysregulation, and Therapeutic Opportunities. *Chest*. 2015;148(5):1300–6.
231. Huang SK, Peters-Golden M. Eicosanoid lipid mediators in fibrotic lung diseases: ready for prime time? *Chest*. 2008;133(6):1442–50.
232. Dagouassat M, Gagliolo JM, Chrusciel S, Bourin MC, Duprez C, Caramelle P, et al. The cyclooxygenase-2-prostaglandin E2 pathway maintains senescence of chronic obstructive pulmonary disease fibroblasts. *Am J Respir Crit Care Med*. 2013;187(7):703–14.
233. Chen Y, Chen P, Hanaoka M, Droma Y, Kubo K. Enhanced levels of prostaglandin E2 and matrix metalloproteinase-2 correlate with the severity of airflow limitation in stable COPD. *Respirology*. 2008;13(7):1014–21.
234. Montuschi P, Kharitonov SA, Ciabattoni G, Barnes PJ. Exhaled leukotrienes and prostaglandins in COPD. *Thorax*. 2003;58(7):585–8.
235. Chen Y, Hanaoka M, Chen P, Droma Y, Voelkel NF, Kubo K. Protective effect of beraprost sodium, a stable prostacyclin analog, in the development of cigarette smoke extract-induced emphysema. *Am J Physiol Lung Cell Mol Physiol*. 2009;296(4):L648–56.
236. Wu X, Bos IST, Conlon TM, Ansari M, Verschut V, van der Koog L, et al. A transcriptomics-guided drug target discovery strategy identifies receptor ligands for lung regeneration. *Sci Adv*. 2022;8(12):eabj9949.
237. Sieber P, Schafer A, Lieberherr R, Le Goff F, Stritt M, Welford RWD, et al. Novel high-throughput myofibroblast assays identify agonists with therapeutic potential in pulmonary fibrosis that act via EP2 and EP4 receptors. *PLoS One*. 2018;13(11):e0207872.
238. Fortier SM, Penke LR, King D, Pham TX, Ligresti G, Peters-Golden M. Myofibroblast dedifferentiation proceeds via distinct transcriptomic and phenotypic transitions. *JCI Insight*. 2021;6(6).
239. Singh Patidar B, Meena A, Kumar M, Menon B, Rohil V, Kumar Bansal S. Adenosine Metabolism in COPD: A Study on Adenosine Levels, 5'-Nucleotidase, Adenosine Deaminase and Its Isoenzymes Activity in Serum, Lymphocytes and Erythrocytes. *COPD*. 2018;15(6):559–71.
240. Zhou Y, Murthy JN, Zeng D, Belardinelli L, Blackburn MR. Alterations in adenosine metabolism and signaling in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *PLoS One*. 2010;5(2):e9224.

241. Polosa R, Blackburn MR. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol Sci*. 2009;30(10):528–35.
242. Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, Mazzoni L. Effects of CGS 21680, a selective adenosine A2A receptor agonist, on allergic airways inflammation in the rat. *Eur J Pharmacol*. 2002;438(3):183–8.
243. Nadeem A, Fan M, Ansari HR, Ledent C, Jamal Mustafa S. Enhanced airway reactivity and inflammation in A2A adenosine receptor-deficient allergic mice. *Am J Physiol Lung Cell Mol Physiol*. 2007;292(6):L1335–44.
244. Mohsenin A, Mi T, Xia Y, Kellems RE, Chen JF, Blackburn MR. Genetic removal of the A2A adenosine receptor enhances pulmonary inflammation, mucin production, and angiogenesis in adenosine deaminase-deficient mice. *Am J Physiol Lung Cell Mol Physiol*. 2007;293(3):L753–61.
245. Varani K, Caramori G, Vincenzi F, Adcock I, Casolari P, Leung E, et al. Alteration of adenosine receptors in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2006;173(4):398–406.
246. Sun CX, Zhong H, Mohsenin A, Morschl E, Chunn JL, Molina JG, et al. Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. *J Clin Invest*. 2006;116(8):2173–82.
247. Roman J, Rivera HN, Roser-Page S, Sitaraman SV, Ritzenthaler JD. Adenosine induces fibronectin expression in lung epithelial cells: implications for airway remodeling. *Am J Physiol Lung Cell Mol Physiol*. 2006;290(2):L317–25.
248. Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS One*. 2007;2(5):e482.
249. Mustafa SJ, Nadeem A, Fan M, Zhong H, Belardinelli L, Zeng D. Effect of a specific and selective A(2B) adenosine receptor antagonist on adenosine agonist AMP and allergen-induced airway responsiveness and cellular influx in a mouse model of asthma. *J Pharmacol Exp Ther*. 2007;320(3):1246–51.
250. Karmouty-Quintana H, Weng T, Garcia-Morales LJ, Chen NY, Pedroza M, Zhong H, et al. Adenosine A2B receptor and hyaluronan modulate pulmonary hypertension associated with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol*. 2013;49(6):1038–47.
251. Crim C, Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, et al. Pneumonia risk in COPD patients receiving inhaled corticosteroids alone or in combination: TORCH study results. *Eur Respir J*. 2009;34(3):641–7.
252. Yawn BP, Li Y, Tian H, Zhang J, Arcona S, Kahler KH. Inhaled corticosteroid use in patients with chronic obstructive pulmonary disease and the risk of pneumonia: a retrospective claims data analysis. *Int J Chron Obstruct Pulmon Dis*. 2013;8:295–304.
253. Suissa S, Patenaude V, Lapi F, Ernst P. Inhaled corticosteroids in COPD and the risk of serious pneumonia. *Thorax*. 2013;68(11):1029–36.
254. Leigh R, Pizzichini MM, Morris MM, Maltais F, Hargreave FE, Pizzichini E. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. *Eur Respir J*. 2006;27(5):964–71.
255. Agusti A, Celli BR, Criner GJ, Halpin D, Anzueto A, Barnes P, et al. Global Initiative for Chronic Obstructive Lung Disease 2023 Report: GOLD Executive Summary. *Eur Respir J*. 2023;61(4).
256. Multiple. Farmacotherapeutisch kompas [Available from: www.farmacotherapeutischkompas.nl/.
257. Barnes PJ. Glucocorticosteroids: current and future directions. *Br J Pharmacol*. 2011;163(1):29–43.
258. Cazzola M, Calzetta L, Rogliani P, Puxeddu E, Facciolo F, Matera MG. Interaction between corticosteroids and muscarinic antagonists in human airways. *Pulm Pharmacol Ther*. 2016;36:1–9.
259. Vettorazzi S, Nalbantoglu D, Gebhardt JCM, Tuckermann J. A guide to changing paradigms of glucocorticoid receptor function-a model system for genome regulation and physiology. *FEBS J*. 2022;289(19):5718–43.
260. Nishi Y, Boswell V, Ansari T, Piprawala F, Satchi S, Page CP. Elastase-induced changes in lung function: relationship to morphometry and effect of drugs. *Pulm Pharmacol Ther*. 2003;16(4):221–9.

261. Dorscheid DR, Patchell BJ, Estrada O, Marroquin B, Tse R, White SR. Effects of corticosteroid-induced apoptosis on airway epithelial wound closure in vitro. *Am J Physiol Lung Cell Mol Physiol*. 2006;291(4):L794–801.

262. Matsumoto D, Toba H, Kenzaki K, Sakiyama S, Sakamoto S, Takashima M, et al. Lung regeneration with rat fetal lung implantation and promotion of alveolar stem cell differentiation by corticosteroids. *Regen Ther*. 2023;24:426–33.

263. Matsumoto K, Tajima H, Okazaki H, Nakamura T. Negative regulation of hepatocyte growth factor gene expression in human lung fibroblasts and leukemic cells by transforming growth factor-beta 1 and glucocorticoids. *J Biol Chem*. 1992;267(35):24917–20.

264. Singh D, Litewka D, Paramo R, Rendon A, Sayiner A, Tanni SE, et al. DElaying Disease Progression In COPD with Early Initiation of Dual Bronchodilator or Triple Inhaled PharmacoTherapy (DEPICT): A Predictive Modelling Approach. *Adv Ther*. 2023;40(10):4282–97.

265. Shaker SB, Dirksen A, Ulrik CS, Hestad M, Stavngaard T, Laursen LC, et al. The effect of inhaled corticosteroids on the development of emphysema in smokers assessed by annual computed tomography. *COPD*. 2009;6(2):104–11.

266. Kunz LIZ, Postma DS, Klooster K, Lapperre TS, Vonk JM, Sont JK, et al. Relapse in FEV1 Decline After Steroid Withdrawal in COPD. *Chest*. 2015;148(2):389–96.

267. Yun JH, Lamb A, Chase R, Singh D, Parker MM, Saferali A, et al. Blood eosinophil count thresholds and exacerbations in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2018;141(6):2037–47 e10.

268. Saito Z, Yoshida M, Kojima A, Tamura K, Hasegawa T, Kuwano K. Benefits and Risks of Inhaled Corticosteroid Treatment in Patients with Chronic Obstructive Pulmonary Disease Classified by Blood Eosinophil Counts. *Lung*. 2020;198(6):925–31.

269. Kunz LI, Strebus J, Budulac SE, Lapperre TS, Sterk PJ, Postma DS, et al. Inhaled steroids modulate extracellular matrix composition in bronchial biopsies of COPD patients: a randomized, controlled trial. *PLoS One*. 2013;8(5):e63430.

270. van den Berge M, Steiling K, Timens W, Hiemstra PS, Sterk PJ, Heijink IH, et al. Airway gene expression in COPD is dynamic with inhaled corticosteroid treatment and reflects biological pathways associated with disease activity. *Thorax*. 2014;69(1):14–23.

271. Wang Y, Ninaber DK, van Schadewijk A, Hiemstra PS. Tiotropium and Fluticasone Inhibit Rhinovirus-Induced Mucin Production via Multiple Mechanisms in Differentiated Airway Epithelial Cells. *Front Cell Infect Microbiol*. 2020;10:278.

272. Rogers DF. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr Opin Pharmacol*. 2004;4(3):241–50.

273. Ohnishi H, Tanimoto T, Inaba R, Eitoku M. Efficacy and safety of mucolytics in patients with stable chronic obstructive pulmonary disease: A systematic review and meta-analysis. *Respir Investig*. 2024;62(6):1168–75.

274. Ward JE, Tan X. Peroxisome proliferator activated receptor ligands as regulators of airway inflammation and remodelling in chronic lung disease. *PPAR Res*. 2007;2007:14983.

275. Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med*. 2002;53:409–35.

276. Scher JU, Pillinger MH. 15d-PGJ2: the anti-inflammatory prostaglandin? *Clin Immunol*. 2005;114(2):100–9.

277. Uhlig S. Who tidies up the lung? Listen to Cox-2 and Nrf2. *Am J Respir Crit Care Med*. 2005;171(11):1198–9.

278. Tseng CH. Pioglitazone and Risk of Chronic Obstructive Pulmonary Disease in Patients with Type 2 Diabetes Mellitus: A Retrospective Cohort Study. *Int J Chron Obstruct Pulmon Dis*. 2022;17:285–95.

279. Rinne ST, Liu CF, Feemster LC, Collins BF, Bryson CL, O'Riordan TG, et al. Thiazolidinediones are associated with a reduced risk of COPD exacerbations. *Int J Chron Obstruct Pulmon Dis*. 2015;10:1591–7.

280. Lea S, Plumb J, Metcalfe H, Spicer D, Woodman P, Fox JC, et al. The effect of peroxisome proliferator-activated receptor-gamma ligands on in vitro and in vivo models of COPD. *Eur Respir J*. 2014;43(2):409–20.

281. Lee IT, Yang CC, Yang CM. Harnessing peroxisome proliferator-activated receptor gamma agonists to induce Heme Oxygenase-1: a promising approach for pulmonary inflammatory disorders. *Cell Commun Signal*. 2024;22(1):125.

282. Li J, Dai A, Hu R, Zhu L, Tan S. Positive correlation between PPARgamma/PGC-1alpha and gamma-GCS in lungs of rats and patients with chronic obstructive pulmonary disease. *Acta Biochim Biophys Sin (Shanghai)*. 2010;42(9):603–14.

283. Ma SX, Xie GF, Fang P, Tang MM, Deng YP, Lu YJ, et al. Low 15d-PGJ2 status is associated with oxidative stress in chronic obstructive pulmonary disease patients. *Inflamm Res*. 2023;72(2):171–80.

284. Reddy AT, Lakshmi SP, Banno A, Reddy RC. Role of GPx3 in PPARgamma-induced protection against COPD-associated oxidative stress. *Free Radic Biol Med*. 2018;126:350–7.

285. He S, Tian R, Zhang X, Yao Q, Chen Q, Liu B, et al. PPARgamma inhibits small airway remodeling through mediating the polarization homeostasis of alveolar macrophages in COPD. *Clin Immunol*. 2023;250:109293.

286. Hou G, Yin Y, Han D, Wang QY, Kang J. Rosiglitazone attenuates the metalloprotease/anti-metalloprotease imbalance in emphysema induced by cigarette smoke: involvement of extracellular signal-regulated kinase and NFkappaB signaling. *Int J Chron Obstruct Pulmon Dis*. 2015;10:715–24.

287. Zhou XM, Hou G, Gu DX, Wang QY, Zhao L. Peroxisome proliferator-activated receptor-gamma in induced sputum is correlated with MMP-9/TIMP-1 imbalance and formation of emphysema in COPD patients. *J Thorac Dis*. 2017;9(10):3703–10.

288. Solleti SK, Simon DM, Srisuma S, Arikan MC, Bhattacharya S, Rangasamy T, et al. Airway epithelial cell PPARgamma modulates cigarette smoke-induced chemokine expression and emphysema susceptibility in mice. *Am J Physiol Lung Cell Mol Physiol*. 2015;309(3):L293–304.

289. Boo HJ, Park SJ, Noh M, Min HY, Jeong LS, Lee HY. LJ-529, a partial peroxisome proliferator-activated receptor gamma (PPARgamma) agonist and adenosine A(3) receptor agonist, ameliorates elastase-induced pulmonary emphysema in mice. *Arch Pharm Res*. 2020;43(5):540–52.

290. Liang J, Huang G, Liu X, Zhang X, Rabata A, Liu N, et al. Lipid Deficiency Contributes to Impaired Alveolar Progenitor Cell Function in Aging and Idiopathic Pulmonary Fibrosis. *Am J Respir Cell Mol Biol*. 2024;71(2):242–53.

291. Li Y, Wongsiriroj N, Blaner WS. The multifaceted nature of retinoid transport and metabolism. *Hepatobiliary Surg Nutr*. 2014;3(3):126–39.

292. Cunningham TJ, Duester G. Mechanisms of retinoic acid signalling and its roles in organ and limb development. *Nat Rev Mol Cell Biol*. 2015;16(2):110–23.

293. Shmarakov IO, Gusarova GA, Islam MN, Marhuenda-Munoz M, Bhattacharya J, Blaner WS. Retinoids stored locally in the lung are required to attenuate the severity of acute lung injury in male mice. *Nat Commun*. 2023;14(1):851.

294. Plantier L, Rochette-Egly C, Goven D, Boutten A, Bonay M, Leseche G, et al. Dysregulation of elastin expression by fibroblasts in pulmonary emphysema: role of cellular retinoic acid binding protein 2. *Thorax*. 2008;63(11):1012–7.

295. Liu B, Harvey CS, McGowan SE. Retinoic acid increases elastin in neonatal rat lung fibroblast cultures. *Am J Physiol*. 1993;265(5 Pt 1):L430–7.

296. Kaartinen L, Nettesheim P, Adler KB, Randell SH. Rat tracheal epithelial cell differentiation in vitro. *In Vitro Cell Dev Biol Anim*. 1993;29A(6):481–92.

297. Ng-Blichfeldt JP, Schrik A, Kortekaas RK, Noordhoek JA, Heijink IH, Hiemstra PS, et al. Retinoic acid signaling balances adult distal lung epithelial progenitor cell growth and differentiation. *EBioMedicine*. 2018;36:461–74.

298. Ng-Blichfeldt JP, Alcada J, Montero MA, Dean CH, Griesenbach U, Griffiths MJ, et al. Deficient retinoid-driven angiogenesis may contribute to failure of adult human lung regeneration in emphysema. *Thorax*. 2017;72(6):510–21.

299. Kunig AM, Balasubramaniam V, Markham NE, Morgan D, Montgomery G, Grover TR, et al. Recombinant human VEGF treatment enhances alveolarization after hyperoxic lung injury in neonatal rats. *Am J Physiol Lung Cell Mol Physiol*. 2005;289(4):L529–35.

300. Cho SJ, George CL, Snyder JM, Acarregui MJ. Retinoic acid and erythropoietin maintain alveolar development in mice treated with an angiogenesis inhibitor. *Am J Respir Cell Mol Biol*. 2005;33(6):622–8.

301. McGowan SE. The lipofibroblast: more than a lipid-storage depot. *Am J Physiol Lung Cell Mol Physiol*. 2019;316(5):L869–L71.
302. Massaro GD, Massaro D, Chambon P. Retinoic acid receptor-alpha regulates pulmonary alveolus formation in mice after, but not during, perinatal period. *Am J Physiol Lung Cell Mol Physiol*. 2003;284(2):L431–3.
303. Massaro GD, Massaro D, Chan WY, Clerch LB, Ghyselinck N, Chambon P, et al. Retinoic acid receptor-beta: an endogenous inhibitor of the perinatal formation of pulmonary alveoli. *Physiol Genomics*. 2000;4(1):51–7.
304. McGowan S, Jackson SK, Jenkins-Moore M, Dai HH, Chambon P, Snyder JM. Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers. *Am J Respir Cell Mol Biol*. 2000;23(2):162–7.
305. Checkley W, West KP, Jr., Wise RA, Baldwin MR, Wu L, LeClerq SC, et al. Maternal vitamin A supplementation and lung function in offspring. *N Engl J Med*. 2010;362(19):1784–94.
306. Portas L, Pereira M, Shaheen SO, Wyss AB, London SJ, Burney PGJ, et al. Lung Development Genes and Adult Lung Function. *Am J Respir Crit Care Med*. 2020;202(6):853–65.
307. Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet*. 2011;43(11):1082–90.
308. Mongey R, van der Plaat DA, Shaheen SO, Portas L, Potts J, Hind MD, et al. Effect of vitamin A on adult lung function: a triangulation of evidence approach. *Thorax*. 2025;80(4):236–44.
309. Zheng L, Yu X, Xia Z, Guo Y, Dai Y. The Associations Between Serum Vitamins and Carotenoids with Chronic Obstructive Pulmonary Disease: Results from the NHANES. *Int J Chron Obstruct Pulmon Dis*. 2023;18:2985–97.
310. Baybutt RC, Hu L, Molteni A. Vitamin A deficiency injures lung and liver parenchyma and impairs function of rat type II pneumocytes. *J Nutr*. 2000;130(5):1159–65.
311. Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat Med*. 1997;3(6):675–7.
312. Hind M, Maden M. Retinoic acid induces alveolar regeneration in the adult mouse lung. *Eur Respir J*. 2004;23(1):20–7.
313. Tepper J, Pfeiffer J, Aldrich M, Tumas D, Kern J, Hoffman E, et al. Can retinoic acid ameliorate the physiologic and morphologic effects of elastase instillation in the rat? *Chest*. 2000;117(5 Suppl 1):242S–4S.
314. Fujita M, Ye Q, Ouchi H, Nakashima N, Hamada N, Hagimoto N, et al. Retinoic acid fails to reverse emphysema in adult mouse models. *Thorax*. 2004;59(3):224–30.
315. Lucey EC, Goldstein RH, Breuer R, Rexer BN, Ong DE, Snider GL. Retinoic acid does not affect alveolar septation in adult FVB mice with elastase-induced emphysema. *Respiration*. 2003;70(2):200–5.
316. Maden M. Retinoids have differing efficacies on alveolar regeneration in a dexamethasone-treated mouse. *Am J Respir Cell Mol Biol*. 2006;35(2):260–7.
317. Stinchcombe SV, Maden M. Retinoic acid induced alveolar regeneration: critical differences in strain sensitivity. *Am J Respir Cell Mol Biol*. 2008;38(2):185–91.
318. Mao JT, Goldin JG, Dermand J, Ibrahim G, Brown MS, Emerick A, et al. A pilot study of all-trans-retinoic acid for the treatment of human emphysema. *Am J Respir Crit Care Med*. 2002;165(5):718–23.
319. Roth MD, Connett JE, D'Armiento JM, Foronjy RF, Friedman PJ, Goldin JG, et al. Feasibility of retinoids for the treatment of emphysema study. *Chest*. 2006;130(5):1334–45.
320. Gokey JJ, Snowball J, Green J, Waltamath M, Spinney JJ, Black KE, et al. Pretreatment of aged mice with retinoic acid supports alveolar regeneration via upregulation of reciprocal PDGFA signalling. *Thorax*. 2021;76(5):456–67.
321. Stolk J, Stockley RA, Stoel BC, Cooper BG, Piitulainen E, Seersholm N, et al. Randomised controlled trial for emphysema with a selective agonist of the gamma-type retinoic acid receptor. *Eur Respir J*. 2012;40(2):306–12.
322. Chisholm DR, Tomlinson CWE, Zhou GL, Holden C, Affleck V, Lamb R, et al. Fluorescent Retinoic Acid Analogues as Probes for Biochemical and Intracellular Characterization of Retinoid Signaling Pathways. *ACS Chem Biol*. 2019;14(3):369–77.

323. Chisholm DR, Whiting A. Design of synthetic retinoids. *Methods Enzymol*. 2020;637:453–91.
324. Roberts C. Regulating Retinoic Acid Availability during Development and Regeneration: The Role of the CYP26 Enzymes. *J Dev Biol*. 2020;8(1).
325. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. *Cell*. 2012;149(6):1192–205.
326. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell*. 2009;17(1):9–26.
327. Frank DB, Peng T, Zepp JA, Snitow M, Vincent TL, Penkala IJ, et al. Emergence of a Wave of Wnt Signaling that Regulates Lung Alveologenesis by Controlling Epithelial Self-Renewal and Differentiation. *Cell Rep*. 2016;17(9):2312–25.
328. Lustig B, Jerchow B, Sachs M, Weiler S, Pietsch T, Karsten U, et al. Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. *Mol Cell Biol*. 2002;22(4):1184–93.
329. Lehmann M, Hu Q, Hu Y, Hafner K, Costa R, van den Berg A, et al. Chronic WNT/beta-catenin signaling induces cellular senescence in lung epithelial cells. *Cell Signal*. 2020;70:109588.
330. Kneidinger N, Yildirim AO, Callegari J, Takenaka S, Stein MM, Dumitrascu R, et al. Activation of the WNT/beta-catenin pathway attenuates experimental emphysema. *Am J Respir Crit Care Med*. 2011;183(6):723–33.
331. Conlon TM, John-Schuster G, Heide D, Pfister D, Lehmann M, Hu Y, et al. Inhibition of LTbetaR signalling activates WNT-induced regeneration in lung. *Nature*. 2020;588(7836):151–6.
332. Hu Y, Ng-Blichfeldt JP, Ota C, Ciminieri C, Ren W, Hiemstra PS, et al. Wnt/beta-catenin signaling is critical for regenerative potential of distal lung epithelial progenitor cells in homeostasis and emphysema. *Stem Cells*. 2020;38(11):1467–78.
333. Verhees KJ, Pansters NA, Baarsma HA, Remels AH, Haegens A, de Theije CC, et al. Pharmacological inhibition of GSK-3 in a guinea pig model of LPS-induced pulmonary inflammation: II. Effects on skeletal muscle atrophy. *Respir Res*. 2013;14(1):117.
334. Baarsma HA, Bos S, Meurs H, Visser KH, Smit M, Schols AM, et al. Pharmacological inhibition of GSK-3 in a guinea pig model of LPS-induced pulmonary inflammation: I. Effects on lung remodeling and pathology. *Respir Res*. 2013;14(1):113.
335. Bao Z, Lim S, Liao W, Lin Y, Thiemermann C, Leung BP, et al. Glycogen synthase kinase-3beta inhibition attenuates asthma in mice. *Am J Respir Crit Care Med*. 2007;176(5):431–8.
336. Baarsma HA, Spanjer AI, Haitsma G, Engelbertink LH, Meurs H, Jonker MR, et al. Activation of WNT/beta-catenin signaling in pulmonary fibroblasts by TGF-beta(1) is increased in chronic obstructive pulmonary disease. *PLoS One*. 2011;6(9):e25450.
337. Zou W, Ye D, Liu S, Hu J, Zhu T, He F, et al. GSK-3beta Inhibitors Attenuate the PM2.5-Induced Inflammatory Response in Bronchial Epithelial Cells. *Int J Chron Obstruct Pulmon Dis*. 2021;16:2845–56.
338. Fernandes R, Barbosa-Matos C, Borges-Pereira C, Carvalho A, Costa S. Glycogen Synthase Kinase-3 Inhibition by CHIR99021 Promotes Alveolar Epithelial Cell Proliferation and Lung Regeneration in the Lipopolysaccharide-Induced Acute Lung Injury Mouse Model. *Int J Mol Sci*. 2024;25(2).
339. de Carvalho A, Strikoudis A, Liu HY, Chen YW, Dantas TJ, Vallee RB, et al. Glycogen synthase kinase 3 induces multilineage maturation of human pluripotent stem cell-derived lung progenitors in 3D culture. *Development*. 2019;146(2).
340. Arciniegas Ruiz SM, Eldar-Finkelman H. Glycogen Synthase Kinase-3 Inhibitors: Preclinical and Clinical Focus on CNS-A Decade Onward. *Front Mol Neurosci*. 2021;14:792364.
341. Costa R, Wagner DE, Doryab A, De Santis MM, Schorpp K, Rothenaigner I, et al. A drug screen with approved compounds identifies amlexanox as a novel Wnt/beta-catenin activator inducing lung epithelial organoid formation. *Br J Pharmacol*. 2021;178(19):4026–41.
342. Kumawat K, Gosens R. WNT-5A: signaling and functions in health and disease. *Cell Mol Life Sci*. 2016;73(3):567–87.
343. Baarsma HA, Skronska-Wasek W, Mutze K, Ciolek F, Wagner DE, John-Schuster G, et al. Noncanonical WNT-5A signaling impairs endogenous lung repair in COPD. *J Exp Med*. 2017;214(1):143–63.

344. Trinh-Minh T, Chen CW, Tran Manh C, Li YN, Zhu H, Zhou X, et al. Noncanonical WNT5A controls the activation of latent TGF-beta to drive fibroblast activation and tissue fibrosis. *J Clin Invest*. 2024;134(10).
345. Patel M, Post Y, Hill N, Sura A, Ye J, Fisher T, et al. A WNT mimetic with broad spectrum FZD-specificity decreases fibrosis and improves function in a pulmonary damage model. *Respir Res*. 2024;25(1):153.
346. Nabhan AN, Webster JD, Adams JJ, Blazer L, Everrett C, Eidenschenk C, et al. Targeted alveolar regeneration with Frizzled-specific agonists. *Cell*. 2023;186(14):2995–3012 e15.
347. Torii K, Nishizawa K, Kawasaki A, Yamashita Y, Katada M, Ito M, et al. Anti-apoptotic action of Wnt5a in dermal fibroblasts is mediated by the PKA signaling pathways. *Cell Signal*. 2008;20(7):1256–66.
348. Ho C, Lee PH, Hsu YC, Wang FS, Huang YT, Lin CL. Sustained Wnt/beta-catenin signaling rescues high glucose induction of transforming growth factor-beta1-mediated renal fibrosis. *Am J Med Sci*. 2012;344(5):374–82.
349. Nishiguchi G, Caine EA, McGowan K, Shi Z, Aggarwal A, Mayasundari A, et al. Structure-Activity Relationship of Potent, Selective, and Orally Bioavailable Molecular Glue Degraders of CK1alpha. *ACS Med Chem Lett*. 2024;15(10):1692–8.
350. Nemec V, Khirsariya P, Janovska P, Moyano PM, Maier L, Prochazkova P, et al. Discovery of Potent and Exquisitely Selective Inhibitors of Kinase CK1 with Tunable Isoform Selectivity. *Angew Chem Int Ed Engl*. 2023;62(11):e202217532.
351. Jiang S, Zhang M, Sun J, Yang X. Casein kinase 1alpha: biological mechanisms and theranostic potential. *Cell Commun Signal*. 2018;16(1):23.
352. Bidere N, Ngo VN, Lee J, Collins C, Zheng L, Wan F, et al. Casein kinase 1alpha governs antigen-receptor-induced NF-kappaB activation and human lymphoma cell survival. *Nature*. 2009;458(7234):92–6.
353. Kim HY, Yoon JY, Yun JH, Cho KW, Lee SH, Rhee YM, et al. CXXC5 is a negative-feedback regulator of the Wnt/beta-catenin pathway involved in osteoblast differentiation. *Cell Death Differ*. 2015;22(6):912–20.
354. Kim HY, Choi S, Yoon JH, Lim HJ, Lee H, Choi J, et al. Small molecule inhibitors of the Dishevelled-CXXC5 interaction are new drug candidates for bone anabolic osteoporosis therapy. *EMBO Mol Med*. 2016;8(4):375–87.
355. Foronjy R, Imai K, Shiomi T, Mercer B, Sklepkiewicz P, Thankachen J, et al. The divergent roles of secreted frizzled related protein-1 (SFRP1) in lung morphogenesis and emphysema. *Am J Pathol*. 2010;177(2):598–607.
356. Wang R, Ahmed J, Wang G, Hassan I, Strulovici-Barel Y, Hackett NR, et al. Down-regulation of the canonical Wnt beta-catenin pathway in the airway epithelium of healthy smokers and smokers with COPD. *PLoS One*. 2011;6(4):e14793.
357. Elzi DJ, Song M, Hakala K, Weintraub ST, Shiio Y. Wnt antagonist SFRP1 functions as a secreted mediator of senescence. *Mol Cell Biol*. 2012;32(21):4388–99.
358. Bodine PV, Stauffer B, Ponce-de-Leon H, Bhat RA, Mangine A, Seestaller-Wehr LM, et al. A small molecule inhibitor of the Wnt antagonist secreted frizzled-related protein-1 stimulates bone formation. *Bone*. 2009;44(6):1063–8.
359. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature*. 2011;476(7360):293–7.
360. Bell SM, Schreiner CM, Wert SE, Mucenski ML, Scott WJ, Whitsett JA. R-spondin 2 is required for normal laryngeal-tracheal, lung and limb morphogenesis. *Development*. 2008;135(6):1049–58.
361. Raslan AA, Oh YJ, Jin YR, Yoon JK. R-Spondin2, a Positive Canonical WNT Signaling Regulator, Controls the Expansion and Differentiation of Distal Lung Epithelial Stem/Progenitor Cells in Mice. *Int J Mol Sci*. 2022;23(6).
362. Qiu D, Xu K, Chung N, Robbins J, Luo R, Lawrence M, et al. Identification and validation of G protein-coupled receptors modulating flow-dependent signaling pathways in vascular endothelial cells. *Front Mol Biosci*. 2023;10:1198079.

363. Stolz D, Mkorombindo T, Schumann DM, Agusti A, Ash SY, Bafadhel M, et al. Towards the elimination of chronic obstructive pulmonary disease: a Lancet Commission. *Lancet*. 2022;400(10356):921–72.
364. Bowdish DME. The Aging Lung: Is Lung Health Good Health for Older Adults? *Chest*. 2019;155(2):391–400.
365. Kirkeleit J, Riise T, Wielscher M, Accordini S, Carsin AE, Dratva J, et al. Early life exposures contributing to accelerated lung function decline in adulthood - a follow-up study of 11,000 adults from the general population. *EClinicalMedicine*. 2023;66:102339.
366. Hopkinson NS, Bush A, Allinson JP, Faner R, Zar HJ, Agusti A. Early Life Exposures and the Development of Chronic Obstructive Pulmonary Disease across the Life Course. *Am J Respir Crit Care Med*. 2024;210(5):572–80.
367. Kroemer G, Maier AB, Cuervo AM, Gladyshev VN, Ferrucci L, Gorbunova V, et al. From geroscience to precision geromedicine: Understanding and managing aging. *Cell*. 2025;188(8):2043–62.
368. Meiners S, Eickelberg O, Konigshoff M. Hallmarks of the ageing lung. *Eur Respir J*. 2015;45(3):807–27.
369. Cottage CT, Peterson N, Kearley J, Berlin A, Xiong X, Huntley A, et al. Targeting p16-induced senescence prevents cigarette smoke-induced emphysema by promoting IGF1/Akt1 signaling in mice. *Commun Biol*. 2019;2:307.
370. Baker JR, Vuppusetty C, Colley T, Hassibi S, Fenwick PS, Donnelly LE, et al. MicroRNA-570 is a novel regulator of cellular senescence and inflammaging. *FASEB J*. 2019;33(2):1605–16.
371. Houssaini A, Breau M, Kebe K, Abid S, Marcos E, Lipskaia L, et al. mTOR pathway activation drives lung cell senescence and emphysema. *JCI Insight*. 2018;3(3).
372. Beaulieu D, Attwe A, Breau M, Lipskaia L, Marcos E, Born E, et al. Phospholipase A2 receptor 1 promotes lung cell senescence and emphysema in obstructive lung disease. *Eur Respir J*. 2021;58(2).
373. Noureddine H, Gary-Bobo G, Alifano M, Marcos E, Saker M, Vienney N, et al. Pulmonary artery smooth muscle cell senescence is a pathogenic mechanism for pulmonary hypertension in chronic lung disease. *Circ Res*. 2011;109(5):543–53.
374. Barnes PJ, Baker J, Donnelly LE. Cellular Senescence as a Mechanism and Target in Chronic Lung Diseases. *Am J Respir Crit Care Med*. 2019;200(5):556–64.
375. Baker JR, Donnelly LE, Barnes PJ. Senotherapy: A New Horizon for COPD Therapy. *Chest*. 2020;158(2):562–70.
376. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature*. 2011;479(7372):232–6.
377. Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell*. 2015;14(4):644–58.
378. Nambiar A, Kellogg D, 3rd, Justice J, Goros M, Gelfond J, Pascual R, et al. Senolytics dasatinib and quercetin in idiopathic pulmonary fibrosis: results of a phase I, single-blind, single-center, randomized, placebo-controlled pilot trial on feasibility and tolerability. *EBioMedicine*. 2023;90:104481.
379. Justice JN, Nambiar AM, Tchkonia T, LeBrasseur NK, Pascual R, Hashmi SK, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. *EBioMedicine*. 2019;40:554–63.
380. Ganesan S, Faris AN, Comstock AT, Chattoraj SS, Chattoraj A, Burgess JR, et al. Quercetin prevents progression of disease in elastase/LPS-exposed mice by negatively regulating MMP expression. *Respir Res*. 2010;11(1):131.
381. Baker JR, Daly L, Hassibi S, Kimura G, Nishimoto Y, Kizawa Y, et al. Senolytic therapy reduces inflammation in epithelial cells from COPD patients and in smoke-exposure mice. *Front Med (Lausanne)*. 2025;12:1451056.
382. Han MK, Barreto TA, Martinez FJ, Comstock AT, Sajjan US. Randomised clinical trial to determine the safety of quercetin supplementation in patients with chronic obstructive pulmonary disease. *BMJ Open Respir Res*. 2020;7(1).

383. Woldhuis RR, Van Den Berge M, Kooistra W, Timens W, Brandsma C-A. Targeting lung fibroblast senescence to restore ECM regulation in COPD. *European Respiratory Journal*.62(suppl 67):PA2372.
384. Guo Y, Fenwick P, Viola P, Al-Sahaf M, J. Barnes P, E. Donnelly L. Navitoclax inhibits senescence in human alveolar type 2 cells from COPD patients. *European Respiratory Journal*.64(suppl 68):PA897.
385. Guerrero A, Herranz N, Sun B, Wagner V, Gallage S, Guiho R, et al. Cardiac glycosides are broad-spectrum senolytics. *Nat Metab*. 2019;1(11):1074–88.
386. Triana-Martinez F, Picallos-Rabina P, Da Silva-Alvarez S, Pietrocola F, Llanos S, Rodilla V, et al. Identification and characterization of Cardiac Glycosides as senolytic compounds. *Nat Commun*. 2019;10(1):4731.
387. Crespo-Garcia S, Fournier F, Diaz-Marin R, Klier S, Ragusa D, Masaki L, et al. Therapeutic targeting of cellular senescence in diabetic macular edema: preclinical and phase 1 trial results. *Nat Med*. 2024;30(2):443–54.
388. Devulder J, Fenwick P, Al Sahaf M, Viola P, Razdan N, Lemaire R, et al. Effect of the novel senolytic drug, BCL-xL PROTAC, on small airway epithelial cells in COPD. *European Respiratory Journal*.64(suppl 68):PA4429.
389. Lee S, Ro H, In HJ, Choi JH, Kim MO, Lee J, et al. Fisetin inhibits TNF-alpha/NF-kappaB-induced IL-8 expression by targeting PKCdelta in human airway epithelial cells. *Cytokine*. 2018;108:247–54.
390. Devulder J, Fenwick P, Al Sahaf M, Viola P, Donnelly L, Barnes P. Effect of the putative senotherapy, Fisetin on small airway epithelial cells in COPD. *European Respiratory Journal*.64(suppl 68):PA4423.
391. Guerin DJ, Kha CX, Tseng KA. From Cell Death to Regeneration: Rebuilding After Injury. *Front Cell Dev Biol*. 2021;9:655048.
392. Hu Y, Hu Q, Ansari M, Riemondy K, Pineda R, Sembrat J, et al. Airway-derived emphysema-specific alveolar type II cells exhibit impaired regenerative potential in COPD. *Eur Respir J*. 2024;64(6).
393. Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respir Res*. 2006;7(1):53.
394. Cai Y, Zhou H, Zhu Y, Sun Q, Ji Y, Xue A, et al. Elimination of senescent cells by beta-galactosidase-targeted prodrug attenuates inflammation and restores physical function in aged mice. *Cell Res*. 2020;30(7):574–89.
395. Guerrero A, Guiho R, Herranz N, Uren A, Withers DJ, Martinez-Barbera JP, et al. Galactose-modified duocarmycin prodrugs as senolytics. *Aging Cell*. 2020;19(4):e13133.
396. Maus M, Lopez-Polo V, Mateo L, Lafarga M, Aguilera M, De Lama E, et al. Iron accumulation drives fibrosis, senescence and the senescence-associated secretory phenotype. *Nat Metab*. 2023;5(12):2111–30.
397. Admasu TD, Kim K, Rae M, Avelar R, Gonciarz RL, Rebbaa A, et al. Selective ablation of primary and paracrine senescent cells by targeting iron dyshomeostasis. *Cell Rep*. 2023;42(2):112058.
398. Cloonan SM, Glass K, Laucho-Contreras ME, Bhashyam AR, Cervo M, Pabon MA, et al. Mitochondrial iron chelation ameliorates cigarette smoke-induced bronchitis and emphysema in mice. *Nat Med*. 2016;22(2):163–74.
399. Baar MP, Brandt RMC, Putavet DA, Klein JDD, Derks KWJ, Bourgeois BRM, et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell*. 2017;169(1):132–47 e16.
400. Han Y, Wu Y, He B, Wu D, Hua J, Qian H, et al. DNA nanoparticles targeting FOXO4 selectively eliminate cigarette smoke-induced senescent lung fibroblasts. *Nanoscale Adv*. 2023;5(21):5965–73.
401. Victorelli S, Salmonowicz H, Chapman J, Martini H, Vizioli MG, Riley JS, et al. Apoptotic stress causes mtDNA release during senescence and drives the SASP. *Nature*. 2023;622(7983):627–36.
402. Hassibi S, Baker J, Daly L, Yamamoto Y, Kyle R, Ito K. P207 Senolytic effects of Telaglenastat, a glutaminase inhibitor, on senescent airway epithelial cells. *Thorax*. 2023;78(Suppl 4):A235–A6.

403. Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, et al. Senolytic CAR T cells reverse senescence-associated pathologies. *Nature*. 2020;583(7814):127–32.
404. Yang D, Sun B, Li S, Wei W, Liu X, Cui X, et al. NKG2D-CAR T cells eliminate senescent cells in aged mice and nonhuman primates. *Sci Transl Med*. 2023;15(709):eadd1951.
405. Yoshida S, Nakagami H, Hayashi H, Ikeda Y, Sun J, Tenma A, et al. The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. *Nat Commun*. 2020;11(1):2482.
406. McHugh D, Gil J. Senescence and aging: Causes, consequences, and therapeutic avenues. *J Cell Biol*. 2018;217(1):65–77.
407. Reyes NS, Krasilnikov M, Allen NC, Lee JY, Hyams B, Zhou M, et al. Sentinel p16(INK4a+) cells in the basement membrane form a reparative niche in the lung. *Science*. 2022;378(6616):192–201.
408. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. *Cell*. 2023;186(2):243–78.
409. Polverino F, Wu TD, Rojas-Quintero J, Wang X, Mayo J, Tomchaney M, et al. Metformin: Experimental and Clinical Evidence for a Potential Role in Emphysema Treatment. *Am J Respir Crit Care Med*. 2021;204(6):651–66.
410. Park CS, Bang BR, Kwon HS, Moon KA, Kim TB, Lee KY, et al. Metformin reduces airway inflammation and remodeling via activation of AMP-activated protein kinase. *Biochem Pharmacol*. 2012;84(12):1660–70.
411. Kahnert K, Andreas S, Kellerer C, Lutter JI, Lucke T, Yildirim O, et al. Reduced decline of lung diffusing capacity in COPD patients with diabetes and metformin treatment. *Sci Rep*. 2022;12(1):1435.
412. Ray A, Paik JM, Wexler DJ, Sreedhara SK, Bykov K, Feldman WB, et al. Glucose-Lowering Medications and Risk of Chronic Obstructive Pulmonary Disease Exacerbations in Patients With Type 2 Diabetes. *JAMA Intern Med*. 2025;185(4):399–410.
413. Xu M, Tchkonia T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci U S A*. 2015;112(46):E6301–10.
414. Cheresh P, Kim SJ, Jablonski R, Watanabe S, Lu Z, Chi M, et al. SIRT3 Overexpression Ameliorates Asbestos-Induced Pulmonary Fibrosis, mt-DNA Damage, and Lung Fibrogenic Monocyte Recruitment. *Int J Mol Sci*. 2021;22(13).
415. Yao H, Chung S, Hwang JW, Rajendrasozhan S, Sundar IK, Dean DA, et al. SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice. *J Clin Invest*. 2012;122(6):2032–45.
416. Baker JR, Vuppusetty C, Colley T, Papaioannou AI, Fenwick P, Donnelly L, et al. Oxidative stress dependent microRNA-34a activation via PI3Kalpha reduces the expression of sirtuin-1 and sirtuin-6 in epithelial cells. *Sci Rep*. 2016;6:35871.
417. Pyo IS, Yun S, Yoon YE, Choi JW, Lee SJ. Mechanisms of Aging and the Preventive Effects of Resveratrol on Age-Related Diseases. *Molecules*. 2020;25(20).
418. Norheim KL, Ben Ezra M, Heckenbach I, Andreasson LM, Eriksen LL, Dyhre-Petersen N, et al. Effect of nicotinamide riboside on airway inflammation in COPD: a randomized, placebo-controlled trial. *Nat Aging*. 2024;4(12):1772–81.
419. Zinovkin RA, Zamyatnin AA. Mitochondria-Targeted Drugs. *Curr Mol Pharmacol*. 2019;12(3):202–14.
420. Bodas M, Vij N. Augmenting autophagy for prognosis based intervention of COPD-pathophysiology. *Respir Res*. 2017;18(1):83.
421. Wiegman CH, Michaeloudes C, Haji G, Narang P, Clarke CJ, Russell KE, et al. Oxidative stress-induced mitochondrial dysfunction drives inflammation and airway smooth muscle remodeling in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2015;136(3):769–80.
422. Ng-Blichfeldt JP, de Jong T, Kortekaas RK, Wu X, Lindner M, Guryev V, et al. TGF-beta activation impairs fibroblast ability to support adult lung epithelial progenitor cell organoid formation. *Am J Physiol Lung Cell Mol Physiol*. 2019;317(1):L14–L28.
423. McQualter JL, McCarty RC, Van der Velden J, O'Donoghue RJ, Asselin-Labat ML, Bozinovski S, et al. TGF-beta signaling in stromal cells acts upstream of FGF-10 to regulate epithelial stem cell growth in the adult lung. *Stem Cell Res*. 2013;11(3):1222–33.

424. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, et al. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med*. 2001;163(6):1476–83.
425. Wu X, Verschut V, Woest ME, Ng-Blichfeldt JP, Matias A, Villetti G, et al. Rho-Kinase 1/2 Inhibition Prevents Transforming Growth Factor-beta-Induced Effects on Pulmonary Remodeling and Repair. *Front Pharmacol*. 2020;11:609509.
426. Wang DZ, Li S, Hockemeyer D, Sutherland L, Wang Z, Schratt G, et al. Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc Natl Acad Sci U S A*. 2002;99(23):14855–60.
427. Koopmans T, Kumawat K, Halayko AJ, Gosens R. Regulation of actin dynamics by WNT-5A: implications for human airway smooth muscle contraction. *Sci Rep*. 2016;6:30676.
428. Kumawat K, Koopmans T, Menzen MH, Prins A, Smit M, Halayko AJ, et al. Cooperative signaling by TGF-beta1 and WNT-11 drives sm-alpha-actin expression in smooth muscle via Rho kinase-actin-MRTF-A signaling. *Am J Physiol Lung Cell Mol Physiol*. 2016;311(3):L529–37.
429. Schaafsma D, Gosens R, Zaagsma J, Halayko AJ, Meurs H. Rho kinase inhibitors: a novel therapeutical intervention in asthma? *Eur J Pharmacol*. 2008;585(2-3):398–406.
430. Mitsuboshi S, Homma J, Sekine H, Takagi R, Shimizu T, Kanzaki M. A novel alveolar epithelial cell sheet fabricated under feeder-free conditions for potential use in pulmonary regenerative therapy. *Regen Ther*. 2022;19:113–21.
431. Schweitzer KS, Hatoum H, Brown MB, Gupta M, Justice MJ, Beteck B, et al. Mechanisms of lung endothelial barrier disruption induced by cigarette smoke: role of oxidative stress and ceramides. *Am J Physiol Lung Cell Mol Physiol*. 2011;301(6):L836–46.
432. McKelvey MC, Brown R, Ryan S, Mall MA, Weldon S, Taggart CC. Proteases, Mucus, and Mucosal Immunity in Chronic Lung Disease. *Int J Mol Sci*. 2021;22(9).
433. Abboud RT, Vimalanathan S. Pathogenesis of COPD. Part I. The role of protease-antiprotease imbalance in emphysema. *Int J Tuberc Lung Dis*. 2008;12(4):361–7.
434. Barnes PJ. Cellular and molecular mechanisms of asthma and COPD. *Clin Sci (Lond)*. 2017;131(13):1541–58.
435. Turner AM, Ficker JH, Vianello A, Clarenbach CF, Janciauskiene S, Chorostowska-Wynimko J, et al. Advancing the understanding and treatment of lung pathologies associated with alpha 1 antitrypsin deficiency. *Ther Adv Respir Dis*. 2025;19:17534666251318841.
436. Hubbard RC, Crystal RG. Alpha-1-antitrypsin augmentation therapy for alpha-1-antitrypsin deficiency. *Am J Med*. 1988;84(6A):52–62.
437. Gadek JE, Klein HG, Holland PV, Crystal RG. Replacement therapy of alpha 1-antitrypsin deficiency. Reversal of protease-antiprotease imbalance within the alveolar structures of PiZ subjects. *J Clin Invest*. 1981;68(5):1158–65.
438. Bianchera A, Alomari E, Bruno S. Augmentation Therapy with Alpha-1 Antitrypsin: Present and Future of Production, Formulation, and Delivery. *Curr Med Chem*. 2022;29(3):385–410.
439. Dirksen A, Dijkman JH, Madsen F, Stoel B, Hutchison DC, Ulrik CS, et al. A randomized clinical trial of alpha(1)-antitrypsin augmentation therapy. *Am J Respir Crit Care Med*. 1999;160(5 Pt 1):1468–72.
440. Dirksen A, Piitulainen E, Parr DG, Deng C, Wencker M, Shaker SB, et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. *Eur Respir J*. 2009;33(6):1345–53.
441. Chapman KR, Burdon JG, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, et al. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;386(9991):360–8.
442. Miravitlles M, Dirksen A, Ferrarotti I, Koblizek V, Lange P, Mahadeva R, et al. European Respiratory Society statement: diagnosis and treatment of pulmonary disease in alpha(1)-antitrypsin deficiency. *Eur Respir J*. 2017;50(5).
443. Pires Ferreira D, Gruntman AM, Flotte TR. Gene therapy for alpha-1 antitrypsin deficiency: an update. *Expert Opin Biol Ther*. 2023;23(3):283–91.
444. Brantly ML, Kuhn BT, Farah HW, Mahadeva R, Cole A, Chang CL, et al. Recombinant Alpha-1 Antitrypsin-Fc Fusion Protein INBRX-101 in Adults With Alpha-1 Antitrypsin Deficiency: A Phase 1 Study. *Chronic Obstr Pulm Dis*. 2024;11(3):282–92.

445. Brigham KL, Lane KB, Meyrick B, Stecenko AA, Strack S, Cannon DR, et al. Transfection of nasal mucosa with a normal alpha1-antitrypsin gene in alpha1-antitrypsin-deficient subjects: comparison with protein therapy. *Hum Gene Ther*. 2000;11(7):1023–32.
446. Yadav AK, Gu W, Zhang T, Xu X, Yu L. Current Perspectives on Biological Therapy for COPD. *COPD*. 2023;20(1):197–209.
447. Manicone AM, Gharib SA, Gong KQ, Eddy WE, Long ME, Frevert CW, et al. Matrix Metalloproteinase-28 Is a Key Contributor to Emphysema Pathogenesis. *Am J Pathol*. 2017;187(6):1288–300.
448. D'Armiento J, Dalal SS, Okada Y, Berg RA, Chada K. Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell*. 1992;71(6):955–61.
449. Gosselink JV, Hayashi S, Elliott WM, Xing L, Chan B, Yang L, et al. Differential expression of tissue repair genes in the pathogenesis of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2010;181(12):1329–35.
450. Navratilova Z, Zatloukal J, Kriegova E, Kolek V, Petrek M. Simultaneous up-regulation of matrix metalloproteinases 1, 2, 3, 7, 8, 9 and tissue inhibitors of metalloproteinases 1, 4 in serum of patients with chronic obstructive pulmonary disease. *Respirology*. 2012;17(6):1006–12.
451. Molet S, Belleguic C, Lena H, Germain N, Bertrand CP, Shapiro SD, et al. Increase in macrophage elastase (MMP-12) in lungs from patients with chronic obstructive pulmonary disease. *Inflamm Res*. 2005;54(1):31–6.
452. Churg A, Wang R, Wang X, Onnervik PO, Thim K, Wright JL. Effect of an MMP-9/MMP-12 inhibitor on smoke-induced emphysema and airway remodelling in guinea pigs. *Thorax*. 2007;62(8):706–13.
453. Dahl R, Titlestad I, Lindqvist A, Wielders P, Wray H, Wang M, et al. Effects of an oral MMP-9 and -12 inhibitor, AZD1236, on biomarkers in moderate/severe COPD: a randomised controlled trial. *Pulm Pharmacol Ther*. 2012;25(2):169–77.
454. Parasaram V, Wang X, Krisanarungson P, Vyavahare N. Targeted delivery of pentagalloyl glucose inhibits matrix metalloproteinase activity and preserves elastin in emphysematous lungs. *Respir Res*. 2021;22(1):249.
455. Nakajima T, Nakamura H, Owen CA, Yoshida S, Tsuduki K, Chubachi S, et al. Plasma Cathepsin S and Cathepsin S/Cystatin C Ratios Are Potential Biomarkers for COPD. *Dis Markers*. 2016;2016:4093870.
456. Zhang X, Shan P, Homer R, Zhang Y, Petrache I, Mannam P, et al. Cathepsin E promotes pulmonary emphysema via mitochondrial fission. *Am J Pathol*. 2014;184(10):2730–41.
457. Chalmers JD, Shteinberg M, Mall MA, O'Donnell AE, Watz H, Gupta A, et al. Cathepsin C (dipeptidyl peptidase 1) inhibition in adults with bronchiectasis: AIRLEAF, a phase II randomised, double-blind, placebo-controlled, dose-finding study. *Eur Respir J*. 2025;65(1).
458. Chen X, Yan Y, Zhang Z, Zhang F, Liu M, Du L, et al. Discovery and In Vivo Anti-inflammatory Activity Evaluation of a Novel Non-peptidyl Non-covalent Cathepsin C Inhibitor. *J Med Chem*. 2021;64(16):11857–85.
459. McDonald PP, Leifer FG, Basso J, Lasala D, Li D, Chen KJ, et al. Brensocatib (an oral, reversible inhibitor of dipeptidyl peptidase-1) attenuates disease progression in two animal models of rheumatoid arthritis. *Front Immunol*. 2023;14:1231047.
460. Cipolla D, Zhang J, Korkmaz B, Chalmers JD, Basso J, Lasala D, et al. Dipeptidyl peptidase-1 inhibition with brensocatib reduces the activity of all major neutrophil serine proteases in patients with bronchiectasis: results from the WILLOW trial. *Respir Res*. 2023;24(1):133.
461. Fujie K, Shinguh Y, Yamazaki A, Hatanaka H, Okamoto M, Okuhara M. Inhibition of elastase-induced acute inflammation and pulmonary emphysema in hamsters by a novel neutrophil elastase inhibitor FR901277. *Inflamm Res*. 1999;48(3):160–7.
462. Feng L, Liu X, Zhu W, Guo F, Wu Y, Wang R, et al. Inhibition of human neutrophil elastase by pentacyclic triterpenes. *PLoS One*. 2013;8(12):e82794.
463. Watz H, Nagelschmitz J, Kirsten A, Pedersen F, van der Mey D, Schwers S, et al. Safety and efficacy of the human neutrophil elastase inhibitor BAY 85-8501 for the treatment of non-cystic fibrosis bronchiectasis: A randomized controlled trial. *Pulm Pharmacol Ther*. 2019;56:86–93.
464. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350(26):2645–53.

465. Bracke KR, Verhamme FM, Seys LJ, Bantsimba-Malanda C, Cunoosamy DM, Herbst R, et al. Role of CXCL13 in cigarette smoke-induced lymphoid follicle formation and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2013;188(3):343–55.

466. Seys LJ, Verhamme FM, Schinwald A, Hammad H, Cunoosamy DM, Bantsimba-Malanda C, et al. Role of B Cell-Activating Factor in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2015;192(6):706–18.

467. van der Strate BW, Postma DS, Brandsma CA, Melgert BN, Luinge MA, Geerlings M, et al. Cigarette smoke-induced emphysema: A role for the B cell? *Am J Respir Crit Care Med*. 2006;173(7):751–8.

468. Polverino F, Cosio BG, Pons J, Laucho-Contreras M, Tejera P, Iglesias A, et al. B Cell-Activating Factor. An Orchestrator of Lymphoid Follicles in Severe Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2015;192(6):695–705.

469. Sullivan JL, Bagevalu B, Glass C, Sholl L, Kraft M, Martinez FD, et al. B Cell-Adaptive Immune Profile in Emphysema-Predominant Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2019;200(11):1434–9.

470. John-Schuster G, Hager K, Conlon TM, Irmler M, Beckers J, Eickelberg O, et al. Cigarette smoke-induced iBALT mediates macrophage activation in a B cell-dependent manner in COPD. *Am J Physiol Lung Cell Mol Physiol*. 2014;307(9):L692–706.

471. Jia J, Conlon TM, Sarker RS, Tasdemir D, Smirnova NF, Srivastava B, et al. Cholesterol metabolism promotes B-cell positioning during immune pathogenesis of chronic obstructive pulmonary disease. *EMBO Mol Med*. 2018;10(5).

472. Faner R, Cruz T, Casserras T, Lopez-Giraldo A, Noell G, Coca I, et al. Network Analysis of Lung Transcriptomics Reveals a Distinct B-Cell Signature in Emphysema. *Am J Respir Crit Care Med*. 2016;193(11):1242–53.

473. Suzuki M, Sze MA, Campbell JD, Brothers JF, 2nd, Lenburg ME, McDonough JE, et al. The cellular and molecular determinants of emphysematous destruction in COPD. *Sci Rep*. 2017;7(1):9562.

474. Rojas-Quintero J, Ochsner SA, Lee HS, Cong C, Waich Cohen A, Colborg AS, et al. Skewed adaptive immune responses are involved in alpha-1 antitrypsin deficiency emphysema. *Eur Respir J*. 2025;65(1).

475. Kratz A, Campos-Neto A, Hanson MS, Ruddle NH. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. *J Exp Med*. 1996;183(4):1461–72.

476. Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. *Nat Immunol*. 2006;7(4):344–53.

477. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*. 2001;104(4):487–501.

478. Mebius RE. Organogenesis of lymphoid tissues. *Nat Rev Immunol*. 2003;3(4):292–303.

479. van de Pavert SA, Mebius RE. New insights into the development of lymphoid tissues. *Nat Rev Immunol*. 2010;10(9):664–74.

480. Hartl D, Tirouvanziam R, Laval J, Greene CM, Habiel D, Sharma L, et al. Innate Immunity of the Lung: From Basic Mechanisms to Translational Medicine. *J Innate Immun*. 2018;10(5-6):487–501.

481. Cupedo T, Jansen W, Kraal G, Mebius RE. Induction of secondary and tertiary lymphoid structures in the skin. *Immunity*. 2004;21(5):655–67.

482. Lochner M, Ohnmacht C, Presley L, Bruhns P, Si-Tahar M, Sawa S, et al. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORgamma t and LTi cells. *J Exp Med*. 2011;208(1):125–34.

483. Wolf MJ, Seleznik GM, Zeller N, Heikenwalder M. The unexpected role of lymphotoxin beta receptor signaling in carcinogenesis: from lymphoid tissue formation to liver and prostate cancer development. *Oncogene*. 2010;29(36):5006–18.

484. Finkin S, Yuan D, Stein I, Taniguchi K, Weber A, Unger K, et al. Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. *Nat Immunol*. 2015;16(12):1235–44.

485. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell*. 2001;7(2):401–9.

3955 486. Dejardin E, Droin NM, Delhase M, Haas E, Cao Y, Makris C, et al. The lymphotoxin-beta
3956 receptor induces different patterns of gene expression via two NF-kappaB pathways. *Immunity*.
3957 2002;17(4):525–35.
3958 487. Miyawaki S, Nakamura Y, Suzuka H, Koba M, Yasumizu R, Ikehara S, et al. A new mutation,
3959 aly, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. *Eur J*
3960 *Immunol*. 1994;24(2):429–34.
3961 488. Koike R, Nishimura T, Yasumizu R, Tanaka H, Hataba Y, Hataba Y, et al. The splenic marginal
3962 zone is absent in alymphoplastic aly mutant mice. *Eur J Immunol*. 1996;26(3):669–75.
3963 489. Karrer U, Althage A, Odermatt B, Roberts CW, Korsmeyer SJ, Miyawaki S, et al. On the key
3964 role of secondary lymphoid organs in antiviral immune responses studied in alymphoplastic (aly/aly)
3965 and spleenless (Hox11(-)/-) mutant mice. *J Exp Med*. 1997;185(12):2157–70.
3966 490. Fava RA, Notidis E, Hunt J, Szanya V, Ratcliffe N, Ngam-Ek A, et al. A role for the
3967 lymphotoxin/LIGHT axis in the pathogenesis of murine collagen-induced arthritis. *J Immunol*.
3968 2003;171(1):115–26.
3969 491. Rennert PD, Browning JL, Mebius R, Mackay F, Hochman PS. Surface lymphotoxin alpha/beta
3970 complex is required for the development of peripheral lymphoid organs. *J Exp Med*. 1996;184(5):1999–
3971 2006.
3972 492. Browning JL, Allaire N, Ngam-Ek A, Notidis E, Hunt J, Perrin S, et al. Lymphotoxin-beta
3973 receptor signaling is required for the homeostatic control of HEV differentiation and function.
3974 *Immunity*. 2005;23(5):539–50.
3975 493. Boutaffala L, Bertrand MJ, Remouchamps C, Seleznik G, Reisinger F, Janas M, et al. NIK
3976 promotes tissue destruction independently of the alternative NF-kappaB pathway through
3977 TNFR1/RIP1-induced apoptosis. *Cell Death Differ*. 2015;22(12):2020–33.
3978 494. de Leon-Boenig G, Bowman KK, Feng JA, Crawford T, Everett C, Franke Y, et al. The crystal
3979 structure of the catalytic domain of the NF-kappaB inducing kinase reveals a narrow but flexible active
3980 site. *Structure*. 2012;20(10):1704–14.
3981 495. Ramadass V, Vaiyapuri T, Tergaonkar V. Small Molecule NF-kappaB Pathway Inhibitors in
3982 Clinic. *Int J Mol Sci*. 2020;21(14).
3983 496. Riley C, Ammar U, Alsfouk A, Anthony NG, Baiget J, Berretta G, et al. Design and Synthesis
3984 of Novel Aminoindazole-pyrrolo[2,3-b]pyridine Inhibitors of IKKalpha That Selectively Perturb
3985 Cellular Non-Canonical NF-kappaB Signalling. *Molecules*. 2024;29(15).
3986 497. Chung KF. Inflammatory mediators in chronic obstructive pulmonary disease. *Curr Drug*
3987 *Targets Inflamm Allergy*. 2005;4(6):619–25.
3988 498. Couillin I, Vasseur V, Charron S, Gasse P, Tavernier M, Guillet J, et al. IL-1R1/MyD88
3989 signaling is critical for elastase-induced lung inflammation and emphysema. *J Immunol*.
3990 2009;183(12):8195–202.
3991 499. Tasaka S, Inoue K, Miyamoto K, Nakano Y, Kamata H, Shinoda H, et al. Role of interleukin-
3992 6 in elastase-induced lung inflammatory changes in mice. *Exp Lung Res*. 2010;36(6):362–72.
3993 500. Kurimoto E, Miyahara N, Kanehiro A, Waseda K, Taniguchi A, Ikeda G, et al. IL-17A is
3994 essential to the development of elastase-induced pulmonary inflammation and emphysema in mice.
3995 *Respir Res*. 2013;14(1):5.
3996 501. Mebratu YA, Tesfaigzi Y. IL-17 Plays a Role in Respiratory Syncytial Virus-induced Lung
3997 Inflammation and Emphysema in Elastase and LPS-injured Mice. *Am J Respir Cell Mol Biol*.
3998 2018;58(6):717–26.
3999 502. Fujii U, Miyahara N, Taniguchi A, Waseda K, Morichika D, Kurimoto E, et al. IL-23 Is
4000 Essential for the Development of Elastase-Induced Pulmonary Inflammation and Emphysema. *Am J*
4001 *Respir Cell Mol Biol*. 2016;55(5):697–707.
4002 503. Bhatt SP, Rabe KF, Hanania NA, Vogelmeier CF, Bafadhel M, Christenson SA, et al.
4003 Dupilumab for COPD with Blood Eosinophil Evidence of Type 2 Inflammation. *N Engl J Med*.
4004 2024;390(24):2274–83.
4005 504. Shibata S, Miyake K, Tateishi T, Yoshikawa S, Yamanishi Y, Miyazaki Y, et al. Basophils
4006 trigger emphysema development in a murine model of COPD through IL-4-mediated generation of
4007 MMP-12-producing macrophages. *Proc Natl Acad Sci U S A*. 2018;115(51):13057–62.

505. Ikeda G, Miyahara N, Koga H, Fuchimoto Y, Waseda K, Kurimoto E, et al. Effect of a cysteinyl leukotriene receptor antagonist on experimental emphysema and asthma combined with emphysema. *Am J Respir Cell Mol Biol*. 2014;50(1):18–29.
506. Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, et al. Angiotensin II Signal Transduction: An Update on Mechanisms of Physiology and Pathophysiology. *Physiol Rev*. 2018;98(3):1627–738.
507. Mei D, Tan WSD, Liao W, Heng CKM, Wong WSF. Activation of angiotensin II type-2 receptor protects against cigarette smoke-induced COPD. *Pharmacol Res*. 2020;161:105223.
508. Bastos AC, Magalhaes GS, Gregorio JF, Matos NA, Motta-Santos D, Bezerra FS, et al. Oral formulation angiotensin-(1-7) therapy attenuates pulmonary and systemic damage in mice with emphysema induced by elastase. *Immunobiology*. 2020;225(2):151893.
509. Kistemaker LE, Gosens R. Acetylcholine beyond bronchoconstriction: roles in inflammation and remodeling. *Trends Pharmacol Sci*. 2015;36(3):164–71.
510. Borkar NA, Thompson MA, Bartman CM, Khalfaoui L, Sine S, Sathish V, et al. Nicotinic receptors in airway disease. *Am J Physiol Lung Cell Mol Physiol*. 2024;326(2):L149–L63.
511. Hollenhorst MI, Krasteva-Christ G. Nicotinic Acetylcholine Receptors in the Respiratory Tract. *Molecules*. 2021;26(20).
512. Banzato R, Pinheiro-Menegasso NM, Novelli F, Olivo CR, Taguchi L, de Oliveira Santos S, et al. Alpha-7 Nicotinic Receptor Agonist Protects Mice Against Pulmonary Emphysema Induced by Elastase. *Inflammation*. 2024;47(3):958–74.
513. Pinheiro NM, Miranda C, Santana FR, Bittencourt-Mernak M, Arantes-Costa FM, Olivo C, et al. Effects of VAChT reduction and alpha7nAChR stimulation by PNU-282987 in lung inflammation in a model of chronic allergic airway inflammation. *Eur J Pharmacol*. 2020;882:173239.
514. Vatrella A, Montagnani S, Calabrese C, Parrella R, Pelaia G, Biscione GL, et al. Neuropeptide expression in the airways of COPD patients and smokers with normal lung function. *J Biol Regul Homeost Agents*. 2010;24(4):425–32.
515. Taniguchi A, Oda N, Morichika D, Senoo S, Itano J, Fujii U, et al. Protective effects of neuropeptide Y against elastase-induced pulmonary emphysema. *Am J Physiol Lung Cell Mol Physiol*. 2022;322(4):L539–L49.
516. Hoonhorst SJ, Lo Tam Loi AT, Pouwels SD, Faiz A, Telenga ED, van den Berge M, et al. Advanced glycation endproducts and their receptor in different body compartments in COPD. *Respir Res*. 2016;17:46.
517. Pouwels SD, Hesse L, Wu X, Allam V, van Oldeniel D, Bhiekharie LJ, et al. LL-37 and HMGB1 induce alveolar damage and reduce lung tissue regeneration via RAGE. *Am J Physiol Lung Cell Mol Physiol*. 2021;321(4):L641–L52.
518. Lee H, Park JR, Kim WJ, Sundar IK, Rahman I, Park SM, et al. Blockade of RAGE ameliorates elastase-induced emphysema development and progression via RAGE-DAMP signaling. *FASEB J*. 2017;31(5):2076–89.
519. Sinclair KA, Yerkovich ST, Chen T, McQualter JL, Hopkins PM, Wells CA, et al. Mesenchymal Stromal Cells are Readily Recoverable from Lung Tissue, but not the Alveolar Space, in Healthy Humans. *Stem Cells*. 2016;34(10):2548–58.
520. Lama VN, Smith L, Badri L, Flint A, Andrei AC, Murray S, et al. Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. *J Clin Invest*. 2007;117(4):989–96.
521. Yuan D, Bao Y, El-Hashash A. Mesenchymal stromal cell-based therapy in lung diseases; from research to clinic. *Am J Stem Cells*. 2024;13(2):37–58.
522. Yuan T, Volckaert T, Chanda D, Thannickal VJ, De Langhe SP. Fgf10 Signaling in Lung Development, Homeostasis, Disease, and Repair After Injury. *Front Genet*. 2018;9:418.
523. El Agha E, Herold S, Al Alam D, Quantius J, MacKenzie B, Carraro G, et al. Fgf10-positive cells represent a progenitor cell population during lung development and postnatally. *Development*. 2014;141(2):296–306.
524. Wisman M, Kruk D, Kooistra W, Heijink IH, Woldhuis RR. Lower levels of senescence in human lung mesenchymal stromal cells compared to lung fibroblasts; implications for tissue regeneration in COPD. *Am J Physiol Lung Cell Mol Physiol*. 2025.

525. Kaspi H, Semo J, Abramov N, Dekel C, Lindborg S, Kern R, et al. MSC-NTF (NurOwn(R)) exosomes: a novel therapeutic modality in the mouse LPS-induced ARDS model. *Stem Cell Res Ther*. 2021;12(1):72.
526. Abreu SC, Lopes-Pacheco M, Weiss DJ, Rocco PRM. Mesenchymal Stromal Cell-Derived Extracellular Vesicles in Lung Diseases: Current Status and Perspectives. *Front Cell Dev Biol*. 2021;9:600711.
527. Lightner AL, Sengupta V, Qian S, Ransom JT, Suzuki S, Park DJ, et al. Bone Marrow Mesenchymal Stem Cell-Derived Extracellular Vesicle Infusion for the Treatment of Respiratory Failure From COVID-19: A Randomized, Placebo-Controlled Dosing Clinical Trial. *Chest*. 2023;164(6):1444–53.
528. Michaeloudes C, Li X, Mak JCW, Bhavsar PK. Study of Mesenchymal Stem Cell-Mediated Mitochondrial Transfer in In Vitro Models of Oxidant-Mediated Airway Epithelial and Smooth Muscle Cell Injury. *Methods Mol Biol*. 2021;2269:93–105.
529. Li X, Michaeloudes C, Zhang Y, Wiegman CH, Adcock IM, Lian Q, et al. Mesenchymal stem cells alleviate oxidative stress-induced mitochondrial dysfunction in the airways. *J Allergy Clin Immunol*. 2018;141(5):1634–45 e5.
530. Li X, Zhang Y, Yeung SC, Liang Y, Liang X, Ding Y, et al. Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage. *Am J Respir Cell Mol Biol*. 2014;51(3):455–65.
531. Kruk D, Heijink IH, Slebos DJ, Timens W, Ten Hacken NH. Mesenchymal Stromal Cells to Regenerate Emphysema: On the Horizon? *Respiration*. 2018;96(2):148–58.
532. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet*. 2008;371(9624):1579–86.
533. Panes J, Garcia-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet*. 2016;388(10051):1281–90.
534. Casiraghi F, Perico N, Remuzzi G. Mesenchymal stromal cells for tolerance induction in organ transplantation. *Hum Immunol*. 2018;79(5):304–13.
535. Weiss DJ, Casaburi R, Flannery R, LeRoux-Williams M, Tashkin DP. A placebo-controlled, randomized trial of mesenchymal stem cells in COPD. *Chest*. 2013;143(6):1590–8.
536. Weiss DJ, Segal K, Casaburi R, Hayes J, Tashkin D. Effect of mesenchymal stromal cell infusions on lung function in COPD patients with high CRP levels. *Respir Res*. 2021;22(1):142.
537. Stolk J, Broekman W, Mauad T, Zwaginga JJ, Roelofs H, Fibbe WE, et al. A phase I study for intravenous autologous mesenchymal stromal cell administration to patients with severe emphysema. *QJM*. 2016;109(5):331–6.
538. Hoffman AM, Paxson JA, Mazan MR, Davis AM, Tyagi S, Murthy S, et al. Lung-derived mesenchymal stromal cell post-transplantation survival, persistence, paracrine expression, and repair of elastase-injured lung. *Stem Cells Dev*. 2011;20(10):1779–92.
539. Antunes MA, Abreu SC, Cruz FF, Teixeira AC, Lopes-Pacheco M, Bandeira E, et al. Effects of different mesenchymal stromal cell sources and delivery routes in experimental emphysema. *Respir Res*. 2014;15(1):118.
540. Kitoko JZ, de Castro LL, Nascimento AP, Abreu SC, Cruz FF, Arantes AC, et al. Therapeutic administration of bone marrow-derived mesenchymal stromal cells reduces airway inflammation without up-regulating Tregs in experimental asthma. *Clin Exp Allergy*. 2018;48(2):205–16.
541. Wang M, Hao Y, He W, Jia H, Zhong Z, Xia S. Nebulized mesenchymal stem cell-derived exosomes attenuate airway inflammation in a rat model of chronic obstructive pulmonary disease. *Cell Immunol*. 2025;409-410:104933.
542. Souza MC, Silva JD, Padua TA, Torres ND, Antunes MA, Xisto DG, et al. Mesenchymal stromal cell therapy attenuated lung and kidney injury but not brain damage in experimental cerebral malaria. *Stem Cell Res Ther*. 2015;6(1):102.
543. Kim YS, Kim JY, Shin DM, Huh JW, Lee SW, Oh YM. Tracking intravenous adipose-derived mesenchymal stem cells in a model of elastase-induced emphysema. *Tuberc Respir Dis (Seoul)*. 2014;77(3):116–23.

544. Silva LHA, Antunes MA, Dos Santos CC, Weiss DJ, Cruz FF, Rocco PRM. Strategies to improve the therapeutic effects of mesenchymal stromal cells in respiratory diseases. *Stem Cell Res Ther*. 2018;9(1):45.
545. Cober ND, Rowe K, Deng Y, Benavente-Babace A, Courtman DW, Godin M, et al. Targeting extracellular vesicle delivery to the lungs by microgel encapsulation. *J Extracell Biol*. 2023;2(6):e94.
546. Kruk D, Wisman M, Bruin HG, Lodewijk ME, Hof DJ, Borghuis T, et al. Abnormalities in reparative function of lung-derived mesenchymal stromal cells in emphysema. *Am J Physiol Lung Cell Mol Physiol*. 2021;320(5):L832–L44.
547. van der Plaat DA, de Jong K, Lahousse L, Faiz A, Vonk JM, van Diemen CC, et al. Genome-wide association study on the FEV(1)/FVC ratio in never-smokers identifies HHIP and FAM13A. *J Allergy Clin Immunol*. 2017;139(2):533–40.
548. Wang C, de Mochel NSR, Christenson SA, Cassandras M, Moon R, Brumwell AN, et al. Expansion of hedgehog disrupts mesenchymal identity and induces emphysema phenotype. *J Clin Invest*. 2018;128(10):4343–58.
549. Wang C, Hyams B, Allen NC, Cautivo K, Monahan K, Zhou M, et al. Dysregulated lung stroma drives emphysema exacerbation by potentiating resident lymphocytes to suppress an epithelial stem cell reservoir. *Immunity*. 2023;56(3):576–91 e10.
550. Cassandras M, Wang C, Kathiriya J, Tsukui T, Matatia P, Matthay M, et al. Gli1(+) mesenchymal stromal cells form a pathological niche to promote airway progenitor metaplasia in the fibrotic lung. *Nat Cell Biol*. 2020;22(11):1295–306.
551. Lahmar Z, Ahmed E, Fort A, Vachier I, Bourdin A, Bergougnoux A. Hedgehog pathway and its inhibitors in chronic obstructive pulmonary disease (COPD). *Pharmacol Ther*. 2022;240:108295.
552. Weiner AI, Jackson SR, Zhao G, Quansah KK, Farshchian JN, Neupauer KM, et al. Mesenchyme-free expansion and transplantation of adult alveolar progenitor cells: steps toward cell-based regenerative therapies. *NPJ Regen Med*. 2019;4:17.
553. Raby KL, Michaeloudes C, Tonkin J, Chung KF, Bhavsar PK. Mechanisms of airway epithelial injury and abnormal repair in asthma and COPD. *Front Immunol*. 2023;14:1201658.
554. Ghosh M, Miller YE, Nakachi I, Kwon JB, Baron AE, Brantley AE, et al. Exhaustion of Airway Basal Progenitor Cells in Early and Established Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2018;197(7):885–96.
555. Hayashi Y, Ohnishi H, Kitano M, Kishimoto Y, Takezawa T, Okuyama H, et al. Comparative Study of Immunodeficient Rat Strains in Engraftment of Human-Induced Pluripotent Stem Cell-Derived Airway Epithelia. *Tissue Eng Part A*. 2024;30(3-4):144–53.
556. Ma L, Thapa BR, Le Suer JA, Tilston-Lunel A, Herriges MJ, Berical A, et al. Airway stem cell reconstitution by the transplantation of primary or pluripotent stem cell-derived basal cells. *Cell Stem Cell*. 2023;30(9):1199–216 e7.
557. Ma L, Thapa BR, Le Suer JA, Tilston-Lunel A, Herriges MJ, Wang F, et al. Life-long functional regeneration of in vivo airway epithelium by the engraftment of airway basal stem cells. *Nat Protoc*. 2025;20(3):810–42.
558. Miller AJ, Hill DR, Nagy MS, Aoki Y, Dye BR, Chin AM, et al. In Vitro Induction and In Vivo Engraftment of Lung Bud Tip Progenitor Cells Derived from Human Pluripotent Stem Cells. *Stem Cell Reports*. 2018;10(1):101–19.
559. Herriges MJ, Yampolskaya M, Thapa BR, Lindstrom-Vautrin J, Wang F, Huang J, et al. Durable alveolar engraftment of PSC-derived lung epithelial cells into immunocompetent mice. *Cell Stem Cell*. 2023;30(9):1217–34 e7.
560. Jacob A, Morley M, Hawkins F, McCauley KB, Jean JC, Heins H, et al. Differentiation of Human Pluripotent Stem Cells into Functional Lung Alveolar Epithelial Cells. *Cell Stem Cell*. 2017;21(4):472–88 e10.
561. Ma Q, Ma Y, Dai X, Ren T, Fu Y, Liu W, et al. Regeneration of functional alveoli by adult human SOX9(+) airway basal cell transplantation. *Protein Cell*. 2018;9(3):267–82.
562. Shi Y, Dong M, Zhou Y, Li W, Gao Y, Han L, et al. Distal airway stem cells ameliorate bleomycin-induced pulmonary fibrosis in mice. *Stem Cell Res Ther*. 2019;10(1):161.
563. Wang X, Zhao Y, Li D, Feng Y, Xie Y, Zhou Y, et al. Intrapulmonary distal airway stem cell transplantation repairs lung injury in chronic obstructive pulmonary disease. *Cell Prolif*. 2021;54(6):e13046.

564. Louie SM, Moye AL, Wong IG, Lu E, Shehaj A, Garcia-de-Alba C, et al. Progenitor potential of lung epithelial organoid cells in a transplantation model. *Cell Rep*. 2022;39(2):110662.
565. Ararat E, Louie SM, Lu E, Paschini M, Raiser DM, Kim CF. Comparison of Transplantation of Lung Organoid Cell Types: One Size Does Not Fit All. *Am J Respir Cell Mol Biol*. 2022;66(3):340–3.
566. Milman Krentsis I, Zheng Y, Rosen C, Shin SY, Blagdon C, Shoshan E, et al. Lung cell transplantation for pulmonary fibrosis. *Sci Adv*. 2024;10(34):eadk2524.
567. Wang Y, Meng Z, Liu M, Zhou Y, Chen D, Zhao Y, et al. Autologous transplantation of P63(+) lung progenitor cells for chronic obstructive pulmonary disease therapy. *Sci Transl Med*. 2024;16(734):eadi3360.
568. Zhang S, Zhou M, Shao C, Zhao Y, Liu M, Ni L, et al. Autologous P63+ lung progenitor cell transplantation in idiopathic pulmonary fibrosis: a phase 1 clinical trial. *Elife*. 2025;13.
569. Kinoshita S, Koizumi N, Ueno M, Okumura N, Imai K, Tanaka H, et al. Injection of Cultured Cells with a ROCK Inhibitor for Bullous Keratopathy. *N Engl J Med*. 2018;378(11):995–1003.
570. Tsai MC, Kureshi A, Daniels JT. Tissue engineered corneal endothelium transplantation in an ex vivo human cornea organ culture model. *Sci Rep*. 2025;15(1):12571.
571. Shen L, Sun P, Zhang C, Yang L, Du L, Wu X. Therapy of corneal endothelial dysfunction with corneal endothelial cell-like cells derived from skin-derived precursors. *Sci Rep*. 2017;7(1):13400.
572. Wang XX, Zhang FR, Shang YP, Zhu JH, Xie XD, Tao QM, et al. Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. *J Am Coll Cardiol*. 2007;49(14):1566–71.
573. Granton J, Langleben D, Kutryk MB, Camack N, Galipeau J, Courtman DW, et al. Endothelial NO-Synthase Gene-Enhanced Progenitor Cell Therapy for Pulmonary Arterial Hypertension: The PHACeT Trial. *Circ Res*. 2015;117(7):645–54.
574. Theodorakopoulou MP, Alexandrou ME, Bakaloudi DR, Pitsiou G, Stanopoulos I, Kontakiotis T, et al. Endothelial dysfunction in COPD: a systematic review and meta-analysis of studies using different functional assessment methods. *ERJ Open Res*. 2021;7(2).
575. Liu S, Yang A, Yu Y, Xu B, Yu G, Wang H. Exercise Prescription Training in Chronic Obstructive Pulmonary Disease: Benefits and Mechanisms. *Int J Chron Obstruct Pulmon Dis*. 2025;20:1071–82.
576. Li YX, Wang HB, Li J, Jin JB, Hu JB, Yang CL. Targeting pulmonary vascular endothelial cells for the treatment of respiratory diseases. *Front Pharmacol*. 2022;13:983816.
577. Krump-Konvalinkova V, Bittinger F, Unger RE, Peters K, Lehr HA, Kirkpatrick CJ. Generation of human pulmonary microvascular endothelial cell lines. *Lab Invest*. 2001;81(12):1717–27.
578. Huertas A, Guignabert C, Barbera JA, Bartsch P, Bhattacharya J, Bhattacharya S, et al. Pulmonary vascular endothelium: the orchestra conductor in respiratory diseases: Highlights from basic research to therapy. *Eur Respir J*. 2018;51(4).
579. Altalhi W, Wu T, Wojtkiewicz GR, Jeffs S, Miki K, Ott HC. Intratracheally injected human-induced pluripotent stem cell-derived pneumocytes and endothelial cells engraft in the distal lung and ameliorate emphysema in a rat model. *J Thorac Cardiovasc Surg*. 2023;166(1):e23–e37.
580. Mohammed AN, Kohram F, Lan YW, Li E, Kolesnichenko OA, Kalin TV, et al. Transplantation of alveolar macrophages improves the efficacy of endothelial progenitor cell therapy in mouse model of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol*. 2024;327(1):L114–L25.
581. Wang W, Peng H, Zeng M, Liu J, Liang G, He Z. Endothelial progenitor cells systemic administration alleviates multi-organ senescence by down-regulating USP7/p300 pathway in chronic obstructive pulmonary disease. *J Transl Med*. 2023;21(1):881.
582. Dhurat R, Sukesh M. Principles and Methods of Preparation of Platelet-Rich Plasma: A Review and Author's Perspective. *J Cutan Aesthet Surg*. 2014;7(4):189–97.
583. Corsini A, Perticarini L, Palermi S, Bettinsoli P, Marchini A. Re-Evaluating Platelet-Rich Plasma Dosing Strategies in Sports Medicine: The Role of the "10 Billion Platelet Dose" in Optimizing Therapeutic Outcomes-A Narrative Review. *J Clin Med*. 2025;14(8).

584. Huang G, Hua S, Yang T, Ma J, Yu W, Chen X. Platelet-rich plasma shows beneficial effects for patients with knee osteoarthritis by suppressing inflammatory factors. *Exp Ther Med*. 2018;15(3):3096–102.
585. Karina K, Christoffel LM, Novariani R, Rosadi I, Rosliana I, Rosidah S, et al. The Effect of Intravenous Autologous Activated Platelet-Rich Plasma Therapy on "Profibrotic Cytokine" IL-1beta Levels in Severe and Critical COVID-19 Patients: A Preliminary Study. *Scientifica (Cairo)*. 2021;2021:9427978.
586. Beitia M, Delgado D, Sanchez P, Vallejo de la Cueva A, Cugat JR, Sanchez M. Platelet Lysate Nebulization Protocol for the Treatment of COVID-19 and Its Sequels: Proof of Concept and Scientific Rationale. *Int J Mol Sci*. 2021;22(4).
587. Mammoto T, Chen Z, Jiang A, Jiang E, Ingber DE, Mammoto A. Acceleration of Lung Regeneration by Platelet-Rich Plasma Extract through the Low-Density Lipoprotein Receptor-Related Protein 5-Tie2 Pathway. *Am J Respir Cell Mol Biol*. 2016;54(1):103–13.
588. Rafii S, Cao Z, Lis R, Siempos, II, Chavez D, Shido K, et al. Platelet-derived SDF-1 primes the pulmonary capillary vascular niche to drive lung alveolar regeneration. *Nat Cell Biol*. 2015;17(2):123–36.
589. Wu M, Lin H, Shi L, Huang L, Zhuang Y, Zeng Y. Bronchoscopic Treatment of Tracheobronchial Fistula With Autologous Platelet-Rich Plasma. *Ann Thorac Surg*. 2021;111(2):e129–e31.
590. Rubio MM. Platelet Rich Plasma- Platelet Concentrate Therapy in COPD: An Observational Cohort Study. *J Regen Med Biol Res*. 2021;2(2):1–10.
591. Anitua E, Troya M, Falcon-Perez JM, Lopez-Sarrio S, Gonzalez E, Alkhraisat MH. Advances in Platelet Rich Plasma-Derived Extracellular Vesicles for Regenerative Medicine: A Systematic-Narrative Review. *Int J Mol Sci*. 2023;24(17).
592. Tao X, Xue F, Xu J, Wang W. Platelet-rich plasma-derived extracellular vesicles inhibit NF-kappaB/NLRP3 pathway-mediated pyroptosis in intervertebral disc degeneration via the MALAT1/microRNA-217/SIRT1 axis. *Cell Signal*. 2024;117:111106.
593. Abbaszadeh H, Ghorbani F, Abbaspour-Aghdam S, Kamrani A, Valizadeh H, Nadiri M, et al. Chronic obstructive pulmonary disease and asthma: mesenchymal stem cells and their extracellular vesicles as potential therapeutic tools. *Stem Cell Res Ther*. 2022;13(1):262.
594. Bari E, Ferrarotti I, Torre ML, Corsico AG, Perteghella S. Mesenchymal stem/stromal cell secretome for lung regeneration: The long way through "pharmaceuticalization" for the best formulation. *J Control Release*. 2019;309:11–24.
595. Antunes MA, Lapa ESJR, Rocco PR. Mesenchymal stromal cell therapy in COPD: from bench to bedside. *Int J Chron Obstruct Pulmon Dis*. 2017;12:3017–27.
596. M HR, Bayraktar E, G KH, Abd-Ellah MF, Amero P, Chavez-Reyes A, et al. Exosomes: From Garbage Bins to Promising Therapeutic Targets. *Int J Mol Sci*. 2017;18(3).
597. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res Ther*. 2018;9(1):63.
598. Nagelkerke A, Ojansivu M, van der Koog L, Whittaker TE, Cunnane EM, Silva AM, et al. Extracellular vesicles for tissue repair and regeneration: Evidence, challenges and opportunities. *Adv Drug Deliv Rev*. 2021;175:113775.
599. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J Extracell Vesicles*. 2024;13(2):e12404.
600. Gandek TB, van der Koog L, Nagelkerke A. A Comparison of Cellular Uptake Mechanisms, Delivery Efficacy, and Intracellular Fate between Liposomes and Extracellular Vesicles. *Adv Healthc Mater*. 2023;12(25):e2300319.
601. Ridzuan N, Zakaria N, Widera D, Sheard J, Morimoto M, Kiyokawa H, et al. Human umbilical cord mesenchymal stem cell-derived extracellular vesicles ameliorate airway inflammation in a rat model of chronic obstructive pulmonary disease (COPD). *Stem Cell Res Ther*. 2021;12(1):54.
602. Antunes MA, Braga CL, Oliveira TB, Kitoko JZ, Castro LL, Xisto DG, et al. Mesenchymal Stromal Cells From Emphysematous Donors and Their Extracellular Vesicles Are Unable to Reverse Cardiorespiratory Dysfunction in Experimental Severe Emphysema. *Front Cell Dev Biol*. 2021;9:661385.

603. Jin T, Liu X, Li G, Sun S, Xie L. Intravenous injection of BMSCs modulate tsRNA expression and ameliorate lung remodeling in COPD mice. *Stem Cell Res Ther*. 2024;15(1):450.
604. Kim YS, Kim JY, Cho R, Shin DM, Lee SW, Oh YM. Adipose stem cell-derived nanovesicles inhibit emphysema primarily via an FGF2-dependent pathway. *Exp Mol Med*. 2017;49(1):e284.
605. Xuan W, Wang S, Alarcon-Calderon A, Bagwell MS, Para R, Wang F, et al. Nebulized platelet-derived extracellular vesicles attenuate chronic cigarette smoke-induced murine emphysema. *Transl Res*. 2024;269:76–93.
606. Stam J, Bartel S, Bischoff R, Wolters JC. Isolation of extracellular vesicles with combined enrichment methods. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2021;1169:122604.
607. Webber J, Clayton A. How pure are your vesicles? *J Extracell Vesicles*. 2013;2.
608. Whittaker TE, Nagelkerke A, Nele V, Kauscher U, Stevens MM. Experimental artefacts can lead to misattribution of bioactivity from soluble mesenchymal stem cell paracrine factors to extracellular vesicles. *J Extracell Vesicles*. 2020;9(1):1807674.
609. Gonzalez H, McCarthy S, Masterson C, Byrnes D, Sallent I, Horan E, et al. Nebulised mesenchymal stem cell derived extracellular vesicles ameliorate E. coli induced pneumonia in a rodent model. *Stem Cell Res Ther*. 2023;14(1):151.
610. Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. *Int J Pharm*. 2010;392(1-2):1–19.
611. Sibum I, Hagedoorn P, de Boer AH, Frijlink HW, Grasmeijer F. Challenges for pulmonary delivery of high powder doses. *Int J Pharm*. 2018;548(1):325–36.
612. Lechanteur A, Evrard B. Influence of Composition and Spray-Drying Process Parameters on Carrier-Free DPI Properties and Behaviors in the Lung: A review. *Pharmaceutics*. 2020;12(1).
613. Vehring R. Pharmaceutical particle engineering via spray drying. *Pharm Res*. 2008;25(5):999–1022.
614. Sharma A, Khamar D, Cullen S, Hayden A, Hughes H. Innovative Drying Technologies for Biopharmaceuticals. *Int J Pharm*. 2021;609:121115.
615. Pisano R, Arsiccio A, Capozzi LC, Trout BL. Achieving continuous manufacturing in lyophilization: Technologies and approaches. *Eur J Pharm Biopharm*. 2019;142:265–79.
616. Jansen EM, van der Koog L, Elferink RAB, Rafie K, Nagelkerke A, Gosens R, et al. Stabilized Extracellular Vesicle Formulations for Inhalable Dry Powder Development. *Small*. 2025:e2411096.
617. Kang WY, Shin EK, Kim EH, Kang MH, Bang CY, Bang OY, et al. Lyoprotectant Constituents Suited for Lyophilization and Reconstitution of Stem-Cell-Derived Extracellular Vesicles. *Biomater Res*. 2024;28:0005.
618. Charoenviriyakul C, Takahashi Y, Nishikawa M, Takakura Y. Preservation of exosomes at room temperature using lyophilization. *Int J Pharm*. 2018;553(1-2):1–7.
619. Frank J, Richter M, de Rossi C, Lehr CM, Fuhrmann K, Fuhrmann G. Extracellular vesicles protect glucuronidase model enzymes during freeze-drying. *Sci Rep*. 2018;8(1):12377.
620. Popowski KD, Moatti A, Scull G, Silkstone D, Lutz H, Lopez de Juan Abad B, et al. Inhalable dry powder mRNA vaccines based on extracellular vesicles. *Matter*. 2022;5(9):2960–74.
621. Harrell CR, Miloradovic D, Sadikot R, Fellabaum C, Markovic BS, Miloradovic D, et al. Molecular and Cellular Mechanisms Responsible for Beneficial Effects of Mesenchymal Stem Cell-Derived Product "Exo-d-MAPPS" in Attenuation of Chronic Airway Inflammation. *Anal Cell Pathol (Amst)*. 2020;2020:3153891.
622. Syromiatnikova V, Prokopeva A, Gomzikova M. Methods of the Large-Scale Production of Extracellular Vesicles. *Int J Mol Sci*. 2022;23(18).
623. Shekari F, Alibhai FJ, Baharvand H, Borger V, Bruno S, Davies O, et al. Cell culture-derived extracellular vesicles: Considerations for reporting cell culturing parameters. *J Extracell Biol*. 2023;2(10):e115.
624. Grangier A, Branchu J, Volatron J, Piffoux M, Gazeau F, Wilhelm C, et al. Technological advances towards extracellular vesicles mass production. *Adv Drug Deliv Rev*. 2021;176:113843.
625. Mendt M, Kamerkar S, Sugimoto H, McAndrews KM, Wu CC, Gagea M, et al. Generation and testing of clinical-grade exosomes for pancreatic cancer. *JCI Insight*. 2018;3(8).
626. Watson DC, Yung BC, Bergamaschi C, Chowdhury B, Bear J, Stellas D, et al. Scalable, cGMP-compatible purification of extracellular vesicles carrying bioactive human heterodimeric IL-15/lactadherin complexes. *J Extracell Vesicles*. 2018;7(1):1442088.

627. Ng CY, Kee LT, Al-Masawa ME, Lee QH, Subramaniam T, Kok D, et al. Scalable Production of Extracellular Vesicles and Its Therapeutic Values: A Review. *Int J Mol Sci*. 2022;23(14).
628. Lasser C, Jang SC, Lotvall J. Subpopulations of extracellular vesicles and their therapeutic potential. *Mol Aspects Med*. 2018;60:1–14.
629. Goetz R, Mohammadi M. Exploring mechanisms of FGF signalling through the lens of structural biology. *Nat Rev Mol Cell Biol*. 2013;14(3):166–80.
630. Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, et al. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem*. 2006;281(10):6120–3.
631. Makarenkova HP, Hoffman MP, Beenken A, Eliseenkova AV, Meech R, Tsau C, et al. Differential interactions of FGFs with heparan sulfate control gradient formation and branching morphogenesis. *Sci Signal*. 2009;2(88):ra55.
632. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv*. 2020;6(28):eaba1983.
633. Kranenburg AR, Willems-Widyastuti A, Mooi WJ, Saxena PR, Sterk PJ, de Boer WI, et al. Chronic obstructive pulmonary disease is associated with enhanced bronchial expression of FGF-1, FGF-2, and FGFR-1. *J Pathol*. 2005;206(1):28–38.
634. Raredon MSB, Adams TS, Suhail Y, Schupp JC, Poli S, Neumark N, et al. Single-cell connectomic analysis of adult mammalian lungs. *Sci Adv*. 2019;5(12):eaaw3851.
635. Brehm JM, Hagiwara K, Tesfaigzi Y, Bruse S, Mariani TJ, Bhattacharya S, et al. Identification of FGF7 as a novel susceptibility locus for chronic obstructive pulmonary disease. *Thorax*. 2011;66(12):1085–90.
636. Upadhyay D, Correa-Meyer E, Sznajder JI, Kamp DW. FGF-10 prevents mechanical stretch-induced alveolar epithelial cell DNA damage via MAPK activation. *Am J Physiol Lung Cell Mol Physiol*. 2003;284(2):L350–9.
637. Waters CM, Savla U. Keratinocyte growth factor accelerates wound closure in airway epithelium during cyclic mechanical strain. *J Cell Physiol*. 1999;181(3):424–32.
638. Shiraishi K, Shichino S, Ueha S, Nakajima T, Hashimoto S, Yamazaki S, et al. Mesenchymal-Epithelial Interactome Analysis Reveals Essential Factors Required for Fibroblast-Free Alveolosphere Formation. *iScience*. 2019;11:318–33.
639. Volckaert T, Dill E, Campbell A, Tiozzo C, Majka S, Bellusci S, et al. Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. *J Clin Invest*. 2011;121(11):4409–19.
640. Ramasamy SK, Mailleux AA, Gupte VV, Mata F, Sala FG, Veltmaat JM, et al. Fgf10 dosage is critical for the amplification of epithelial cell progenitors and for the formation of multiple mesenchymal lineages during lung development. *Dev Biol*. 2007;307(2):237–47.
641. Cardoso WV, Itoh A, Nogawa H, Mason I, Brody JS. FGF-1 and FGF-7 induce distinct patterns of growth and differentiation in embryonic lung epithelium. *Dev Dyn*. 1997;208(3):398–405.
642. Xu X, McCormick-Shannon K, Voelker DR, Mason RJ. KGF increases SP-A and SP-D mRNA levels and secretion in cultured rat alveolar type II cells. *Am J Respir Cell Mol Biol*. 1998;18(2):168–78.
643. Chelly N, Mouhieddine-Gueddiche OB, Barlier-Mur AM, Chailley-Heu B, Bourbon JR. Keratinocyte growth factor enhances maturation of fetal rat lung type II cells. *Am J Respir Cell Mol Biol*. 1999;20(3):423–32.
644. Ray P, Devaux Y, Stolz DB, Yarlagadda M, Watkins SC, Lu Y, et al. Inducible expression of keratinocyte growth factor (KGF) in mice inhibits lung epithelial cell death induced by hyperoxia. *Proc Natl Acad Sci U S A*. 2003;100(10):6098–103.
645. Srisuma S, Bhattacharya S, Simon DM, Solleti SK, Tyagi S, Starcher B, et al. Fibroblast growth factor receptors control epithelial-mesenchymal interactions necessary for alveolar elastogenesis. *Am J Respir Crit Care Med*. 2010;181(8):838–50.
646. Guzy RD, Stoilov I, Elton TJ, Mecham RP, Ornitz DM. Fibroblast growth factor 2 is required for epithelial recovery, but not for pulmonary fibrosis, in response to bleomycin. *Am J Respir Cell Mol Biol*. 2015;52(1):116–28.
647. Pan X, Xu S, Zhou Z, Wang F, Mao L, Li H, et al. Fibroblast growth factor-2 alleviates the capillary leakage and inflammation in sepsis. *Mol Med*. 2020;26(1):108.

4390 648. Jang S, Lee H, Park J, Cha SR, Lee J, Park Y, et al. PTD-FGF2 Attenuates Elastase Induced
4391 Emphysema in Mice and Alveolar Epithelial Cell Injury. *COPD*. 2023;20(1):109–18.
4392 649. Wang X, Hou X, Zhao Y, Zhao R, Dai J, Dai H, et al. The early and late intervention effects of
4393 collagen-binding FGF2 on elastase-induced lung injury. *Biomed Pharmacother*. 2023;158:114147.
4394 650. Panoskaltsis-Mortari A, Ingbar DH, Jung P, Haddad IY, Bitterman PB, Wangensteen OD, et
4395 al. KGF pretreatment decreases B7 and granzyme B expression and hastens repair in lungs of mice after
4396 allogeneic BMT. *Am J Physiol Lung Cell Mol Physiol*. 2000;278(5):L988–99.
4397 651. Atabai K, Ishigaki M, Geiser T, Ueki I, Matthay MA, Ware LB. Keratinocyte growth factor
4398 can enhance alveolar epithelial repair by nonmitogenic mechanisms. *Am J Physiol Lung Cell Mol*
4399 *Physiol*. 2002;283(1):L163–9.
4400 652. Ulrich K, Stern M, Goddard ME, Williams J, Zhu J, Dewar A, et al. Keratinocyte growth factor
4401 therapy in murine oleic acid-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol*.
4402 2005;288(6):L1179–92.
4403 653. Chen J, Li C, Gao X, Li C, Liang Z, Yu L, et al. Keratinocyte growth factor gene delivery via
4404 mesenchymal stem cells protects against lipopolysaccharide-induced acute lung injury in mice. *PLoS*
4405 *One*. 2013;8(12):e83303.
4406 654. Plantier L, Marchand-Adam S, Antico Arciuch VG, Boyer L, De Coster C, Marchal J, et al.
4407 Keratinocyte growth factor protects against elastase-induced pulmonary emphysema in mice. *Am J*
4408 *Physiol Lung Cell Mol Physiol*. 2007;293(5):L1230–9.
4409 655. Muyal JP, Kotnala S, Bhardwaj H, Tyagi A. Effect of recombinant human keratinocyte growth
4410 factor in inducing Ras-Raf-Erk pathway-mediated cell proliferation in emphysematous mice lung. *Inhal*
4411 *Toxicol*. 2014;26(13):761–71.
4412 656. Shyamsundar M, McAuley DF, Ingram RJ, Gibson DS, O'Kane D, McKeown ST, et al.
4413 Keratinocyte growth factor promotes epithelial survival and resolution in a human model of lung injury.
4414 *Am J Respir Crit Care Med*. 2014;189(12):1520–9.
4415 657. Qian Y, Shi Q, Zhou W, He B, Xu H, Liu B, et al. FGF10 protects against particulate matter-
4416 induced lung injury by inhibiting ferroptosis via Nrf2-dependent signaling. *Int Immunopharmacol*.
4417 2024;134:112165.
4418 658. Liu L, Shi Q, Wang K, Qian Y, Zhou L, Bellusci S, et al. Fibroblast growth factor 10 protects
4419 against particulate matter-induced lung injury by inhibiting oxidative stress-mediated pyroptosis via the
4420 PI3K/Akt/Nrf2 signaling pathway. *Int Immunopharmacol*. 2022;113(Pt A):109398.
4421 659. Hadzic S, Wu CY, Gredic M, Pak O, Loku E, Kraut S, et al. Fibroblast growth factor 10 reverses
4422 cigarette smoke- and elastase-induced emphysema and pulmonary hypertension in mice. *Eur Respir J*.
4423 2023;62(5).
4424 660. Jiang T, Hu W, Zhang S, Ren C, Lin S, Zhou Z, et al. Fibroblast growth factor 10 attenuates
4425 chronic obstructive pulmonary disease by protecting against glycocalyx impairment and endothelial
4426 apoptosis. *Respir Res*. 2022;23(1):269.
4427 661. Miranda O, Farooqui M, Siegfried JM. Status of Agents Targeting the HGF/c-Met Axis in Lung
4428 Cancer. *Cancers (Basel)*. 2018;10(9).
4429 662. Habermann AC, Gutierrez AJ, Bui LT, Yahn SL, Winters NI, Calvi CL, et al. Single-cell RNA
4430 sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary
4431 fibrosis. *Sci Adv*. 2020;6(28):eaba1972.
4432 663. Lee D, Sung ES, Ahn JH, An S, Huh J, You WK. Development of antibody-based c-Met
4433 inhibitors for targeted cancer therapy. *Immunotargets Ther*. 2015;4:35–44.
4434 664. Jung AL, Han M, Griss K, Bertrams W, Nell C, Greulich T, et al. Novel protein biomarkers for
4435 pneumonia and acute exacerbations in COPD: a pilot study. *Front Med (Lausanne)*. 2023;10:1180746.
4436 665. Kanazawa H, Tochino Y, Asai K, Hirata K. Simultaneous assessment of hepatocyte growth
4437 factor and vascular endothelial growth factor in epithelial lining fluid from patients with COPD. *Chest*.
4438 2014;146(5):1159–65.
4439 666. Sauleda J, Noguera A, Blanquer D, Pons J, Lopez M, Villena C, et al. Pulmonary and systemic
4440 hepatocyte and keratinocyte growth factors in patients with chronic obstructive pulmonary disease. *Int*
4441 *J Chron Obstruct Pulmon Dis*. 2008;3(4):719–25.
4442 667. Acevedo N, Escamilla-Gil JM, Espinoza H, Regino R, Ramirez J, Florez de Arco L, et al.
4443 Chronic Obstructive Pulmonary Disease Patients Have Increased Levels of Plasma Inflammatory
4444 Mediators Reported Upregulated in Severe COVID-19. *Front Immunol*. 2021;12:678661.

668. Calvi C, Podowski M, Lopez-Mercado A, Metzger S, Misono K, Malinina A, et al. Hepatocyte growth factor, a determinant of airspace homeostasis in the murine lung. *PLoS Genet*. 2013;9(2):e1003228.
669. Shigemura N, Sawa Y, Mizuno S, Ono M, Ohta M, Nakamura T, et al. Amelioration of pulmonary emphysema by in vivo gene transfection with hepatocyte growth factor in rats. *Circulation*. 2005;111(11):1407–14.
670. Hegab AE, Kubo H, Yamaya M, Asada M, He M, Fujino N, et al. Intranasal HGF administration ameliorates the physiologic and morphologic changes in lung emphysema. *Mol Ther*. 2008;16(8):1417–26.
671. Kennelly H, Mahon BP, English K. Human mesenchymal stromal cells exert HGF dependent cytoprotective effects in a human relevant pre-clinical model of COPD. *Sci Rep*. 2016;6:38207.
672. Nita I, Hostettler K, Tamo L, Medova M, Bombaci G, Zhong J, et al. Hepatocyte growth factor secreted by bone marrow stem cell reduce ER stress and improves repair in alveolar epithelial II cells. *Sci Rep*. 2017;7:41901.
673. Itakura A, Kurauchi O, Morikawa S, Okamura M, Furugori K, Mizutani S. Involvement of hepatocyte growth factor in formation of bronchoalveolar structures in embryonic rat lung in primary culture. *Biochem Biophys Res Commun*. 1997;241(1):98–103.
674. Kalal AA, Mohapatra S. A Comprehensive Review Exploring the Role of Bone Morphogenetic Proteins [BMP]: Biological Mechanisms. *Curr Issues Mol Biol*. 2025;47(3).
675. Wu DH, Hatzopoulos AK. Bone morphogenetic protein signaling in inflammation. *Exp Biol Med (Maywood)*. 2019;244(2):147–56.
676. Aluganti Narasimhulu C, Singla DK. The Role of Bone Morphogenetic Protein 7 (BMP-7) in Inflammation in Heart Diseases. *Cells*. 2020;9(2).
677. Chung MI, Bujnis M, Barkauskas CE, Kobayashi Y, Hogan BLM. Correction: Niche-mediated BMP/SMAD signaling regulates lung alveolar stem cell proliferation and differentiation. *Development*. 2024;151(15).
678. Yanagihara T, Zhou Q, Tsubouchi K, Revill S, Ayoub A, Gholiof M, et al. Intrinsic BMP inhibitor Gremlin regulates alveolar epithelial type II cell proliferation and differentiation. *Biochem Biophys Res Commun*. 2023;656:53–62.
679. Shafiquzzaman M, Biswas S, Li P, Mishina Y, Li B, Liu H. The noncanonical BMP signaling pathway plays an important role in club cell regeneration. *Stem Cells*. 2020;38(3):437–50.
680. Zheng X, Chen X, Hu X, Chen L, Mi N, Zhong Q, et al. Downregulated BMP-Smad1/5/8 signaling causes emphysema via dysfunction of alveolar type II epithelial cells. *J Pathol*. 2024;262(3):320–33.
681. Verhamme FM, De Smet EG, Van Hooste W, Delanghe J, Verleden SE, Joos GF, et al. Bone morphogenetic protein 6 (BMP-6) modulates lung function, pulmonary iron levels and cigarette smoke-induced inflammation. *Mucosal Immunol*. 2019;12(2):340–51.
682. Loth DW, Soler Artigas M, Gharib SA, Wain LV, Franceschini N, Koch B, et al. Genome-wide association analysis identifies six new loci associated with forced vital capacity. *Nat Genet*. 2014;46(7):669–77.
683. Barta I, Paska C, Antus B. Sputum Cytokine Profiling in COPD: Comparison Between Stable Disease and Exacerbation. *Int J Chron Obstruct Pulmon Dis*. 2022;17:1897–908.
684. Liu L, Tang Z, Zeng Q, Qi W, Zhou Z, Chen D, et al. Transcriptomic Insights into Different Stimulation Intensity of Electroacupuncture in Treating COPD in Rat Models. *J Inflamm Res*. 2024;17:2873–87.
685. Andriopoulos B, Jr., Corradini E, Xia Y, Faasse SA, Chen S, Grgurevic L, et al. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat Genet*. 2009;41(4):482–7.
686. Benn A, Hiepen C, Osterland M, Schutte C, Zwijsen A, Knaus P. Role of bone morphogenetic proteins in sprouting angiogenesis: differential BMP receptor-dependent signaling pathways balance stalk vs. tip cell competence. *FASEB J*. 2017;31(11):4720–33.
687. Langenfeld EM, Langenfeld J. Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. *Mol Cancer Res*. 2004;2(3):141–9.
688. Melincovici CS, Bosca AB, Susman S, Marginean M, Mihu C, Istrate M, et al. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol*. 2018;59(2):455–67.

689. Poto R, Loffredo S, Palestra F, Marone G, Patella V, Varricchi G. Angiogenesis, Lymphangiogenesis, and Inflammation in Chronic Obstructive Pulmonary Disease (COPD): Few Certainties and Many Outstanding Questions. *Cells*. 2022;11(10).
690. Zhao W, McCallum SA, Xiao Z, Zhang F, Linhardt RJ. Binding affinities of vascular endothelial growth factor (VEGF) for heparin-derived oligosaccharides. *Biosci Rep*. 2012;32(1):71–81.
691. Claesson-Welsh L. VEGF receptor signal transduction - A brief update. *Vascul Pharmacol*. 2016;86:14–7.
692. Kanazawa H, Hirata K, Yoshikawa J. Imbalance between vascular endothelial growth factor and endostatin in emphysema. *Eur Respir J*. 2003;22(4):609–12.
693. Tuder RM, Yun JH. Vascular endothelial growth factor of the lung: friend or foe. *Curr Opin Pharmacol*. 2008;8(3):255–60.
694. Wang L, Xu Z, Chen B, He W, Hu J, Zhang L, et al. The Role of Vascular Endothelial Growth Factor in Small-airway Remodelling in a Rat Model of Chronic Obstructive Pulmonary Disease. *Sci Rep*. 2017;7:41202.
695. Nguyen Hai C, Bui Duc T, Nguyen Minh T, Trinh Duc L, Tran Quyet T. Quantitative chest computed tomography in chronic obstructive pulmonary disease: assessing the role of emphysema severity and its correlation with clinical characteristics, lung function, and plasma levels of VEGF and IL-1beta. *Ther Adv Respir Dis*. 2025;19:17534666251332469.
696. Chis AF, Rajnoveanu RM, Man MA, Todea DA, Chis BA, Stancu B, et al. Increased Vascular Endothelial Growth Factor Serum Level and the Role of +936C/T Gene Polymorphism in Chronic Obstructive Pulmonary Disease. *Medicina (Kaunas)*. 2021;57(12).
697. Pinto-Plata V, Toso J, Lee K, Park D, Bilello J, Mullerova H, et al. Profiling serum biomarkers in patients with COPD: associations with clinical parameters. *Thorax*. 2007;62(7):595–601.
698. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest*. 2000;106(11):1311–9.
699. Adini A, Ko VH, Puder M, Louie SM, Kim CF, Baron J, et al. PR1P, a VEGF-stabilizing peptide, reduces injury and inflammation in acute lung injury and ulcerative colitis animal models. *Front Immunol*. 2023;14:1168676.
700. Adini A, Wu H, Dao DT, Ko VH, Yu LJ, Pan A, et al. PR1P Stabilizes VEGF and Upregulates Its Signaling to Reduce Elastase-induced Murine Emphysema. *Am J Respir Cell Mol Biol*. 2020;63(4):452–63.
701. Deckx S, Heymans S, Papageorgiou AP. The diverse functions of osteoglycin: a deceitful dwarf, or a master regulator of disease? *FASEB J*. 2016;30(8):2651–61.
702. Nulali J, Zhan M, Zhang K, Tu P, Liu Y, Song H. Osteoglycin: An ECM Factor Regulating Fibrosis and Tumorigenesis. *Biomolecules*. 2022;12(11).
703. van der Koog L, Woest ME, Gorter IC, Verschut V, Elferink RAB, Zuidhof AB, et al. Fibroblast-derived osteoglycin promotes epithelial cell repair. *NPJ Regen Med*. 2025;10(1):16.
704. Koloko Ngassie ML, De Vries M, Borghuis T, Timens W, Sin DD, Nickle D, et al. Age-associated differences in the human lung extracellular matrix. *Am J Physiol Lung Cell Mol Physiol*. 2023;324(6):L799–L814.
705. Lin YZ, Zhong XN, Chen X, Liang Y, Zhang H, Zhu DL. Roundabout signaling pathway involved in the pathogenesis of COPD by integrative bioinformatics analysis. *Int J Chron Obstruct Pulmon Dis*. 2019;14:2145–62.
706. Shi S, Li H. Overexpressed microRNA-140 inhibits pulmonary fibrosis in interstitial lung disease via the Wnt signaling pathway by downregulating osteoglycin. *Am J Physiol Cell Physiol*. 2020;319(5):C895–C905.
707. Zuo C, Li X, Huang J, Chen D, Ji K, Yang Y, et al. Osteoglycin attenuates cardiac fibrosis by suppressing cardiac myofibroblast proliferation and migration through antagonizing lysophosphatidic acid 3/matrix metalloproteinase 2/epidermal growth factor receptor signalling. *Cardiovasc Res*. 2018;114(5):703–12.
708. Xu T, Zhang R, Dong M, Zhang Z, Li H, Zhan C, et al. Osteoglycin (OGN) Inhibits Cell Proliferation and Invasiveness in Breast Cancer via PI3K/Akt/mTOR Signaling Pathway. *Onco Targets Ther*. 2019;12:10639–50.

709. Lee NJ, Ali N, Zhang L, Qi Y, Clarke I, Enriquez RF, et al. Osteoglycin, a novel coordinator of bone and glucose homeostasis. *Mol Metab*. 2018;13:30–44.
710. Piao Y, Yun SY, Fu Z, Jang JM, Back MJ, Kim HH, et al. Recombinant Human HAPLN1 Mitigates Pulmonary Emphysema by Increasing TGF-beta Receptor I and Sirtuins Levels in Human Alveolar Epithelial Cells. *Mol Cells*. 2023;46(9):558–72.
711. Gao C, Fujinawa R, Yoshida T, Ueno M, Ota F, Kizuka Y, et al. A keratan sulfate disaccharide prevents inflammation and the progression of emphysema in murine models. *Am J Physiol Lung Cell Mol Physiol*. 2017;312(2):L268–L76.
712. Obeng-Nyarkoh PI, Valentin-Figueroa ME, Cadham CJ, Cobb NK, Han MK, Levy DT, et al. Lung Cancer Risk in US Adults with COPD: A Systematic Review and Meta-Analysis. *Int J Chron Obstruct Pulmon Dis*. 2025;20:3399–421.
713. Dou D, Lu J, Dou J, Huo Y, Gong X, Zhang X, et al. Global regulatory considerations and practices for tumorigenicity evaluation of cell-based therapy. *Regul Toxicol Pharmacol*. 2025;156:105769.
714. Lai S, Guo Z. Stem cell therapies for chronic obstructive pulmonary disease: mesenchymal stem cells as a promising treatment option. *Stem Cell Res Ther*. 2024;15(1):312.
715. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. *Signal Transduct Target Ther*. 2022;7(1):272.
716. RespiratoryDraftingGroup. Guideline on clinical investigation of medicinal products in the treatment of chronic obstructive pulmonary disease (COPD) 2012 [Available from: https://www.tga.gov.au/sites/default/files/2024-06/guideline-clinical-investigation-medicinal-products-treatment-chronic-obstructive-pulmonary-disease-copd-ema.pdf.
717. Jones PW, Beeh KM, Chapman KR, Decramer M, Mahler DA, Wedzicha JA. Minimal clinically important differences in pharmacological trials. *Am J Respir Crit Care Med*. 2014;189(3):250–5.
718. Donohue JF. Minimal clinically important differences in COPD lung function. *COPD*. 2005;2(1):111–24.
719. Dankers M, Nelissen-Vrancken M, Surminski SMK, Lambooij AC, Schermer TR, van Dijk L. Healthcare Professionals' Preferred Efficacy Endpoints and Minimal Clinically Important Differences in the Assessment of New Medicines for Chronic Obstructive Pulmonary Disease. *Front Pharmacol*. 2019;10:1519.
720. Make B. How can we assess outcomes of clinical trials: the MCID approach. *COPD*. 2007;4(3):191–4.
721. Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. *Eur Respir J*. 2008;31(2):416–69.
722. Cazzola M, Rogliani P, Barnes PJ, Blasi F, Celli B, Hanania NA, et al. An Update on Outcomes for COPD Pharmacological Trials: A COPD Investigators Report - Reassessment of the 2008 American Thoracic Society/European Respiratory Society Statement on Outcomes for COPD Pharmacological Trials. *Am J Respir Crit Care Med*. 2023;208(4):374–94.
723. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med*. 2008;359(15):1543–54.
724. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med*. 2007;356(8):775–89.
725. Miller BE, Tal-Singer R, Rennard SI, Furtwaengler A, Leidy N, Lowings M, et al. Plasma Fibrinogen Qualification as a Drug Development Tool in Chronic Obstructive Pulmonary Disease. Perspective of the Chronic Obstructive Pulmonary Disease Biomarker Qualification Consortium. *Am J Respir Crit Care Med*. 2016;193(6):607–13.
726. Groenewegen KH, Postma DS, Hop WC, Wielders PL, Schlosser NJ, Wouters EF, et al. Increased systemic inflammation is a risk factor for COPD exacerbations. *Chest*. 2008;133(2):350–7.
727. Hurst JR, Vestbo J, Anzueto A, Locantore N, Mullerova H, Tal-Singer R, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med*. 2010;363(12):1128–38.
728. Fibrinogen Studies C, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA*. 2005;294(14):1799–809.

729. Mannino DM, Valvi D, Mullerova H, Tal-Singer R. Fibrinogen, COPD and mortality in a nationally representative U.S. cohort. *COPD*. 2012;9(4):359–66.
730. Zuur-Telgen MC, Citgez E, Zuur AT, VanderValk P, van der Palen J, Kerstjens HAM, et al. Predicting Mortality in COPD with Validated and Sensitive Biomarkers; Fibrinogen and Mid-Range-Proadrenomedullin (MR-proADM). *COPD*. 2021;18(6):643–9.
731. Hou Y, Yao H, Lin JM. Recent advancements in single-cell metabolic analysis for pharmacological research. *J Pharm Anal*. 2023;13(10):1102–16.
732. Lee S, Vu HM, Lee JH, Lim H, Kim MS. Advances in Mass Spectrometry-Based Single Cell Analysis. *Biology (Basel)*. 2023;12(3).
733. Chen C, Wang J, Pan D, Wang X, Xu Y, Yan J, et al. Applications of multi-omics analysis in human diseases. *MedComm (2020)*. 2023;4(4):e315.
734. Van de Sande B, Lee JS, Mutasa-Gottgens E, Naughton B, Bacon W, Manning J, et al. Applications of single-cell RNA sequencing in drug discovery and development. *Nat Rev Drug Discov*. 2023;22(6):496–520.
735. Yonchuk JG, Silverman EK, Bowler RP, Agusti A, Lomas DA, Miller BE, et al. Circulating soluble receptor for advanced glycation end products (sRAGE) as a biomarker of emphysema and the RAGE axis in the lung. *Am J Respir Crit Care Med*. 2015;192(7):785–92.
736. Kakishita M, Nishikimi T, Okano Y, Satoh T, Kyotani S, Nagaya N, et al. Increased plasma levels of adrenomedullin in patients with pulmonary hypertension. *Clin Sci (Lond)*. 1999;96(1):33–9.
737. Stolz D, Christ-Crain M, Morgenthaler NG, Miedinger D, Leuppi J, Muller C, et al. Plasma pro-adrenomedullin but not plasma pro-endothelin predicts survival in exacerbations of COPD. *Chest*. 2008;134(2):263–72.
738. Zien Alaabden A, Mohammad Y, Fahoum S. The role of serum surfactant protein D as a biomarker of exacerbation of chronic obstructive pulmonary disease. *Qatar Med J*. 2015;2015(2):18.
739. Sin DD, Leung R, Gan WQ, Man SP. Circulating surfactant protein D as a potential lung-specific biomarker of health outcomes in COPD: a pilot study. *BMC Pulm Med*. 2007;7:13.
740. Bowler RP. Surfactant protein D as a biomarker for chronic obstructive pulmonary disease. *COPD*. 2012;9(6):651–3.
741. Moreno D, Garcia A, Lema D, De Sanctis JB. Surfactant protein D in chornic obstructive pulmonary disease (COPD). *Recent Pat Endocr Metab Immune Drug Discov*. 2014;8(1):42–7.
742. Pantazopoulos I, Magounaki K, Kotsiou O, Rouka E, Perlikos F, Kakavas S, et al. Incorporating Biomarkers in COPD Management: The Research Keeps Going. *J Pers Med*. 2022;12(3).
743. Mannino DM. Biomarkers for chronic obstructive pulmonary disease diagnosis and progression: insights, disappointments and promise. *Curr Opin Pulm Med*. 2019;25(2):144–9.
744. Zemans RL, Jacobson S, Keene J, Kechris K, Miller BE, Tal-Singer R, et al. Multiple biomarkers predict disease severity, progression and mortality in COPD. *Respir Res*. 2017;18(1):117.
745. Celli BR, Anderson JA, Brook R, Calverley P, Cowans NJ, Crim C, et al. Serum biomarkers and outcomes in patients with moderate COPD: a substudy of the randomised SUMMIT trial. *BMJ Open Respir Res*. 2019;6(1):e000431.
746. Hlapcic I, Belamaric D, Bosnar M, Kifer D, Vukic Dugac A, Rumora L. Combination of Systemic Inflammatory Biomarkers in Assessment of Chronic Obstructive Pulmonary Disease: Diagnostic Performance and Identification of Networks and Clusters. *Diagnostics (Basel)*. 2020;10(12).
747. Vanfleteren L, Weidner J, Franssen FME, Gaffron S, Reynaert NL, Wouters EFM, et al. Biomarker-based clustering of patients with chronic obstructive pulmonary disease. *ERJ Open Res*. 2023;9(1).
748. Yousuf A, McAuley H, Elneima O, Brightling CE. The different phenotypes of COPD. *Br Med Bull*. 2021;137(1):82–97.
749. Guarnier LP, Moro LG, Livero F, de Faria CA, Azevedo MF, Roma BP, et al. Regenerative and translational medicine in COPD: hype and hope. *Eur Respir Rev*. 2023;32(169).
750. Zhang Z, Wang J, Li Y, Liu F, Chen L, He S, et al. Proteomics and metabolomics profiling reveal panels of circulating diagnostic biomarkers and molecular subtypes in stable COPD. *Respir Res*. 2023;24(1):73.
751. Kan M, Shumyatcher M, Himes BE. Using omics approaches to understand pulmonary diseases. *Respir Res*. 2017;18(1):149.

752. Sikkema L, Ramirez-Suastegui C, Strobl DC, Gillett TE, Zappia L, Madissoon E, et al. An integrated cell atlas of the lung in health and disease. *Nat Med*. 2023;29(6):1563–77.
753. Cardoso J, Ferreira AJ, Guimaraes M, Oliveira AS, Simao P, Sucena M. Treatable Traits in COPD - A Proposed Approach. *Int J Chron Obstruct Pulmon Dis*. 2021;16:3167–82.
754. Cazzola M, Rogliani P, Blasi F. Can Treatable Traits Be the Approach to Addressing the Complexity and Heterogeneity of COPD? *Int J Chron Obstruct Pulmon Dis*. 2023;18:1959–64.
755. Devulder JV. Unveiling mechanisms of lung aging in COPD: A promising target for therapeutics development. *Chin Med J Pulm Crit Care Med*. 2024;2(3):133–41.
756. Roche N, Chanez P. Bronchodilator combinations for COPD: real hopes or a new Pandora's box? *Eur Respir J*. 2013;42(6):1441–5.
757. Wu X, Chen D, Gu X, Su X, Song Y, Shi Y. Prevalence and risk of viral infection in patients with acute exacerbation of chronic obstructive pulmonary disease: a meta-analysis. *Mol Biol Rep*. 2014;41(7):4743–51.
758. Jafarinejad H, Moghoofei M, Mostafaei S, Salimian J, Azimzadeh Jamalkandi S, Ahmadi A. Worldwide prevalence of viral infection in AECOPD patients: A meta-analysis. *Microb Pathog*. 2017;113:190–6.
759. Hurst JR, Han MK, Singh B, Sharma S, Kaur G, de Nigris E, et al. Prognostic risk factors for moderate-to-severe exacerbations in patients with chronic obstructive pulmonary disease: a systematic literature review. *Respir Res*. 2022;23(1):213.
760. Dransfield MT, Kunisaki KM, Strand MJ, Anzueto A, Bhatt SP, Bowler RP, et al. Acute Exacerbations and Lung Function Loss in Smokers with and without Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2017;195(3):324–30.
761. Ray S, Chiba N, Yao C, Guan X, McConnell AM, Brockway B, et al. Rare SOX2(+) Airway Progenitor Cells Generate KRT5(+) Cells that Repopulate Damaged Alveolar Parenchyma following Influenza Virus Infection. *Stem Cell Reports*. 2016;7(5):817–25.
762. Vaughan AE, Brumwell AN, Xi Y, Gotts JE, Brownfield DG, Treutlein B, et al. Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature*. 2015;517(7536):621–5.
763. Sadiq MW, Holz O, Ellinghusen BD, Faulenbach C, Muller M, Badorrek P, et al. Lung pharmacokinetics of inhaled and systemic drugs: A clinical evaluation. *Br J Pharmacol*. 2021;178(22):4440–51.
764. Zijlstra GS, Wolting J, Prop J, Petersen AH, Hinrichs WL, Uges DR, et al. Efficacy of a new pulmonary cyclosporine a powder formulation for prevention of transplant rejection in rats. *J Heart Lung Transplant*. 2009;28(5):486–92.
765. Irwin SM, Prideaux B, Lyon ER, Zimmerman MD, Brooks EJ, Schrupp CA, et al. Bedaquiline and Pyrazinamide Treatment Responses Are Affected by Pulmonary Lesion Heterogeneity in Mycobacterium tuberculosis Infected C3HeB/FeJ Mice. *ACS Infect Dis*. 2016;2(4):251–67.
766. Anderson S, Atkins P, Backman P, Cipolla D, Clark A, Daviskas E, et al. Inhaled Medicines: Past, Present, and Future. *Pharmacol Rev*. 2022;74(1):48–118.
767. Forbes B, Backman P, Cabal A, Clark A, Ehrhardt C, Hastedt JE, et al. iBCS: 4. Application of the Inhalation Biopharmaceutics Classification System to the Development of Orally Inhaled Drug Products. *Mol Pharm*. 2025;22(4):1740–51.
768. Thakral S, Sonje J, Munjal B, Suryanarayanan R. Stabilizers and their interaction with formulation components in frozen and freeze-dried protein formulations. *Adv Drug Deliv Rev*. 2021;173:1–19.
769. Tonnis WF, Mensink MA, de Jager A, van der Voort Maarschalk K, Frijlink HW, Hinrichs WL. Size and molecular flexibility of sugars determine the storage stability of freeze-dried proteins. *Mol Pharm*. 2015;12(3):684–94.
770. Mensink MA, Frijlink HW, van der Voort Maarschalk K, Hinrichs WL. How sugars protect proteins in the solid state and during drying (review): Mechanisms of stabilization in relation to stress conditions. *Eur J Pharm Biopharm*. 2017;114:288–95.
771. Matthews AA, Ee PLR, Ge R. Developing inhaled protein therapeutics for lung diseases. *Mol Biomed*. 2020;1(1):11.

772. Berkenfeld K, Carneiro S, Corzo C, Laffleur F, Salar-Behzadi S, Winkeljann B, et al. Formulation strategies, preparation methods, and devices for pulmonary delivery of biologics. *Eur J Pharm Biopharm*. 2024;204:114530.
773. Patton JS, Fishburn CS, Weers JG. The lungs as a portal of entry for systemic drug delivery. *Proc Am Thorac Soc*. 2004;1(4):338–44.
774. Bader J, Brigger F, Leroux JC. Extracellular vesicles versus lipid nanoparticles for the delivery of nucleic acids. *Adv Drug Deliv Rev*. 2024;215:115461.
775. Jansen EM, Frijlink HW, Hinrichs WL, Ruigrok MJ. Are inhaled mRNA vaccines safe and effective? A review of preclinical studies. *Expert Opin Drug Deliv*. 2022;19(11):1471–85.
776. Costabile G, Conte G, Brusco S, Savadi P, Miro A, Quaglia F, et al. State-of-the-Art Review on Inhalable Lipid and Polymer Nanocarriers: Design and Development Perspectives. *Pharmaceutics*. 2024;16(3).
777. Zhao G, Xue L, Weiner AI, Gong N, Adams-Tzivelekidis S, Wong J, et al. TGF-betaR2 signaling coordinates pulmonary vascular repair after viral injury in mice and human tissue. *Sci Transl Med*. 2024;16(732):eadg6229.
778. Meshanni JA, Stevenson ER, Zhang D, Sun R, Ona NA, Reagan EK, et al. Targeted delivery of TGF-beta mRNA to murine lung parenchyma using one-component ionizable amphiphilic Janus Dendrimers. *Nat Commun*. 2025;16(1):1806.
779. Pasero L, Susa F, Limongi T, Pisano R. A Review on Micro and Nanoengineering in Powder-Based Pulmonary Drug Delivery. *Int J Pharm*. 2024;659:124248.
780. Yildirim AO, Conlon TM, Adcock IM, Gosens R, Lehmann M, Kapellos TS, et al. COPD-iNET: a call to the lung community for action to combat the global epidemic of COPD. *Eur Respir J*. 2024;64(3).

Figure legends

Figure 1. The healthy and disrupted alveolar niche in COPD. Alveolar epithelial progenitor cells do not function in isolation but reside within a specialized microenvironment, the alveolar progenitor niche, that orchestrates their behavior. This niche supports progenitor cell survival, regulates their proliferation and differentiation, and integrates repair signals following injury. In COPD, however, these niche interactions are disrupted, impairing repair capacity and contributing to progressive alveolar damage. Created in BioRender. Van der koog, L. (2025) https://BioRender.com/cslgb1t.

Figure 2. Activation of cAMP signaling by GPCRs including EP_2, EP_4 and IP prostanoid, A_{2A}, A_{2B} adenosine and β_2 adrenoceptors. Downstream effects have been linked to regeneration.

Figure 3: Canonical Wnt/β-catenin signaling: mechanisms of activation, degradation and pharmacological modulation. In the absence of Wnt ligand (left), cytosolic β-catenin is sequentially phosphorylated by casein kinase 1 alpha (CK1α) and glycogen synthase kinase-3β (GSK3β) within the β-catenin destruction complex, which also includes AXIN and adenomatous polyposis coli (APC). Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase adaptor β-transducin repeat–containing protein (β-TrCP), leading to polyubiquitination and proteasomal degradation.

Upon Wnt ligand engagement (right), the co-receptors Frizzled (FZD) and LRP5/6 cluster at the membrane, promoting CK1α-dependent phosphorylation and activation of DVL. This results in AXIN recruitment to the membrane, disassembly of the destruction complex, and stabilization of unphosphorylated β-catenin. Stabilized β-catenin accumulates in the cytosol, translocates into the nucleus, and interacts with T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to activate Wnt target genes.

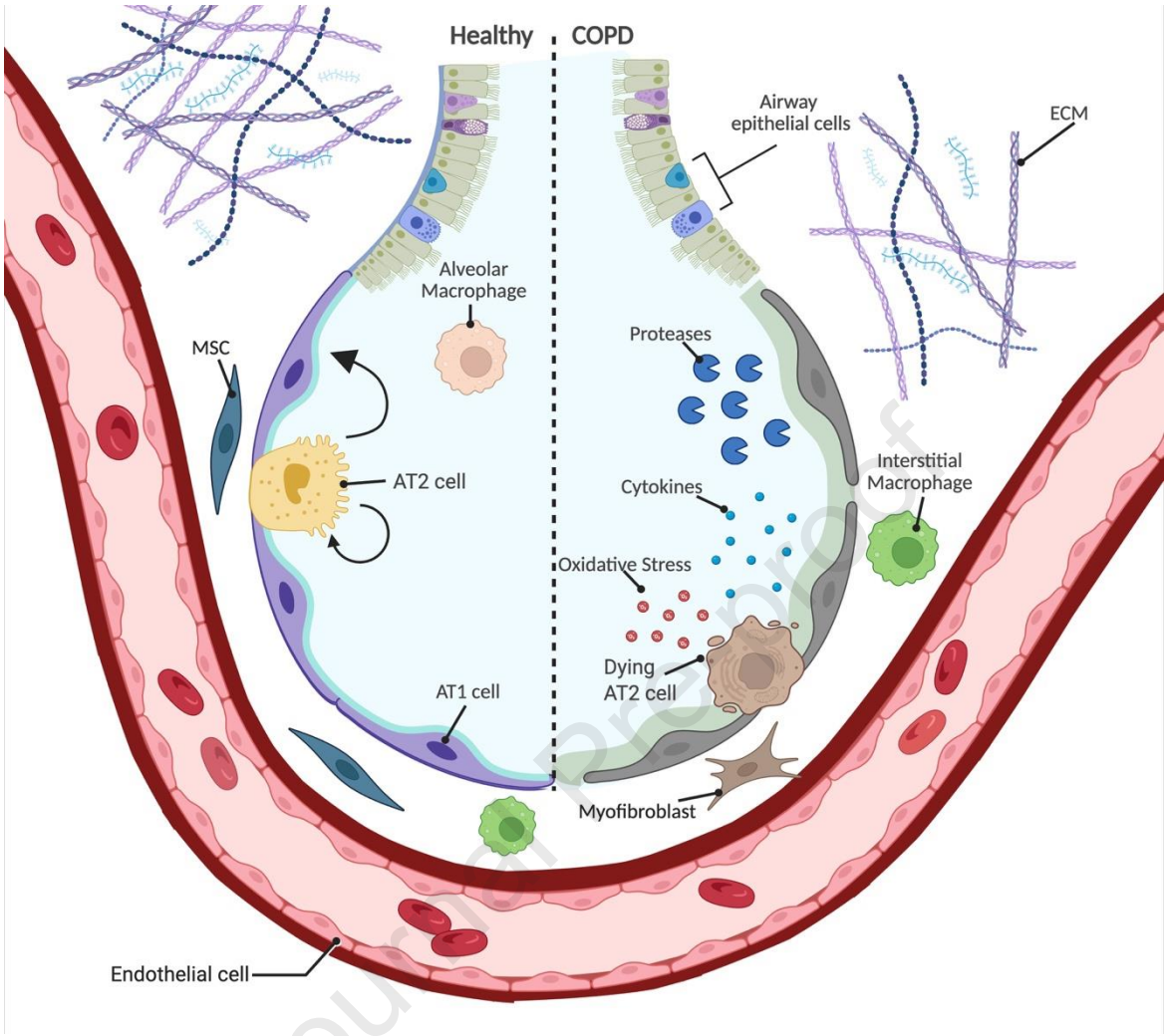
Figure 4. Mode of action of senotherapeutics. Cellular senescence is characterized by changes in mTOR signaling, NF-κB signaling, cGAS/STING signaling, mitochoncrial dysfunction and oxidative stress. Commonly used senotherapeutics target one or more of these pathways to balance cellular signaling (senomorphics) or to eliminate senescent cells (senolytics).

Figure 5. Interplay of persistent inflammation with defective repair. In otherwise healthy subjects without COPD (left panel), bacterial or viral infections will trigger NLRP3 inflammasome-dependent inflammation and a drop in lung function; this is followed by a resolution phase during which epithelial injury is repaired by signals such as WNT, or IL-1β. In COPD, there is a background of persistent inflammation that interferes with repair signals and/or negatively imprints on epithelial progenitors, leading to incomplete repair.

Figure 6. Mining the niche for regenerative therapeutics. The alveolar niche has well-established roles in guiding epithelial repair and regeneration, and many secreted factors derived from lung fibroblasts, endothelial cells and immune cells have been identified. Some of these have been utilized as drugs or as leads for drug development. Created in BioRender. Van der koog, L. (2025) https://BioRender.com/o0uhysv.

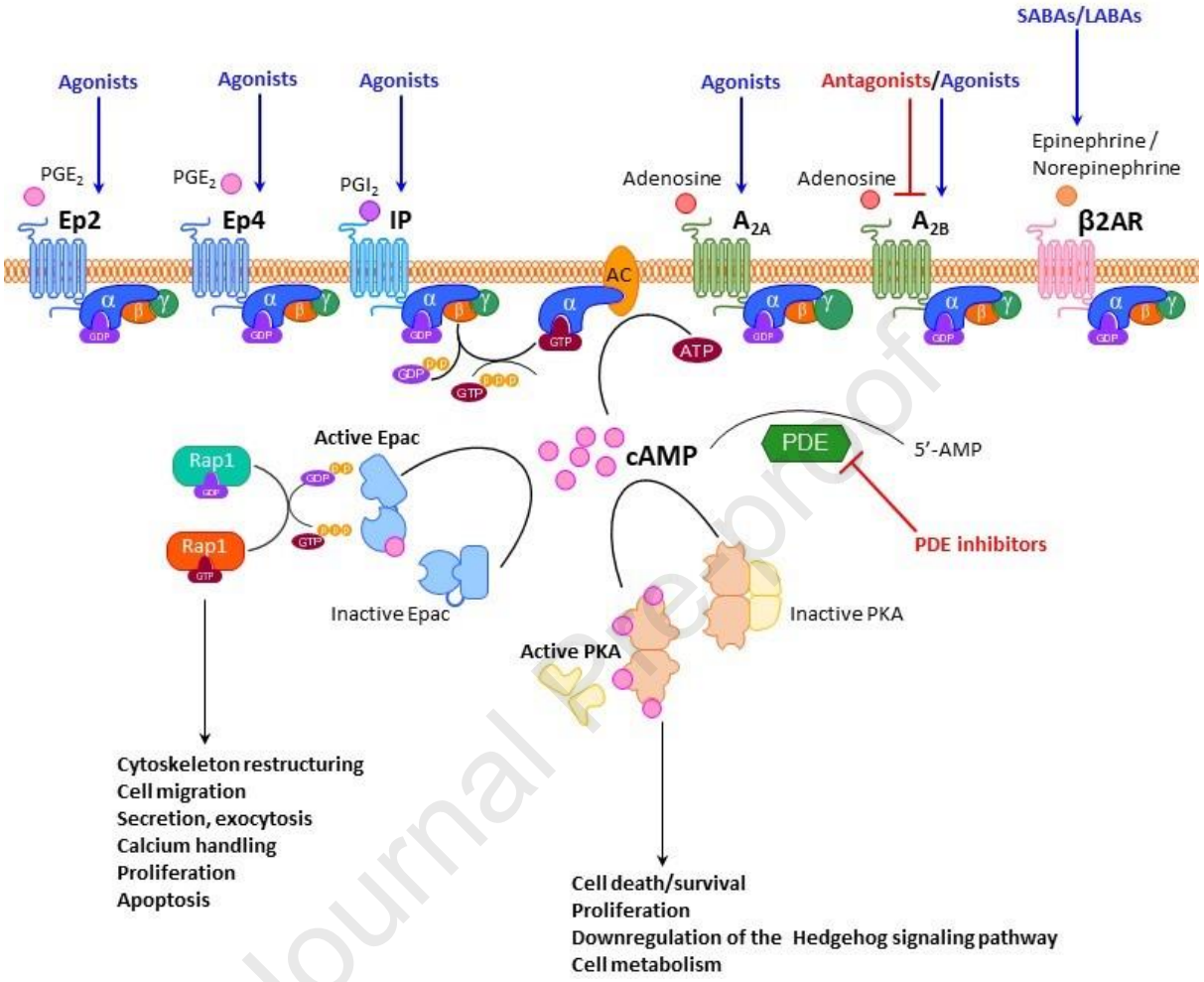
Figure 7. Progressive lung function loss in patients with COPD throughout life. A: Exposure to smoke, air pollution and toxins can cause lung injury, which – if not adequately repaired – contribute to loss of lung function, which is progressive and results in systemic comorbidities, and eventually respiratory failure and death. B: Revised view on lung function loss in COPD. At present, lung function loss in COPD is no longer viewed as a gradual decline as depicted in panel A, but as an intermittent process driven by episodes of disease worsening (exacerbations). These episodes are often associated with bacterial and viral infections and represent opportunities for targeted pharmacological treatment.

4792 **Figure 1**



4793

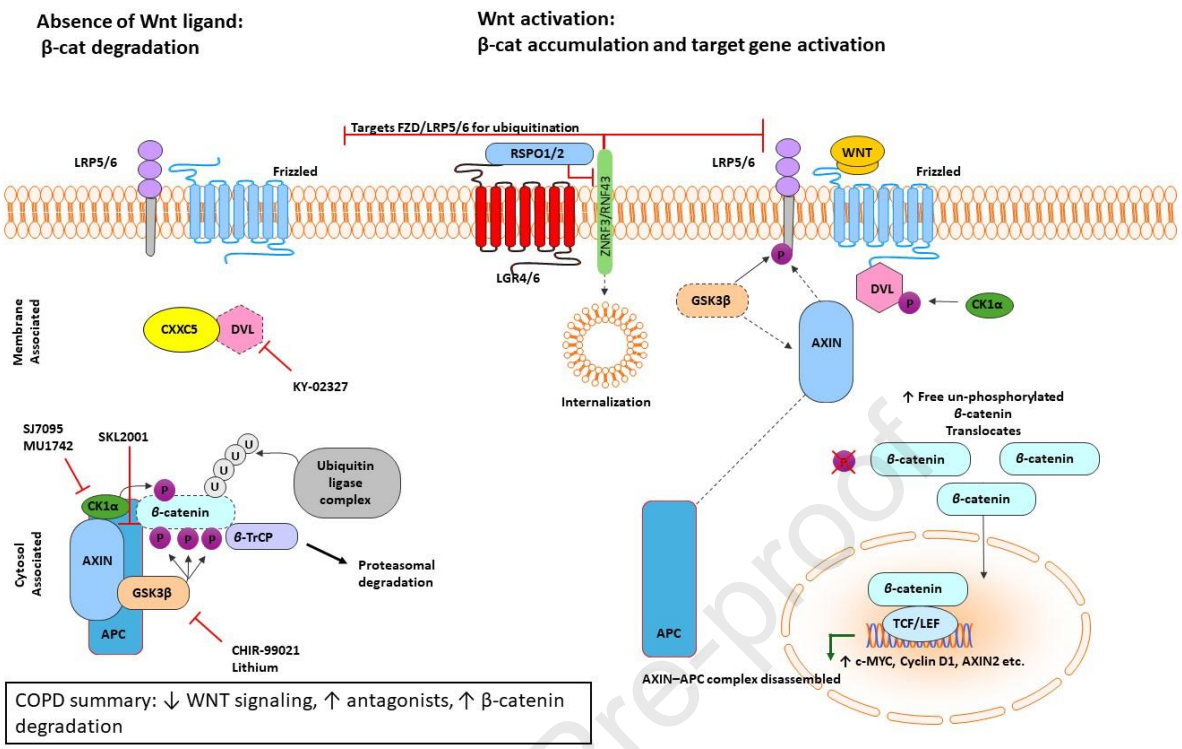
4794 **Figure 2**



4795

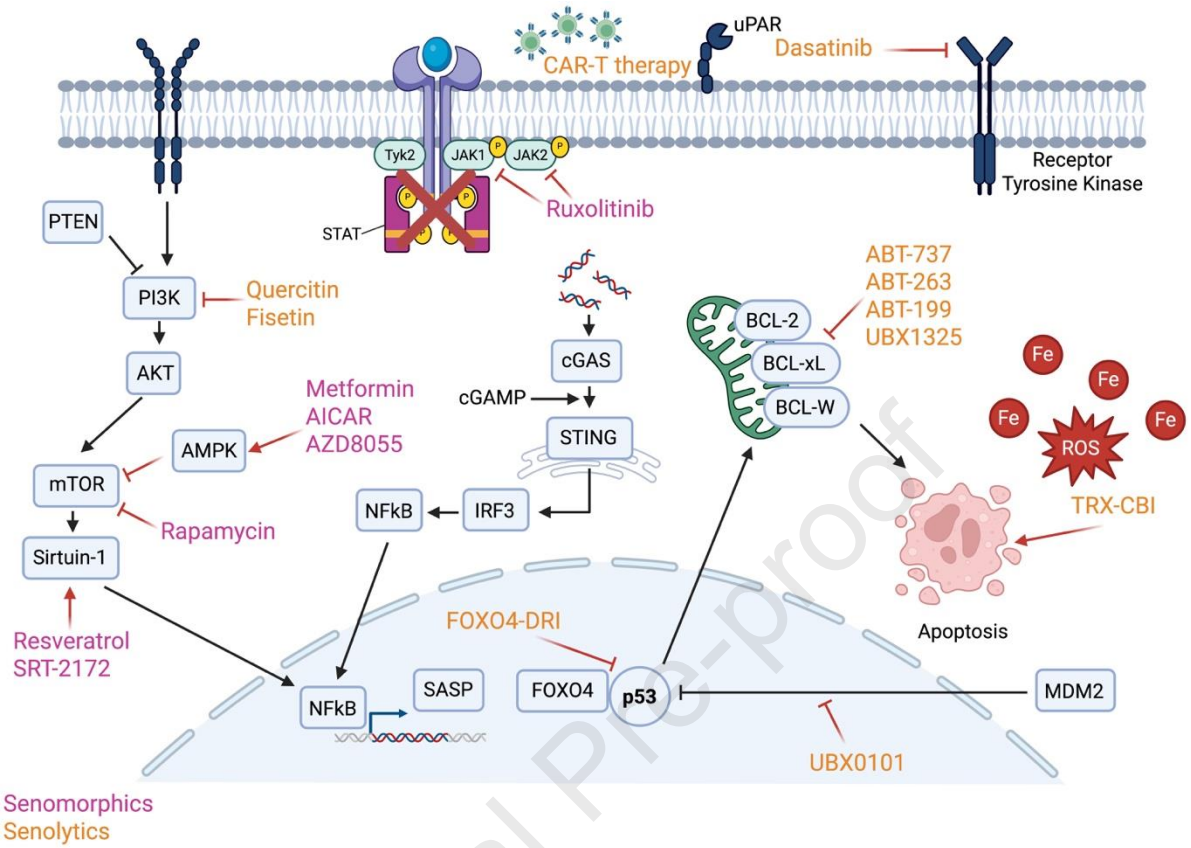
4796

4797 **Figure 3**

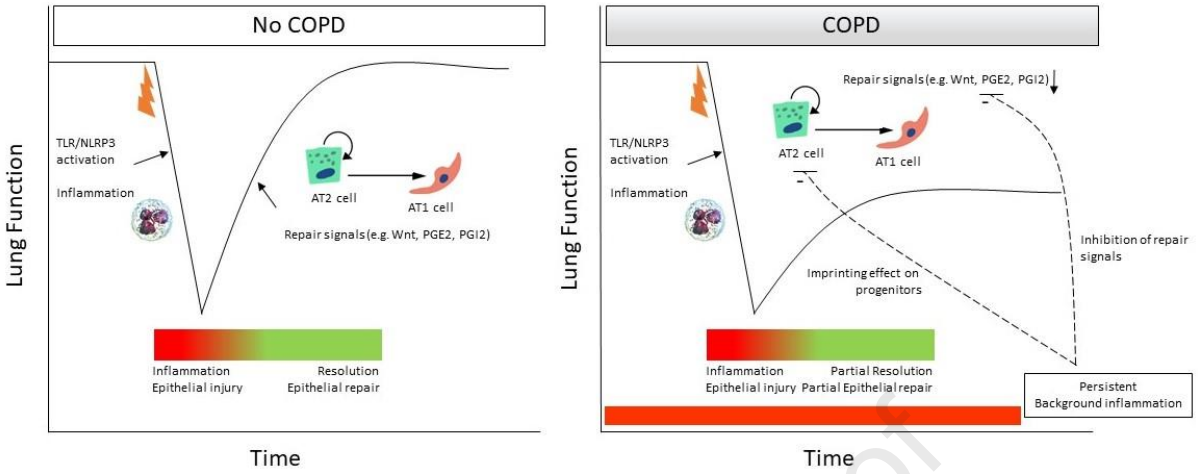


4798

Figure 4



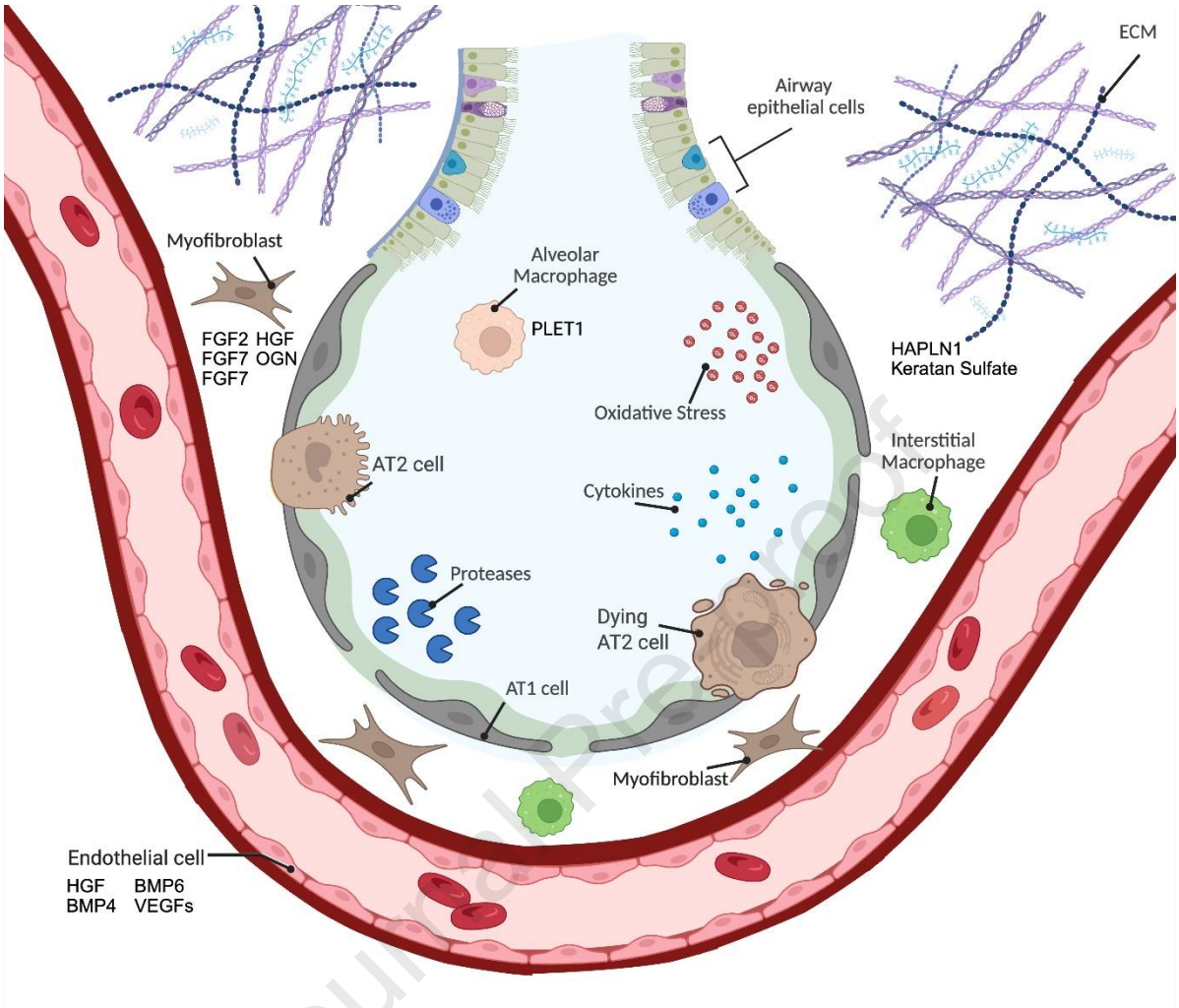
4801 **Figure 5**



4802

4803

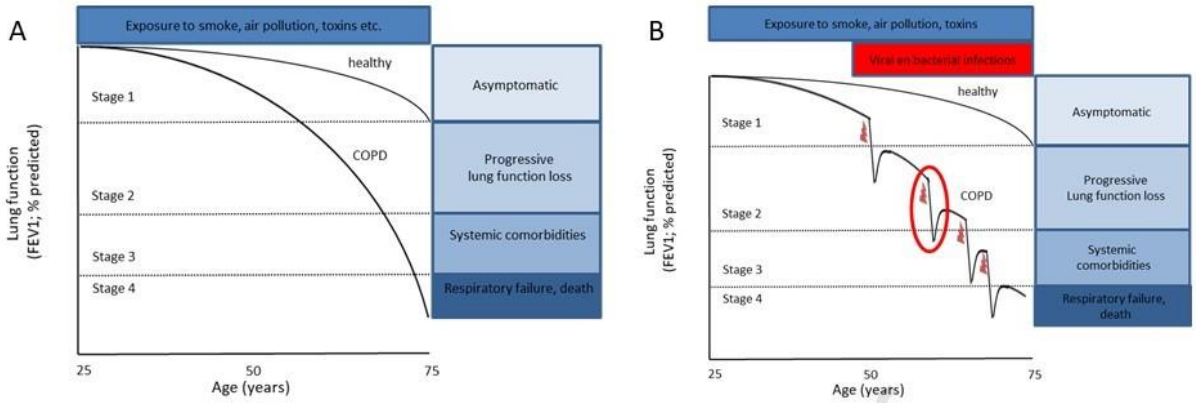
4804 **Figure 6**



4805

4806

4807 **Figure 7**



4808

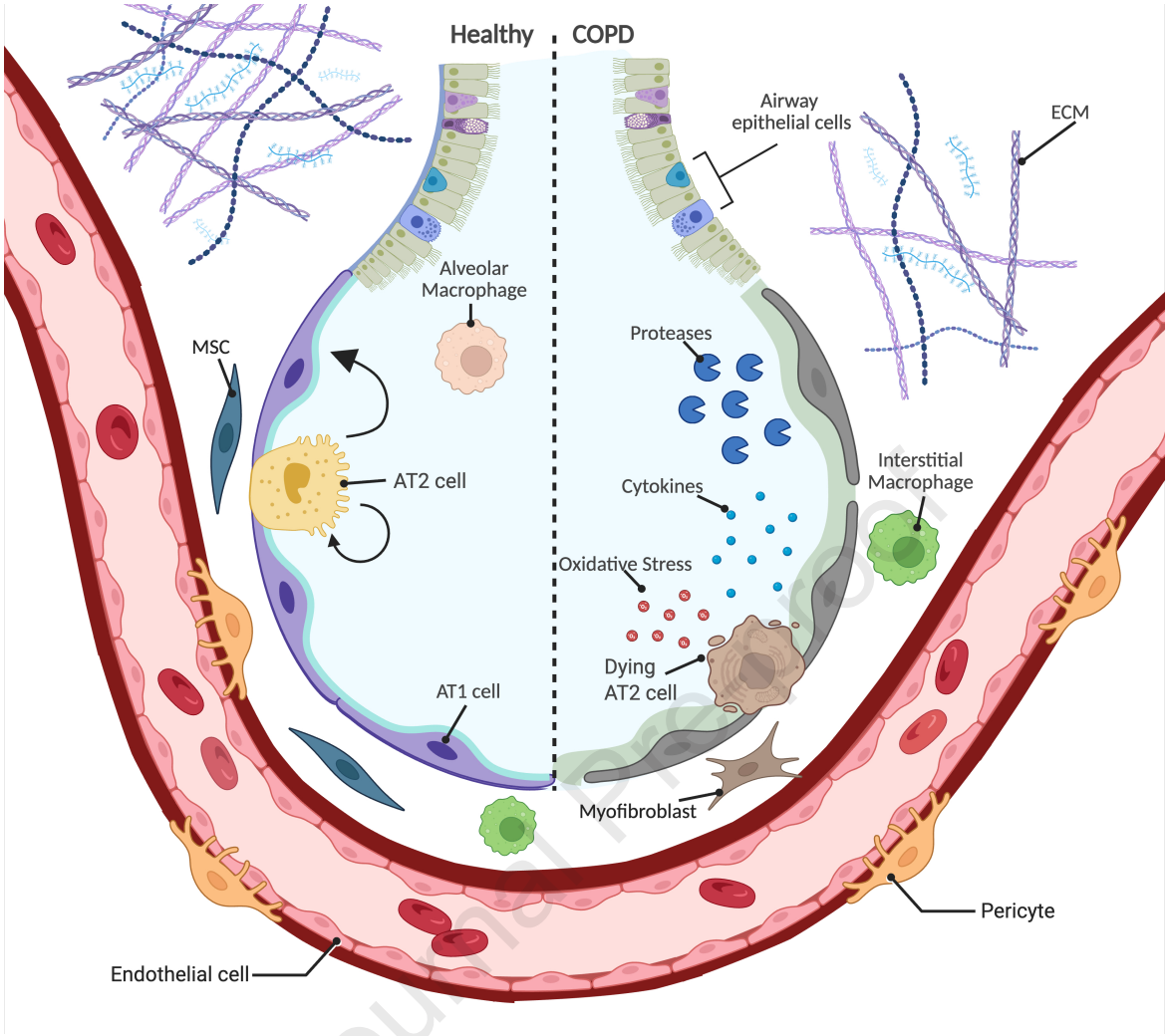
4809 **Table 1: EVs as mediators of tissue repair and regeneration in COPD**

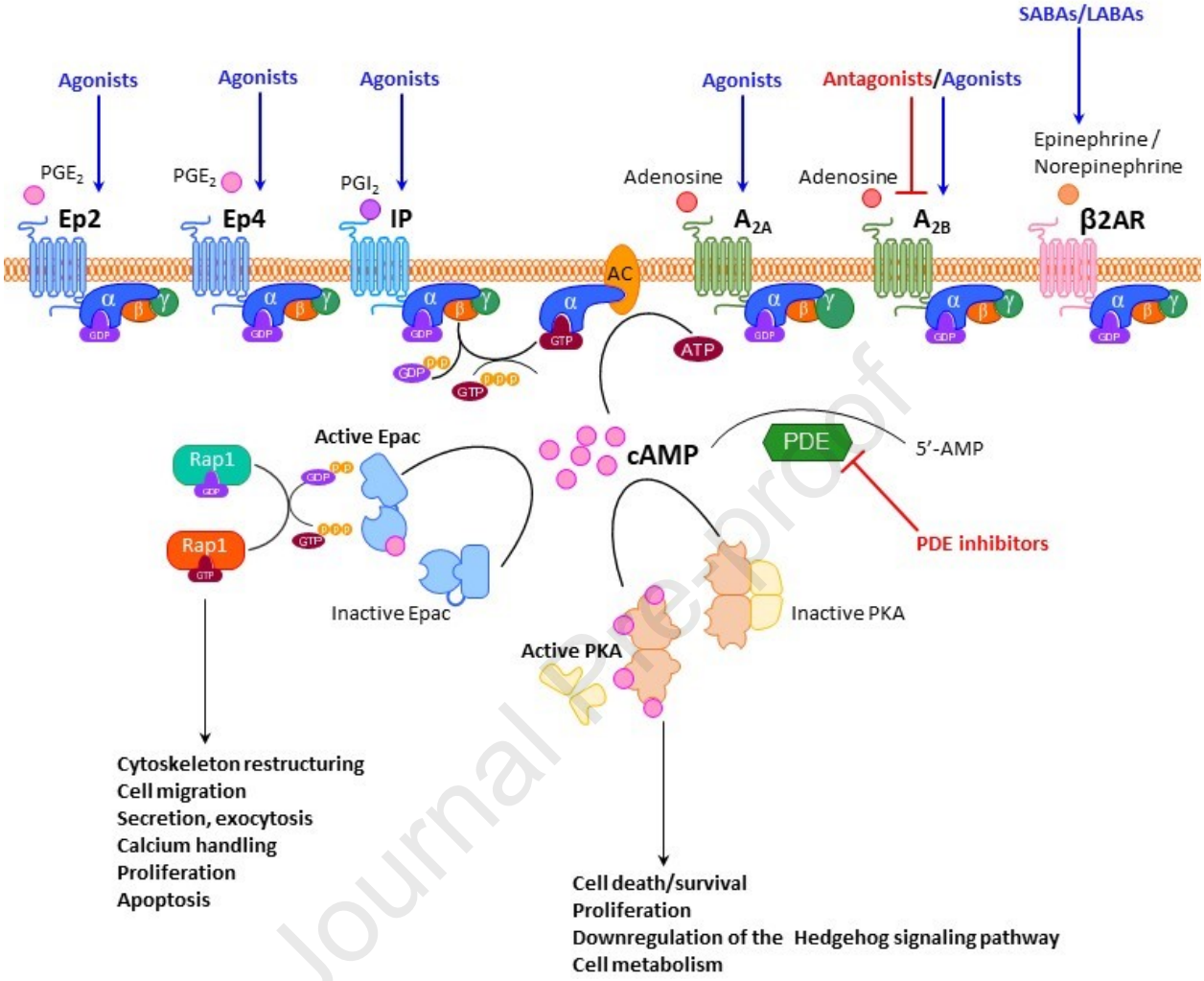
EV source	Isolation method	Concentration / Dose / Time frame	Preclinical COPD model employed	Route of administration	Effects observed	Mechanism of action	Reference
Human lung fibroblasts (MRC5)	Ultrafiltration and SEC	$1.5x10^9$ and $4.5x10^9$ EVs, 5 doses, 8 days	Elastase-induced lung injury in mice	Intratracheal	Improved lung function and reduced lung injury	Not mentioned	[149]
Human umbilical cord MSCs	Ultracentrifugation	EVs isolated from $2.5x10^6$ cells, once	12-week CS model in rats	Intratracheal	Reduced inflammation and decreased emphysema	Modulation of the NF-κB pathway	[601]
Transfected (WNT-3A) HEK293T cells	Ultracentrifugation	$2x10^9$ EVs, 4 doses, 14 days	Elastase-induced lung injury in mice	Intravenous	Improved lung function and reduced lung injury	WNT-3A signaling	[65]

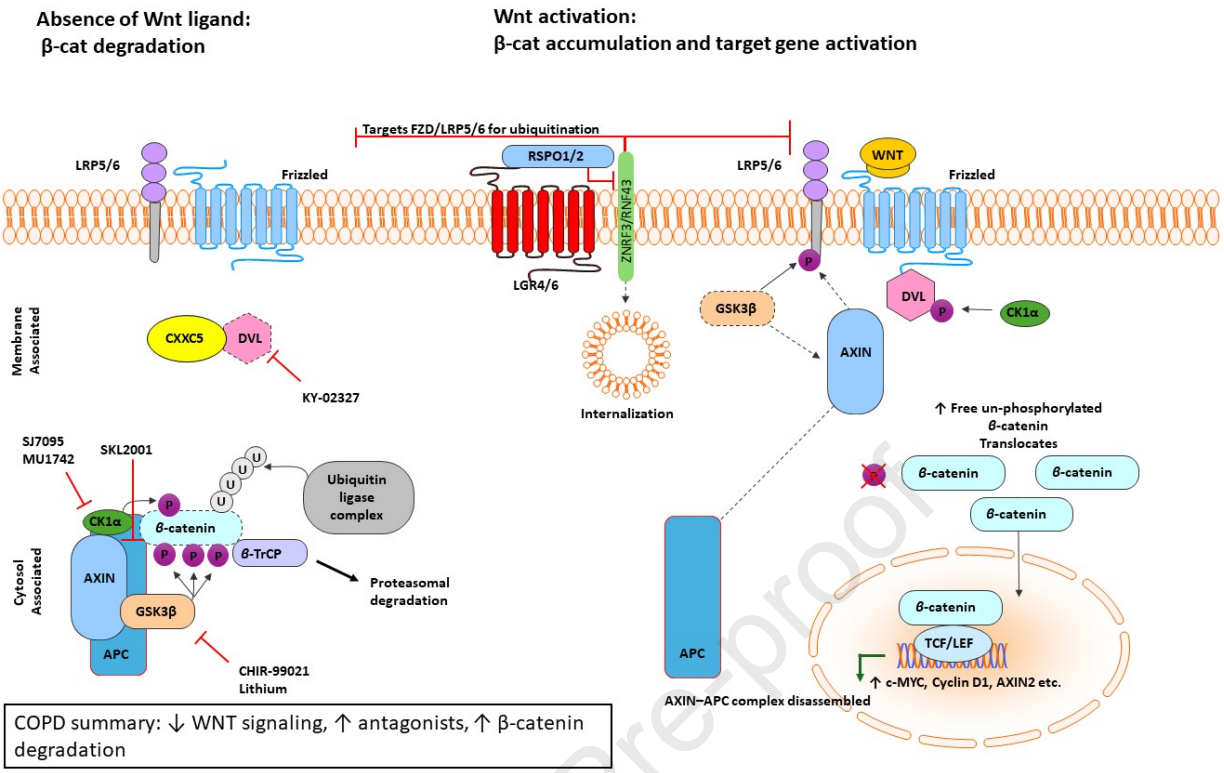
Healthy and emphysematous MSCs	Ultracentrifugation	EVs isolated from $1x10^6$ cells, once	Elastase-induced lung injury in mice	Intravenous	Healthy EVs reduced lung injury and inflammation	Reductio of pro-inflammatory cytokines (IL-1β, TGF-β, and IL-10)	[602]
Human adipose-derived stem cells	Ultracentrifugation	Dose unclear (based on protein concentration), once, 14 days	Elastase-induced lung injury in mice	Intratracheal	No improvement on lung injury	FGF2 signaling	[604]
Human platelets	Not mentioned	$2.5x10^{10}$ and $5.0x10^{10}$ EVs/mL, 12 doses	16-week CS model in mice	Nebulized	Improved lung function and reduced lung injury	Reduced NF-κB activation and apoptosis	[605]
Human bone marrow-derived MSCs	Ultracentrifugation	EVs isolated from $4x10^6$ cells, once	16-week CS model in mice	Intratracheal and intravenous	Intratracheal administration reduced lung injury, intravenous not	Not mentioned	[603]

Human bone marrow-derived MSCs	Size exclusion chromatography and affinity chromatography	$0.5x10^8$, $1.0x10^8$ or $1.5x10^8$ EVs/kg, 5 doses	4-week CS model + intratracheal LPS in rats	Nebulized and intravenous	Improved lung function, reduced inflammation. Most pronounced with lowest dose, no effect intravenous	Suppression of the WNT/β-catenin signaling pathway	[541]

4810







COPD summary: ↓ WNT signaling, ↑ antagonists, ↑ β-catenin degradation