Preclinical models of COPD: a state of the art review

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COPD is a progressive heterogeneous disease with no disease-modifying treatments. *In vivo*, *ex vivo* and *in silico* preclinical models have helped gain insight into the disease. We need to improve and integrate human-relevant models for better COPD endotyping. https://bit.ly/3QZUzVu

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Abstract

COPD is a progressive and heterogeneous condition characterised by varying combinations of emphysema, small airway disease, chronic bronchitis and exacerbations. Although multiple symptomatic therapies exist, no disease-modifying treatments are available. This gap highlights the need for improved preclinical models with greater translational relevance. Large human cohorts and single-cell/multi-omics studies have informed the development of current COPD models. We provide a state of the art review of the major experimental platforms: *in vivo* (small and large animals, genetic and injury models, environmental exposures), *ex vivo* (precision-cut lung slices, organoids, co-cultures, lung-on-a-chip systems) and *in silico* (aerosol dispersion and computational tools). While each approach has yielded important mechanistic insights, none fully captures the complexity of COPD progression, comorbidities, gene–environment interactions or heterogeneous clinical endotypes. Future progress will depend on the development of more integrated, human-relevant modelling systems, alongside advanced exposure platforms and artificial intelligence-driven multi-omics integration to identify biologically meaningful endotypes and speed the creation of phenotype-specific therapies. We propose a phenotype-driven, cross-platform framework in which hypotheses emerging from human clinical and omics data are validated in *in vitro* and *ex vivo* systems, evaluated in phenotype-specific *in vivo* models, and ultimately confirmed through clinical studies.

Introduction

COPD is a progressive, irreversible and multifaceted disease, frequently characterised by rapid loss of lung function [1, 2]. It currently affects over 400 million people and remains steadily the third leading cause of

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death worldwide [1]. The term COPD serves as an umbrella for several clinical phenotypes defined by architectural lung damage, emphysema, airway disease and chronic bronchitis, and the superimposition of exacerbations that further deteriorate lung function [3]. COPD complexity is amplified by comorbidities such as cardiovascular disease, metabolic syndrome, osteoporosis, sarcopenia and an array of mental health issues that complicate holistic management [2].

Several advances have been made in developing symptomatic treatments, including bronchodilators, inhaled and oral corticosteroids, pulmonary rehabilitation and biological therapies. However, clinicians are still facing unmet therapeutic needs, especially the lack of disease-modifying agents that can either halt or reverse disease progression [4–6]. These knowledge gaps remain while COPD continues to pose a public health emergency [1, 7]. To effectively address this emergency, refined and enhanced preclinical models that can improve translational efficacy and fast-track discoveries of innovative therapies are urgently required.

Although COPD is a heterogeneous and immunologically complex disorder with multiple phenotypes, most models oversimplify its biology and fail to reflect the chronic, cumulative and systemic features observed in patients. Ideally, a preclinical animal model must align with the human disease it simulates. Such alignment is done using direct pathological information and/or cell culture and organoid experimental results. However, for COPD, owing to its complexity of endo-phenotypes, it has been large longitudinal observational studies with their rich datasets containing potential genetic traits [8] and biomarkers that have informed animal model development. These patient cohorts have facilitated the discovery of putative biomarkers of disease trajectories [9], including those of exacerbations [10–14], lung function decline [15–19] and mortality [20–22]. As powerful as results of these large cohorts have been, they have inherent limitations. Notably, the findings have been predominantly associative and not predictive, and even with multi-omics analysis mechanistic insights have been limited. Hence, a complementary role for animal and next-generation models continues to exist.

Modelling COPD beyond cigarette smoke

COPD modelling is complicated by the reality that active cigarette smoke (CS) is only one of several aetiologic drivers, including environmental exposures. Biomass is an important modifiable lifestyle factor in low-income countries [23–26]. Biomass or total particulate matter is the product of burning solid organic fuel [23–26], either derived from plants (such as wood smoke from wood stoves or forest fires) or from animal sources (like fossil fuels). The Tasmanian Longitudinal Health Study showed an association between wood stove smoke, smoking and lung function decline in 3314 study participants over 10 years of follow-up [27]. In the Lovelace Smoker's Cohort, being a current smoker and reporting exposure to wood smoke demonstrated an additive effect on reduced forced expiratory volume in 1 s (FEV_1), chronic bronchitis and COPD [28], along with a measurable decline in lung function over time [29]. Similarly, in SPIROMICS, former smokers exposed to high levels (95th percentile) of total particulate matter experienced accelerated FEV_1 decline [30]. These human studies consistently demonstrate strong associations between particulate exposures and disease progression; however, they are inherently limited in their ability to define causality or dissect underlying biological mechanisms, highlighting the need for controlled preclinical models.

Insights from animal models have begun to clarify how different inhaled exposures shape distinct COPD phenotypes. For instance, particulate matter exposure often drives more prominent small airway remodelling, characterised by airway wall thickening and fibrosis, whereas CS exposure more consistently produces severe emphysematous destruction of the lung parenchyma [31]. Importantly, combined exposure to CS and particulate matter produces synergistic effects, resulting in more extensive lung damage than either exposure alone [32]. These distinctions are difficult to resolve in human observational cohorts, reinforcing the value of animal and *ex vivo* systems in identifying exposure-specific mechanisms and pathways.

Dust and fumes derived from biomass combustion contain toxic pollutants that also affect lung function integrity. The toxicity is attributed to direct chemical injury from volatile organic compounds, carbon monoxide, nitrogen oxides and other reactive species. Despite the accumulating epidemiological evidence demonstrating the detrimental effects of biomass and environmental pollutants [33], substantial gaps remain in our understanding of the mechanistic basis of these observations. Bridging this gap requires integration of human cohort findings with mechanistic data derived from preclinical and *ex vivo* models.

E-cigarette or vaping models introduce an additional layer of complexity. These models typically involve exposing mice [34, 35] or occasionally rats [36] to aerosols generated by vaping devices in controlled inhalation systems [34, 35]. The aerosol composition is highly variable and may include propylene glycol, vegetable glycerin, vitamin E acetate, flavouring agents, formaldehyde, heavy metals, carbonyl compounds and differing concentrations of nicotine [34, 35]. This heterogeneity contributes to variable pulmonary

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toxicity across exposure conditions and smoking statuses [37]. Experimental findings suggest that e-cigarette exposure induces airway fibrosis, mucus hypersecretion and inflammatory remodelling, consistent with early COPD-like changes [38]. However, the severity of disease in these models is generally milder than that observed with traditional CS exposure [38]. Despite this, the presence of structural and inflammatory alterations indicates that e-cigarettes are not benign; rather, they represent a lower-intensity but still pathogenic exposure that is likely to result in clinically significant COPD-like phenotypes with sufficient duration and cumulative exposure.

The experimental design becomes even more complex when vaping is combined with conventional CS or cannabis products such as tetrahydrocannabinol and cannabidiol [39, 40]. In such models, animals are exposed to combinations of nicotine-containing aerosols, vitamin E acetate and/or tetrahydrocannabinol formulations [39], resulting in enhanced airway hyperresponsiveness, inflammatory cell infiltration, and even emphysematous changes [41, 42].

Ultimately, the biology of a person chronically exposed to biomass fuel smoke in a low-income setting is not equivalent to that of a long-term cigarette smoker in a high-income country, nor does it mirror the disease observed in individuals with recurrent early-life infections or occupational exposures. Thus, each exposure history imprints a distinct inflammatory signature, structural trajectory and therapeutic response.

Experimental models of COPD: *in vivo*

Preclinical models for COPD have been designed to recapitulate either emphysema or airway or pulmonary vascular disease, but despite genetic modification tool advancements they have failed to fully capture the heterogeneous nature of the human phenotype (tables 1 and 2). Smaller animals are excellent for replicating spontaneous emphysema in genetically modified models or induced emphysema in CS-based or elastase-based models. However, these offer limited airway pathology because they lack human-like anatomical structures such as the absence of respiratory bronchioles [5, 50]. Therefore, complementary *ex vivo* and *in silico* tools are being developed and perfected to overcome the limitations we observe in the *in vivo* models.

Animal models of COPD

Experimental models are essential for advancing COPD research (table 1 and 2). However, it should be acknowledged that our experimental approaches to date have largely failed to result in any disease-modifying therapies for COPD. One explanation may lie in the inherent complexity of COPD making it difficult to fully emulate in animals (table 3).

For over 30 years, CS exposure in animals or across relevant tissue *in vitro* has served as the canonical approach to model COPD [43]. In 1952, the development of CS-exposure chambers changed the panorama of basic science research in COPD [63]. CS-exposed mice have been the cornerstone of preclinical models for COPD [5, 51, 62, 64–72]. Technology has since evolved with models that assess mainstream (nose-only), side-stream (whole-body) smoke exposures and mixed models, even including prenatal fetal exposures using a side-stream protocol [44]. Time of exposure has been classified into acute and chronic exposure protocols. CS exposure lasting between 3 days and 4 weeks is generally considered acute, whereas exposures of 16–24 weeks are considered chronic and lead to the development of emphysema, chronic bronchitis and mucus metaplasia accompanied by adaptive immune inflammatory infiltrates. The middle ground, between 5 to 15 weeks, is often called subacute and its use depends on smoking device used, the murine strain and the biological hypothesis.

Administration of porcine pancreatic elastase (PPE) has been widely used to induce rapid-onset emphysema, offering a complementary, injury-focused model that isolates the structural destruction of alveolar networks [73]. In this model, a single or repeated intratracheal administration of PPE leads to rapid degradation of elastin and other extracellular matrix (ECM) components within the alveolar walls, resulting in emphysema-like pathology characterised by enlarged airspaces, reduced elastic recoil and impaired lung mechanics [74, 75]. Important limitations must be acknowledged. Elastase causes an abrupt, non-physiological injury that bypasses the chronic, low-grade epithelial and endothelial stress imposed by CS and ageing [74–76]. It lacks progressive exposure, systemic effects and the time-lapse heterogeneity that define human COPD. As a result, while the model is powerful for establishing causality and testing targeted interventions for emphysema, it incompletely recapitulates COPD initiation and progression.

In parallel to PPE, a recent model of proteolytic extracellular vesicles (EVs) has provided a robust and reproducible model of pulmonary emphysema in mice, with significant structural lung disease within a 2-week period [77]. The EVs are also found to be central to a chronic lipopolysaccharide (LPS)-induced

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TABLE 1 Animals models, small and large, used in COPD research

Animal	Method	Emphysema	Mucus metaplasia	SAR	Lung function deterioration	Prenatal model available	Modifiable for humanised models
Mouse (*Mus musculus*) [5, 43, 44]	Smoke-based Elastase-based Biomass-based Models with LPS Vaping model [34, 42] HDM/OVA/IL-13 models [45–48]	Yes	Yes Smoke-induced goblet hyperplasia Rarely occlusive mucus plugs	Yes	Yes	Yes Maternal smoke model [49]	Yes Routine customisation
Rat (*Rattus norvegicus*) [5, 43, 50]	Smoke-based Elastase-based Biomass-based Models with LPS Vaping model [34, 36] Starvation model [50]	Yes	Yes Some models develop hyperplasia	Yes	Yes	No	Yes Some knockouts are available
Hamster (*Mesocricetus auratus*) [5, 51]	Elastase-based Very limited smoke-based	Yes	Yes Mild goblet metaplasia	Yes Mild	Yes	No	No
Guinea pig (*Cavia porcellus*) [5, 43]	Primarily smoke-based	Yes	Yes Strong goblet hyperplasia Mucus plugs observed	Yes Robust	Yes	No	No
Ferret (*Mustela putorius furo*) [5, 43]	Primarily smoke-based	Yes	Yes Strong goblet hyperplasia Mucus plugs observed	Yes Strong	Yes	No	Yes Some CF models
Pig (*Sus scrofa*) [5, 52]	Primarily elastase-based Models with LPS	Yes	No	Yes Mild	Yes	No	Yes, but costly
Sheep (*Ovis aries*) [5]	Allergen challenges Some smoke or pollutant models	Limited	Yes Allergic and chronic models	Yes Strong	Yes	No	No
NHP (*Macaca fascicularis*/*mulatta*) [5]	Smoke-based	No	Yes Robust gland hypertrophy and hyperplasia	Yes Robust	Yes	No	No Possible *via* CRISPR but rare and costly

CF: cystic fibrosis; HDM: house dust mite; IL-13: interleukin-13; LPS: lipopolysaccharide; OVA: ovalbumin; NHP: non-human primate; SAR: small airway remodelling.

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TABLE 2 Comparative anatomy of the respiratory tract across animal models and their relevance to COPD

Animal	Anatomical differences	Limitations in replication	Emphysema assessment	Emphysema subtypes
Mouse (*Mus musculus*) [5, 43, 53]	Mice have fewer bronchial generations compared to humans (13–16 *versus* ~23) Monopodial branching No respiratory bronchioles Submucosal glands in trachea only	Pros: robust emphysema, especially elastase-based, and extended genetic modification tools available Cons: smoke-based SAR and mucus hyperplasia are mild/moderate at best	Histological (MLI) Micro-CT MRI with hyperpolarised gas [54]	Centrilobular: mild with smoke-based Panlobular: strong with elastase-based Paraseptal: strain-specific like in Marfan models [55]
Rat (*Rattus norvegicus*) [5, 43, 50]	Monopodial branching No respiratory bronchioles Few submucosal glands beyond upper airways	Pros: robust emphysema, especially elastase-based Cons: poor airway phenotype	Histological (MLI) Micro-CT MRI (functional proton MRI) [56]	Centrilobular: mild with smoke-based Panlobular: strong with elastase-based Paraseptal: no
Hamster (*Mesocricetus auratus*) [5, 51]	Monopodial branching No respiratory bronchioles Minimal submucosal glands	Pros: severe diffuse emphysema with elastase Cons: infrequent use in research and limited genetic manipulation tools	Histological (MLI) Micro-CT	Centrilobular: limited Panlobular: strong with elastase-based Paraseptal: no
Guinea pig (*Cavia porcellus*) [5, 43, 57]	Some dichotomous branching Submucosal glands in bronchi Partial respiratory bronchioles	Pros: airway hyperresponsiveness and airway disease with smoke-based Cons: very mild emphysema development and very few genetic tools for manipulation	Histological (MLI) Micro-CT	Centrilobular: mild with smoke-based Panlobular: possible with elastase-based Paraseptal: no
Ferret (*Mustela putorius furo*) [5, 43, 58]	Bronchioles have submucosal glands Airway epithelium is pseudostratified Similar MCC mechanisms	Pros: excellent model for smoke-based chronic bronchitis and mucus hypersecretion Cons: very mild emphysema development and very few genetic tools for manipulation	Histological (MLI) Micro-CT	Centrilobular: mixed most likely Panlobular: probably with elastase-based Paraseptal: no
Pig (*Sus scrofa*) [5, 52, 59]	Dichotomous branching Submucosal glands in bronchi Respiratory bronchioles present	Pros: translational physiology and functional emphysema assessment model Cons: elastase-based models are mainly used [60]	Histological (MLI) CT and MRI (possible due to size)	Centrilobular: mixed model with mutated pig phenotype [59] Panlobular: with elastase-based Paraseptal: no
Sheep (*Ovis aries*) [5, 61]	Lobulated lungs with dichotomous branching Submucosal glands into small airways Presence of respiratory bronchioles	Pros: great for hyperreactive allergen models Cons: COPD-type changes are rarely seen	Histological (MLI) CT and MRI (possible due to size)	Centrilobular/panlobular mixed: limited smoke-based models established; papain protocol [61] Paraseptal: no
NHP (*Macaca fascicularis*/*mulatta*) [5, 62]	Highly human-like anatomy Dichotomous branching Submucal glands into small airways Presence of respiratory bronchioles	Pros: closest translation to humans and good model for airway disease Cons: no emphysema in smoke-based model; ethical issues and costs	Histological (MLI) CT and MRI (possible due to size) Micro-CT in *ex vivo* samples	Centrilobular: mild at best [62] Panlobular: no Paraseptal: no

CT: computed tomography; MLI: mean linear intercept; MRI: magnetic resonance imaging; NHP: non-human primate.

model of emphysema and smoking models of emphysema [78]. The proteolytic EVs were observed in human COPD clinical samples and these EVs can be transferred into mice to generate emphysema.

Infections play a critical role in COPD by driving exacerbations and accelerated lung function decline. Several infection models in combination with CS exposure have been used to mimic or accelerate COPD, like CS+LPS [79, 80], CS+*Streptococcus pneumoniae* [81] and CS+virus [82–84]. Persistence of the emphysema induced by CS+virus exposure has not been investigated because there are no long-term follow-up studies after CS cessation [85, 86]. Each of these dual-hit systems yields a distinct inflammatory phenotype and robustly mimics clinical scenarios of immune dysregulation and impaired resolution.

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TABLE 3 Advantages and disadvantages of mouse models of COPD and potential solutions

Advantages	Disadvantages	Details	Potential solutions
Low cost, easy availability	Different airway anatomy than humans	Mice lack respiratory bronchioles and have fewer bronchial branches than humans	Generate orthogonal data based on human lung tissue including use of lung organoids, PCLS and 2D human tissue models
Extensive genetic tools including gene editing technologies	Simple immunity	Humans have a complex immune system with frequent redundancies and differential immune response to stimuli	Genetically link relevant phenotypes to putative genes/pathways in humans using patient lung samples and single-cell omics technologies
Well-described murine genome with similarities to humans	Relatively simple genome and genome–phenotype relations	Humans have complex, heterogeneous genome–phenotype relations with horizontal and vertical pleiotropy	Focus on one major COPD trait (*e.g.* emphysema) per experiment and use "humanised" animals, where possible (knockout and knockins)
Readouts for biological processes such as airway inflammation and remodelling well established	Findings may not translate to humans	Owing to simplicity of respiratory tract and immunity of rodents, biological signals may not be recapitulated in humans	Validate animal findings in human tissues using multiple orthogonal approaches; use biomarkers where feasible
Exposure timelines are truncated	Smoke exposures are typically <6 months	Human smoking requires 30+ years of exposure for advent of disease	Acknowledge that animal models mimic at best GOLD 1 disease
Proteolytic models produce rapid emphysema	Elastase/protease instillations induce emphysema within a few weeks	Humans develop emphysema mostly through cigarette smoke exposure not through this mechanism	This model should only be used to evaluate novel therapies to treat emphysema; prevention strategies cannot be evaluated

GOLD: Global Initiative for Chronic Obstructive Lung Disease; PCLS: precision-cut lung slices.

However, to date, these approaches have predominantly provided the exposure first followed by the infection trigger while the reciprocal sequence has largely been neglected, despite evidence that pre-existing infection can prime the airway for exaggerated injury [87, 88].

To better align with human disease, future models should incorporate multi-hit and sequential exposure designs, including repeated or chronic infections, polymicrobial challenges, and paradigms where infection precedes or co-occurs with CS exposure. Approaches that model persistent colonisation, extend follow-up duration and account for host susceptibility factors may further improve translational relevance and enable investigation of how pre-existing infections prime the airway for exacerbations and progressive injury.

Genetic models of COPD

Spontaneous emphysema development in mice has been observed in mouse models with genetic deficiency targeting genes that are important for alveolar homeostasis (figure 1). There are at least three groups of these genetic COPD mouse models:

1) Mouse models targeting genes supported by human genetic evidence including genome-wide association studies (GWAS). Among these, hedgehog interacting protein $(Hhip)^{+/-}$ mice develop spontaneous age-dependent emphysema and increased lung compliance [89], likely due to the exuberant activation of CD8 effector T-cells [90], also detected in early human COPD [91]. Recently, *Adam19*-deficient mice were also reported to demonstrate spontaneous lung function decline at 2 months old [92]. *Serpina1*-deficient mice exhibit protease–antiprotease imbalance, contributing to spontaneous progressive emphysema [93], closely mirroring human α1-antitrypsin deficiency-associated COPD. Other emphysema-specific models are matrix metallopeptidase-12 (*Mmp12*)-deficient mice [94], iron chelation murine models [95] and BCL2 interacting killer (*Bik*)-deficient mice [96].
2) Genes implicated in the disruption of epithelial–matrix signalling are exemplified in the integrin-β6 (*Itgb6*) knockout mouse, which develops age-related emphysema driven by impaired activation of transforming growth factor-β (TGF-β) and increased MMP-12-mediated matrix degradation [97]. Interestingly, despite a lack of direct evidence implicating Itgβ6 in human COPD GWAS, other family members such *Itga1*, *Itga2*, *Itga8* and *Itgb8* have been suggested by lung function GWAS [98].
3) Genes important for innate immunity and oxidative stress: toll-like receptor-4 (*Tlr4*)-deficient mice exhibit spontaneous emphysema associated with increased oxidant production and elastin fragmentation. Triple *Nos* (nitric oxide synthase) knockout mice (iNos, nNos, eNos) demonstrated significant airspace enlargement, indicating an essential role of nitric oxide synthesis for maintaining lung homeostasis [99].

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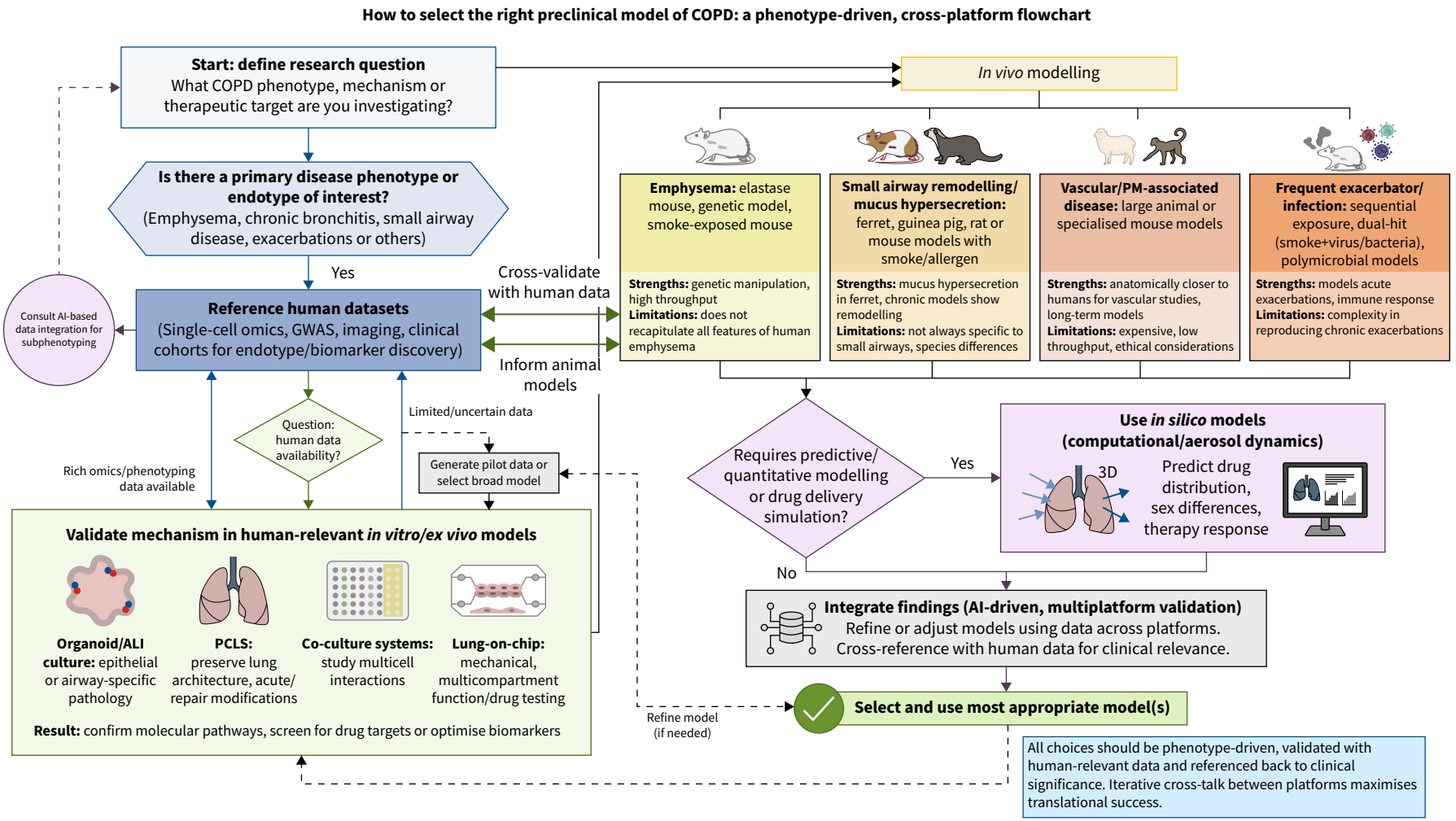


FIGURE 1 Simplified diagram showing experimental animals used in COPD and generalised intervention models. AI: artificial intelligence; ALI: air–liquid interface; GWAS: genome-wide association study; PCLS: precision-cut lung slices; PM: particulate matter. Figure generated with FigureLabs Workspace.

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Collectively, these genetic models demonstrate that COPD-associated emphysema can emerge from diverse yet convergent mechanisms, spanning genetic susceptibility, protease imbalance, immune dysregulation and cell–matrix interaction, and provide mechanistically druggable targets for future therapy design in human COPD.

Immunological considerations

Modelling chronic inflammatory diseases like COPD in mice has some shortcomings, related to the differences in their immune system compared to humans [100]. Mice have a lymphocyte-dominant blood profile, in comparison to a neutrophil-dominant one in humans; mice have 12 TLRs compared to 10 in humans; and murine natural killer (NK) cells work *via* Ly49 while humans rely on the killer cell immunoglobulin receptor (KIR) family. Murine defensins have a different pattern of secretion and there are several differences in murine T-cells' homing location and subsets. Therefore, murine immunity-related genes may provide some clues, but the human immune system has redundancy that is not fully recapitulated in animal models [101].

Furthermore, there are strain-dependent immune responses. Classically, C57BL/6 mice show skewed Th1-inflammation, while BALB/c mice show Th2-dominant inflammation [102, 103], adding to the complexity of mouse models of emphysema. Finally, we must consider the Th1/Th17 neutrophil-dominant *versus* the Th2 eosinophil-dominant COPD phenotypes. Emphysema pathogenesis is strongly associated with a Th17/Th1-driven inflammation in humans and mice that drives parenchymal destruction [104]. Notably, recent findings suggest that Th2 inflammation is also present in patients with COPD [105, 106]. This complicates the exposure design, because smoke-based models (predominantly a Th1-prone model [5, 51, 62, 64, 65–71]) need to be coupled with an allergen (*e.g.* house dust mite) [45, 46] or interleukin-13 (IL-13) overexpression models [47, 48] to skew lung inflammation towards Th2 inflammation (table 1).

In silico *models of COPD*

Computational simulations, or *in silico* models, are evolving tools that replicate lung physiology to test mechanisms of disease, drug intervention and outcome prediction without inherent invasive procedures on animal or human subjects (figure 1). Lung deposition models are computer-generated three-dimensional (3D) models, constructed by reverse-engineering high-resolution computed tomography (CT) scans [107] or murine-derived micro-CT scans [108]. These analyses rely on computational power and imaging software applications that allow modelling and segmentation for further analysis of aerosol dispersion and fluid dynamics [107–110]. Given their simulation nature, these models have been used to test double and triple inhalation therapy combinations, assessing respiratory mechanics [107]. Moreover, fluid dynamics has been proposed to predict pulmonary vascular resistance and remodelling in COPD [111], potentially aiding patient stratification and targeted therapy. Finally, computational simulation of aerosol deposition in lungs is currently used to explore particulate matter distribution between sexes and severity of disease, further clarifying the importance of sexual dimorphism in COPD [112, 113].

Experimental models of COPD: *ex vivo* and other models

Currently, there is limited objective evidence that platforms such as organoids, precision-cut lung slices (PCLS), lung-on-a-chip or artificial intelligence (AI) outperform traditional models in predicting clinical efficacy. Their value instead lies in better alignment with human biology, particularly when combined with patient-derived samples, omics data and clinically relevant biomarkers, and their potential scalability. Thus, their translational potential is not intrinsic but depends on appropriate integration with human-relevant end-points and validation strategies.

Here we summarise the most used *ex vivo* and *in vitro* COPD models.

PCLS

PCLS are an *ex vivo* model that preserves the cellular composition and native architecture of lung tissue [114]. They are produced by filling lungs with agarose, solidifying the tissue and then slicing them into thin sections (100–500 µm), allowing real-time and longitudinal analyses of native cell–cell and cell–ECM interactions in cell culture. However, this method has its limitations, such as limited viability (7–14 days), slicing-induced injury to the samples, donor variability, technical difficulty in assessing airway lumen changes, and loss of immune cells during lung core perfusion.

For COPD research, PCLS are generated from lung tissue of patients with COPD or from rodent models (figure 1) [115]. They preserve disease-defining features such as emphysema and display relevant alterations in bronchoconstriction, repair capacity and inflammation [49, 114, 115]. Most COPD PCLS

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studies use tissue from patients with end-stage disease, which may not reflect early disease. Specific early mechanisms can be studied using PCLS from tumour resections or non-diseased lung tissue. To model specific COPD-related pathways, PCLS have been exposed to CS, CS extract, LPS, polyinosinic:polycytidylic acid, viral infections or elastase [116–121].

PCLS enable detailed analysis of regional heterogeneity in COPD and support functional assessments of ECM stiffness, airway and vascular constriction, endothelial dysfunction and regenerative capacity [115]. Moreover, PCLS allow evaluation of the effects of systemic mediators and therapeutic agents on alveolar and small airway regions [49, 114]. Preclinical PCLS studies have identified promising therapeutic strategies for COPD [115, 116, 122]. Advances such as long-term culture, cryopreservation and integration with imaging and single-cell omics now permit deep, real-time cellular analyses [114, 123]. Linking clinical metadata with multi-omic data and treatment response in PCLS has the potential to improve COPD patient endotyping and personalised therapy development [114].

Organoids and induced pluripotent stem cell-derived systems

Lung organoids are 3D epithelial stem or progenitor cell-derived structures that mimic lung development and architecture, providing a powerful model to study COPD pathogenesis and regeneration (figure 2) [124, 125]. COPD involves distinct proximal and distal epithelial pathologies [126, 127], many of which can be recapitulated in patient- and mouse-derived organoids [124–126, 128].

Airway organoids reproduce bronchiolar structures with ciliated and secretory cells, supporting studies on mucus secretion, ciliary function and epithelial repair [124, 126, 128], and have revealed changes in

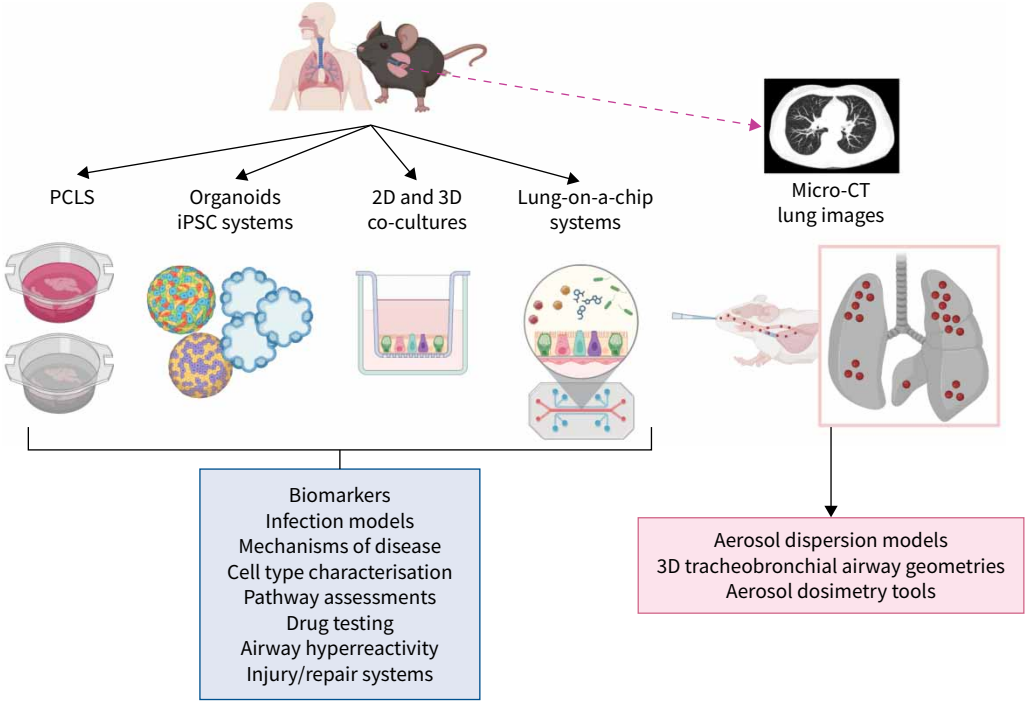


FIGURE 2 Simplified diagram showing *ex vivo* and *in silico* models for COPD. Human airway and lung murine models have been the perfect samples sources for all the major *in vitro* and *ex vivo* technological developments. Precision-cut lung slices (PCLS) provide complete native lung architecture for further biological and physiological studies. Organoids and inducible-pluripotent stem cell (iPSC) systems and complex two- and three-dimensional co-cultures allow the dissection of specific cell–cell microenvironments that can mimic lung structures. Microphysiological systems like lung-on-a-chip are advanced microfluidic systems that recreate functional features of organs at a very small scale. These four strategies allow dissection of molecular pathways, cell subtyping and drug testing at a higher resolution than other simpler *in vitro* methods. Finally, *in silico* models of aerosol dispersion derived from micro-computed tomography (CT) scans offer fundamental advantages in the assessment of particulate dispersion and accumulation, which is essential for aerosol dosimetry in inhalant pharmacodelivery.

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emphysema-associated epithelial subtypes [129, 130]. Alveolar organoids from induced pluripotent stem cells (iPSCs) or adult stem cells replicate distal lung features, including marker expression, surfactant secretion and injury response [131]. iPSC-derived models have elucidated how COPD GWAS genes affect alveolar type 2 cell function [132, 133]. Alveolar organoids from mice or non-COPD donor lungs can be exposed to disease-relevant stimuli, including growth factors, particles or CS extracts, and have identified major pathways dysregulated in COPD pathogenesis [134, 135]. Alveolar organoids have also been used to identify novel pro-regenerative therapies [116, 118, 136] and to model defective repair under CS exposure, serving as a medium-throughput platform for COPD drug discovery [116].

Current lung organoid models capture selected COPD features such as progenitor activity and mucus hyperplasia. Patient-derived organoids retain disease-specific and genetic traits, enabling personalised modelling and drug testing. This model has the potential to be used for more complex interaction studies, including combination exposures to pollutants, or pollutants and infections. Based on the findings from these models, one could design more complex models in *in vivo* models. Because organoids lack full disease complexity, active immune systems, certain functional readouts, and representation of terminal and respiratory bronchiolar regions, follow-up studies to determine the validity of the findings in animal models would be appropriate.

2D and 3D co-culture models

Interaction of epithelial cells with structural and immune cells is essential for maintaining homeostasis and regeneration and is disrupted in COPD [137]. Modelling these interactions remains challenging but provides valuable mechanistic insight by allowing controlled analysis of multicellular communication. Co-culture systems in 2D and 3D formats have been developed to address this (figure 2).

Reductionist 2D co-cultures of epithelial cells with fibroblasts have enabled the dissection of pro-inflammatory pathways in COPD [138]. 2D air–liquid interface (ALI) cultures are commonly used to differentiate basal airway stem cells into polarised, pseudostratified mucociliary epithelia resembling native airways [139]. These models allow the study of disease-specific features such as barrier dysfunction, defective mucociliary clearance, inflammation and remodelling [140]. Notably, ALIs from COPD-derived epithelial stem cells retain disease phenotypes, including goblet cell hyperplasia [139, 141]. They have been used to investigate pathogen-induced exacerbations [142, 143] and epithelial interactions with fibroblasts or immune cells, revealing roles in repair, regeneration and B-cell maturation [144, 145]. Organoid-type models have limitations for modelling COPD, such as lack of vasculature and immune milieu, reversed epithelial polarity and impaired ciliogenesis, and lack of mechanical forces influencing the culture (breathing mechanisms) [146].

More complex 2D approaches, such as lung-on-a-chip systems, incorporate endothelial, stromal and circulating immune cells under haemodynamic conditions [147, 148], though they remain limited by their artificial 2D geometry [149]. Recent advances include mesenchymal co-culture with alveolar organoids to model distal lung regeneration relevant to emphysema [150, 151] and immune–epithelial interactions driving inflammation and repair [152, 153]. More advanced 3D systems, such as perfusable bronchioid models with accessible lumina, now offer promising opportunities to integrate ALI and 3D culture complexity with different cell types [154].

Lung-on-a-chip systems

Organs-on-chips, also known as microphysiological systems (MPSs), are microengineered devices containing perfused microchannels or chambers lined with living human cells that recapitulate key features of tissue–tissue interfaces, mechanical cues and vascular perfusion *in vitro* (figure 3) [155–159]. Lung-on-a-chip platforms extend this concept to the respiratory tract by co-culturing differentiated airway or alveolar epithelium with microvascular endothelium under flow and cyclic stretch, thereby emulating *in vivo*-like vascular and air flow, respiratory cycle–associated mechanical strain, mucociliary function, barrier properties and leukocyte recruitment more faithfully than other models [155–161]. In COPD, small airway-on-a-chip models can reproduce goblet cell hyperplasia, ciliary dysfunction, cytokine hypersecretion, neutrophil recruitment under shear and smoke-induced injury using breathing–smoking lung chips [147, 162, 163], whereas alveolus-on-a-chip systems capture parenchymal and vascular responses, including oedema and thrombosis [164–166].

These MPSs support mechanistic dissection of inflammation and preclinical drug testing by enabling quantitative analysis of epithelial–endothelial crosstalk, immune cell adhesion and modulation of COPD-relevant biomarkers (*e.g.* IL-8, macrophage colony-stimulating factor, MMP1) in response to cytokines, pathogen mimics, CS and candidate anti-inflammatory or pathway-targeted therapies.

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Despite this promise, MPS limitations include device complexity, limited throughput, incomplete capture of chronic remodelling and restricted cellular diversity [167]. Future directions should include incorporating stromal and immune compartments, *e.g.* by integrating 3D matrix-embedded blood channels with physiologically relevant lumen geometry, in which endothelial cells are cultured on lung-specific ECM compositions and stiffnesses and directly interact with fibroblasts.

Inherent strengths and limitations of existing experimental models

The strength and success of an experimental design depend on choosing a specific animal strain and injury model for a specific biological question. COPD has multiple major contributors to development and acceleration: CS and other toxicant exposures (including vaping [37] and cannabis exposure [168]), indoor and outdoor pollution [23–27], mechanical strain, accelerated ageing, genetic factors [8] and developmental origins in early life [44] (figure 1). Animal models have recapitulated, to an extent, each of these or their combinations; yet, challenges still limit their utility.

CS-induced COPD animal models have contributed to immunoinflammatory mechanisms of disease [62, 64–66]. Most studies use three inbred strains, Bl/6, AKR or DBA2 [50]; other susceptible strains include A/J, C3H/HeJ, P/J and BALB/c [169]. Murine CS exposure for 22–24 weeks represents <20–30 years of CS exposure in humans [67]. Whole-body exposure chambers fail to mimic direct inhalation, because mice are obligate nose breathers and filter large particles [68, 69]. Further, diurnal CS exposure reduces smoke inhalation [64]. CS exposure facilities are expensive and time-consuming [68, 170]. Models take months to develop and the induced disease is often mild [51, 70, 71], equivalent to Global Initiative for Chronic Obstructive Lung Disease grade 1 [72, 171], and partially resolves upon cessation [172]. Therefore, not all animal-derived findings can be translated to patients, and they require multiple rounds of *in vitro* and *ex vivo* confirmation experiments to make them relevant for clinical trials development (figure 1).

Lack of translation is a serious problem in animal models. To date, the majority of, if not all, models used target CS-induced emphysema, leaving small airway disease in the proverbial air [173]. This inevitable lack of translation derives from different angles: some emphysema-producing models revolve around developmental pathway genes that cannot be targeted in humans owing to ethical considerations (like *Hhip*$^{+/-}$ mouse [89]); some models cannot be replicated in humans owing to either lack of orthologues in humans and/or incompatibility for pharmacological intervention (like the triple *Nos* knockout mouse [99]); and inhibiting a potentially pharmacological target does more harm than good to humans, like inhibiting circulating CD20^{+} B-cells, which was associated with increased risk of lung infection [174], or TLR inhibition therapies, which weaken the immune response and increase the risk of infection [175].

Therapy testing

Preclinical COPD models are important for therapy testing because they allow controlled CS exposure or other noxious stimuli, longitudinal sampling, toxicity assessment and invasive lung readouts that are not feasible in patients. However, these models only approximate human COPD, typically overrepresent neutrophilic inflammation and rely on young, inbred mice that do not capture patient diversity or disease complexity, thereby limiting translational success. Despite decades of work, preclinical models, particularly small animal models used in drug development, have had limited success in translating into effective therapies. The central issue, however, is not solely the intrinsic inadequacy of individual models, but rather the persistent misalignment between preclinical systems and the biology of human COPD, compounded by the incomplete understanding of COPD endotypes. At the same time, it is important to recognise that these models have generated meaningful mechanistic insights when used to interrogate specific disease components. Experimental studies using knockout models have identified MMP-12 (macrophage elastase) as a critical mediator of emphysema through elastin degradation, while neutrophil elastase deficiency confers protection against CS-induced emphysema, underscoring its role in ECM destruction [176, 177]. Small airway fibrosis has been mechanistically linked to TGF-β signalling, and CS-induced goblet cell metaplasia has been associated with IL-13 upregulation, providing insight into mucus hypersecretion [47, 178]. In addition, animal models have been instrumental in demonstrating corticosteroid resistance and elucidating underlying mechanisms, a hallmark feature of COPD that has direct clinical relevance [179]. *Ex vivo* human systems, including PCLS and airway or alveolar organoids, partly bridge the translational gap but remain constrained by tissue availability, donor variability and the absence of a functional immune system [180].

However, many anti-inflammatory targets have failed in clinical COPD trials. For example, inhibition of the CCR2/CCL2 axis effectively reduces monocyte recruitment and lung inflammation in animal models [181], yet has not translated into meaningful improvements in symptoms or exacerbation rates in patients, while raising safety concerns such as increased infection risk. Similarly, therapies such as α1-antitrypsin

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augmentation [182–184] and peroxisome proliferator-activated receptor-γ agonists have shown inconsistent effects on lung function decline and exacerbation outcomes [185]. By contrast, bronchodilators and inhaled corticosteroids, despite being only partially recapitulated in preclinical systems, remain the cornerstone of COPD management [5, 186]. These discrepancies highlight a broader issue: an overemphasis on inflammatory pathways in drug development, often at the expense of targeting structural and tissue remodelling processes that are more directly linked to disease progression.

A major barrier to improving translation is the lack of biomarkers that can be consistently measured and aligned across preclinical models and human disease. This limitation reflects both biological heterogeneity and insufficient integration between experimental and clinical research. Rather than viewing preclinical models as standalone predictors of therapeutic efficacy, they should be positioned as tools to dissect discrete biological pathways relevant to specific COPD phenotypes or endotypes. In this context, future progress will depend on frameworks that integrate human omics data, such as GWAS, single-cell and spatial transcriptomics, and deeply phenotyped clinical cohorts, with targeted experimental validation in *in vitro*, *ex vivo* and *in vivo* systems. Such an approach would enable hypothesis generation rooted in human disease biology, followed by mechanistic interrogation in appropriately designed models. Ultimately, the key limitation is not the absence of models, but the incomplete understanding of COPD as a spectrum of distinct endotypes, which makes faithful recapitulation in any single model inherently challenging.

Why COPD drug development keeps failing: a broken translational pipeline

Compared to other chronic highly morbid lung disorders like asthma, the therapeutic pipeline for COPD is alarmingly anaemic. Animal models exist that replicate clinical exposures or physiological contexts contributing to COPD development [5, 43, 50]. Cell platforms have been developed that incorporate the structural cell types involved in COPD pathophysiology [114, 123, 139, 141]. While drug development strategies refined by machine learning and medicinal chemistry have provided many options for targeting mechanisms, pathways and molecules, the translation into COPD treatments has been cumbersome [187].

One critical element is the acceptance of preclinical platforms for validation. The platforms should be available to many laboratories, require minimal optimisation for establishment and deliver reliable readouts that approximate elements of COPD disease establishment or progression. Organoids and PCLS provide a heightened level of tissue complexity and can be subject to CS or particulate exposures albeit within a narrow time scale [115, 116, 118, 122, 136].

A battery of cell injury behaviours and tissue organisational measures can be assessed and quantified, allowing determinations of progression or regression with introduction of pharmacological agents. The elevation of therapeutic candidates to *in vivo* testing using animal models provides a second level of validation that ideally should employ a post-phenotypic context [188].

Clinical trials of candidate therapeutics are currently constrained by metrics that imperfectly reflect disease burden and require prolonged monitoring to establish reliable trajectories (*i.e.* FEV_1 or COPD exacerbations). The incorporation of quantitative imaging (like micro-CT for lung density and structure) and composite readouts might provide more informative signals of efficacy [189, 190]. The use of surrogate readouts for specific subtypes of COPD (like models that describe virus-exacerbated, protease-driven or iron dysregulation models) could shorten timelines for drug pharmacological assessments. Moreover, the exploration of multifactorial adaptive and pragmatic trial designs that includes preclinical platforms (advanced organoids, PCLS or lung-on-a-chip) could allow flexible testing of multiple compounds in a recruited COPD cohort [191–193].

AI and data-driven integration

AI and data-driven approaches are increasingly essential for extracting biological and clinical insight from the high-dimensional datasets generated by single-cell, spatial, proteomic and imaging studies in COPD [194, 195]. As human cohorts grow in size and complexity, AI-based methods enable multiscale subphenotyping by identifying patient clusters defined by shared cellular compositions, transcriptional programmes and spatial microenvironments rather than by conventional clinical labels. In this context, AI provides a scalable framework for redefining COPD heterogeneity, moving beyond spirometry-based classifications towards biologically grounded endotypes that may better predict disease progression and therapeutic response.

A major strength of AI lies in its ability to integrate multi-modal data across scales. Machine-learning models can jointly analyse lung single-cell profiles, blood-based biomarkers, quantitative CT features and clinical trajectories to uncover latent structures that are not apparent in any single dataset [196, 197]. Early

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work combining imaging and blood transcriptomics demonstrates that AI can distinguish airway-predominant from emphysema-predominant disease axes, suggesting that similar approaches could link specific cellular microenvironments to radiographic patterns and systemic readouts [198]. These integrative models also create opportunities for hypothesis generation, identifying candidate pathways or cell states that are associated with distinct clinical phenotypes and can be prioritised for experimental validation. However, data that are provided for AI-driven analyses need to include reliable measurement of environmental exposures. Because these measurements are lacking in most population-based studies, data from controlled animal and *in vitro* models could provide the missing link to provide more reliable predictions of changes observed in human tissues and populations. In fact, while AI-driven approaches show strong promise, their effectiveness relies heavily on access to high-quality, longitudinal datasets with well-annotated exposure information. In addition, endotypes identified through AI must undergo rigorous experimental validation before they can inform model design or therapeutic strategies. Despite AI's powerful ability to integrate complex data, its performance is fundamentally constrained by the quality of the underlying datasets. Therefore, controlled experimental models remain indispensable for generating reliable training data and for validating hypotheses derived from AI analyses.

Opportunities and limitations of omics in understanding COPD heterogeneity

Transcriptomic profiling at individual-cell resolution has enabled marker-free discovery of cell types and states redefining complex diseases such as COPD [199]. Even in modestly sized studies, single-cell approaches have been transformative, revealing cellular heterogeneity and disease-associated programmes that were previously unappreciated using bulk methods [200].

For example, in COPD, single-cell and spatial transcriptomic studies have shown that chronic injury induces remodelling of the lung through coordinated epithelial, immune and stromal programmes, instead of isolated single cellular defects [201, 202]. A distinct secretoglobin family 3A member 2^{+} distal-airway population, recently identified in terminal and respiratory bronchioles, often co-expressing surfactant protein B [129, 203, 204], which has been labelled as a respiratory airway secretory (RAS) progenitor, is positioned at the bronchiole–alveolar interface and exhibits stem-like features including the putative capacity to contribute to the AT2 cell pool during repair. In COPD, owing to oxidative stress and small airway remodelling, RAS cells may become progressively exhausted and diminished.

Among immune cells, increased recruitment and activation of pro-inflammatory states of circulating monocytes and monocyte-derived macrophages have been described [180, 205]. Whereas in the healthy adult lungs, resident macrophages that show M1- or M2-like features predominate in the airways, in the COPD lung, monocyte-derived macrophages and macrophages without cell surface markers of M1 or M2 pathways fill most of the alveolar spaces [206, 207]. These macrophages are pro-inflammatory and exhibit poor phagocytic function. Why these cells accumulate in the COPD lung and what their role is in the pathogenesis of COPD progression or exacerbations are unknown.

COPD single-cell datasets commonly show altered T-cell composition consistent with persistent antigenic stimulation in the injured lung environment [104]. Non-immune structural compartments also shift as epithelial, endothelial and fibroblast populations acquire inflammatory programmes (*e.g.* NF-κB/interferon-driven states) [208, 209]. Collectively, these observations support a model in which COPD pathobiology emerges from interactions among coordinated pathogenic cells and define COPD as a disease of pathological microenvironments.

Integrating lung single-cell data with imaging and blood-based omics is beginning to link these microenvironments to clinically measurable phenotypes [194, 195]. It should be noted that single-cell data offer a snapshot in time, and could provide insights into cell fate and trajectories [210]. Most existing studies rely on convenience cohorts and end-stage lung tissue, and therefore lack longitudinal and spatial depth or disease heterogeneity, providing limited causal insight. Moving forwards, adequately designed, hypothesis-driven, longitudinal studies are essential to translate single-cell insights into clinically actionable frameworks for COPD development and progression.

A framework for next-generation, human-relevant preclinical modelling in COPD

A pragmatic takeaway

Recent advances in human-focused technologies are transforming our understanding of COPD pathogenesis. Single-cell RNA sequencing, spatial transcriptomics and large-scale expression quantitative trait loci and protein quantitative trait loci analyses, combined with increasingly sophisticated clinical phenotyping tools such as high-resolution imaging, are providing unprecedented insight into the cellular and molecular architecture of COPD [67, 211]. These approaches are uncovering novel genes, pathways

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and cell populations closely linked to specific disease phenotypes, offering a far more granular and data-driven view of COPD heterogeneity than was previously possible. However, identifying candidate mechanisms from human datasets is only the starting point. A persistent challenge in the field is that many targets emerging from preclinical or discovery pipelines fail to translate into meaningful clinical outcomes, underscoring the need for a more rigorous and bidirectional validation strategy.

To address this gap, we recommend that experimental designs explicitly define a primary phenotype of interest and select models accordingly. The goal should not be to recreate COPD in its entirety, but to interrogate specific disease components with precision.

We advocate for an integrated, iterative framework in which findings from human omics and clinical data are validated in *in vitro*, *ex vivo* and *in vivo* systems, with insights from these models used to refine and better interpret human data.

Within this bidirectional paradigm, we propose a phenotype-driven, cross-platform approach in which hypotheses originate from human clinical and omics datasets, are validated in *in vitro* and *ex vivo* systems, are interrogated in phenotype-specific *in vivo* models, and are ultimately confirmed through clinical investigation (figure 1). In this framework, preclinical models are not viewed as standalone predictors of therapeutic success, but as complementary tools for dissecting discrete biological pathways within defined disease contexts. By placing experimental models within an iterative framework grounded in human data, COPD research can better align with clinical reality and support the development of more meaningful and effective therapies.

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