Review Article

Journal of Innate Immunity

J Innate Immun 2020;12:203–225
DOI: 10.1159/000502489

Received: July 18, 2019
Accepted after revision: July 25, 2019
Published online: September 17, 2019

Animal Models Reflecting Chronic Obstructive Pulmonary Disease and Related Respiratory Disorders: Translating Pre-Clinical Data into Clinical Relevance

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Keywords
Chronic obstructive pulmonary disease · Animal models · Treatment · Fibrosis · Inflammation

Abstract
Chronic obstructive pulmonary disease (COPD) affects the lives of an ever-growing number of people worldwide. The lack of understanding surrounding the pathophysiology of the disease and its progression has led to COPD becoming the third leading cause of death worldwide. COPD is incurable, with current treatments only addressing associated symptoms and sometimes slowing its progression, thus highlighting the need to develop novel treatments. However, this has been limited by the lack of experimental standardization within the respiratory disease research area. A lack of coherent animal models that accurately represent all aspects of COPD clinical presentation makes the translation of promising in vitro data to human clinical trials exceptionally challenging. Here, we review current knowledge within the COPD research field, with a focus on current COPD animal models. Moreover, we include a set of advantages and disadvantages for the selection of pre-clinical models for the identification of novel COPD treatments.

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease syndrome characterized by an inflammatory airway state induced by environmental stimuli, primarily tobacco smoke and household or industrial air pollution [1–3]. COPD is believed to be a combination of disorders, including chronic bronchitis and emphysema, both of which cause diminished airflow at expiration and are associated with obstructed bronchi [1, 4, 5]. The disease has become a silent killer in low- and middle-income countries because of its slow progression in many patients [6–8]. Currently estimated to affect 328 million people worldwide, COPD is expected to become one of the leading causes of death within the next 2 decades, although data from outside the USA and European Union are still limited [9–11]. There has been a lack of funding for COPD research compared to that provided for other causes of global mortality and morbidity, despite the major public health problem COPD poses [12].

COPD is characterized by a progressive decline in lung function with intermittent periods of inflammatory bouts, termed exacerbations. The exacerbations are often triggered by infections and result in a significant increase in both morbidity and mortality [3, 13, 14]. These exacerbations are responsible for the majority of

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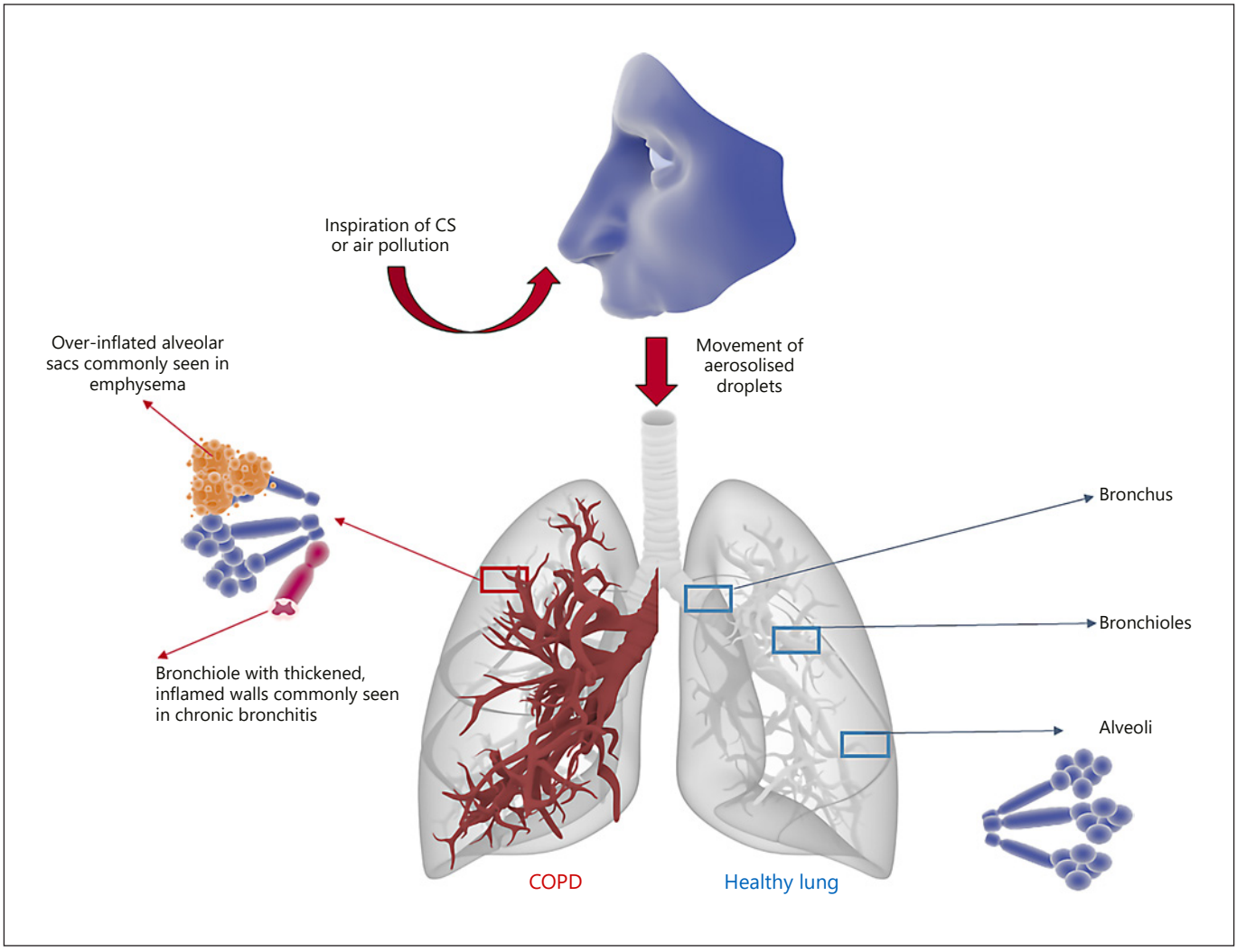

Fig. 1. COPD state versus healthy lung tissue. Inhaled irritants (predominantly CS or fumes from biomass fuels) cause a chronic inflammatory state at different levels of the airways. In the bronchi this may lead to chronic bronchitis, in the bronchioles it causes bronchiolitis, and in the terminal airways and alveoli causes impaired gas exchange due to emphysema. High proteolytic activity results in mucus-hypersecretion and remodeling. The latter causes emphysema (reducing the area available for gas exchange) and a lack of recoil (limiting the expiratory airflow and causing hyperinflation of the lungs).

direct COPD-associated health costs amounting to an estimated USD 30 billion in the USA alone in 2010 [15–17]. The economic impact of COPD is not only associated with direct treatment costs, but stretches further as patients are unable to work, resulting in an estimated loss of USD 20 billion to the US economy alone [18]. These costs are expected to increase; as the recently released American Lung Association's "State of the Air Report" highlights, more than 40% of US citizens live in areas containing "unhealthy" levels of particulate matter [19, 20].

The implications of COPD are compounded by the absence of novel drug targets in the area of pulmonary medicine, largely resulting from a lack of knowledge surrounding progression of the disease [21, 22]. Furthermore, disease modeling has proved difficult, as no single in vivo model of COPD fully captures the breadth of symptoms experienced by patients, particularly in the later stages of disease [23–26]. It is important to survey the current COPD research landscape to promote successful models for understanding disease pathology and developing more effective treatment options.

Methods

We searched the available literature using various scientific databases including PubMed, ScienceDirect, Google Scholar, Mendeley, Scopus, and Web of Science for English language articles published until the start of July 2019. Articles were excluded if they were unpublished or non-English articles. The number of articles deemed to meet these criteria and included in this review was 312.

Clinical Presentation

Clinical presentation of COPD often includes a chronic cough and increased sputum production, and dyspnea, which are all common features amongst patients [27]. These symptoms result from the development of chronic bronchitis and/or emphysema, which are associated with inflamed bronchioles and distended alveolar sacs, respectively (Fig. 1). A strong indication of COPD involves the symptoms above, patient history of predisposing risk factors, and a post-bronchodilator forced expiratory volume after 1 s (FEV_1) divided by the forced vital capacity (FVC) value <0.7 [28, 29]. Some have argued that spirometry alone is not an accurate enough predictor of COPD [30–32]. In addition, the use of a FEV_1/FVC ratio <0.7 to define COPD has shortcomings. For example, it may be prone to over-diagnosis of COPD in individuals over 70 years of age, an age-span where COPD has its highest prevalence. Instead, age-adjusted ratios (lower limit of normal) better reflect COPD among the elderly population [33].

Chronic bronchitis (productive cough that lasts for 3 months or more per year, for at least 2 years) can occur separately or combined with emphysema in COPD, where the latter diagnosis is based on spirometry. Decline in respiratory function is heterogeneous amongst patients, with some experiencing rapid, almost immediate reduction in lung function whilst others experience a more gradual decline [34–39]. The resultant airway damage, lack of elastic lung recoil, and pulmonary obstruction is irreversible, with no cure currently available. Progression of COPD is poorly understood, with patients often requiring supportive medical intervention, such as bronchodilating therapy, corticosteroids, antibiotics, and in severe cases, long-term oxygen therapy. Changes that promote exacerbations are often associated with bacterial or viral infections that cause worsening of the patient's symptoms beyond day-to-day variation, and leave much to interpretation by the prescribing doctor [32, 40]. This has highlighted the need for personalized precision medical treatment for patients and the development of robust biomarkers for the diagnosis and prediction of these events [5, 32, 41]. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 report proposed an updated assessment tool to assign categories of disease severity based on respiratory symptoms and exacerbation history, with treatment options and escalations proposed for each group based on specific clinical presentation [40, 42].

Risk Factors for COPD Development

Smoking is one of the major risk factors associated with COPD development [43, 44]. Smokers have a higher prevalence of respiratory function irregularities, greater rates of FEV_1 decline, and higher rates of mortality than non-smokers [45]. Furthermore, tobacco smoke exposure at prenatal or postnatal stages of human development is detrimental to lung development, and associated with lung remodeling and risk of pulmonary disease development [46–49]. Air pollution is another reported cause of COPD, implicated in approximately 15–20% of cases [50]. Exposure to workplace air pollutants such as organic or inorganic dusts, chemical products, and fumes are not fully appreciated as causes of COPD [51]. Sources of household air pollution include chemical/coal fires coupled with poorly ventilated living spaces, leading to an accumulation of polluted air and exposing individuals to prolonged airway insult [52, 53]. Chronic COPD sufferers experience bacterial or viral infections of the lower respiratory tract, with several studies displaying correlations between COPD exacerbations and infection [54, 55]. Virus detection rates in patients who have experienced previous exacerbations have been reported to be as high as 64% [55].

It is not only external factors that influence the COPD development [56]. Host factors including genetic make-up, lung cell hyperresponsiveness to stimuli, and poor lung growth during childhood play crucial roles in the development and severity of COPD [57, 58]. Additionally, several comorbidities afflict patients with COPD, including cardiovascular disease, muscle weakness, lung cancer, and depression [59–62]. These comorbidities are often associated with smoking and/or genetic predispositions and may appear in the absence of respiratory decline [27, 63].

Treatment Options

Current COPD treatment approaches rely on minimizing exposure to smoke inhalation and reducing the occurrence and severity of exacerbations [64, 65]. Early-

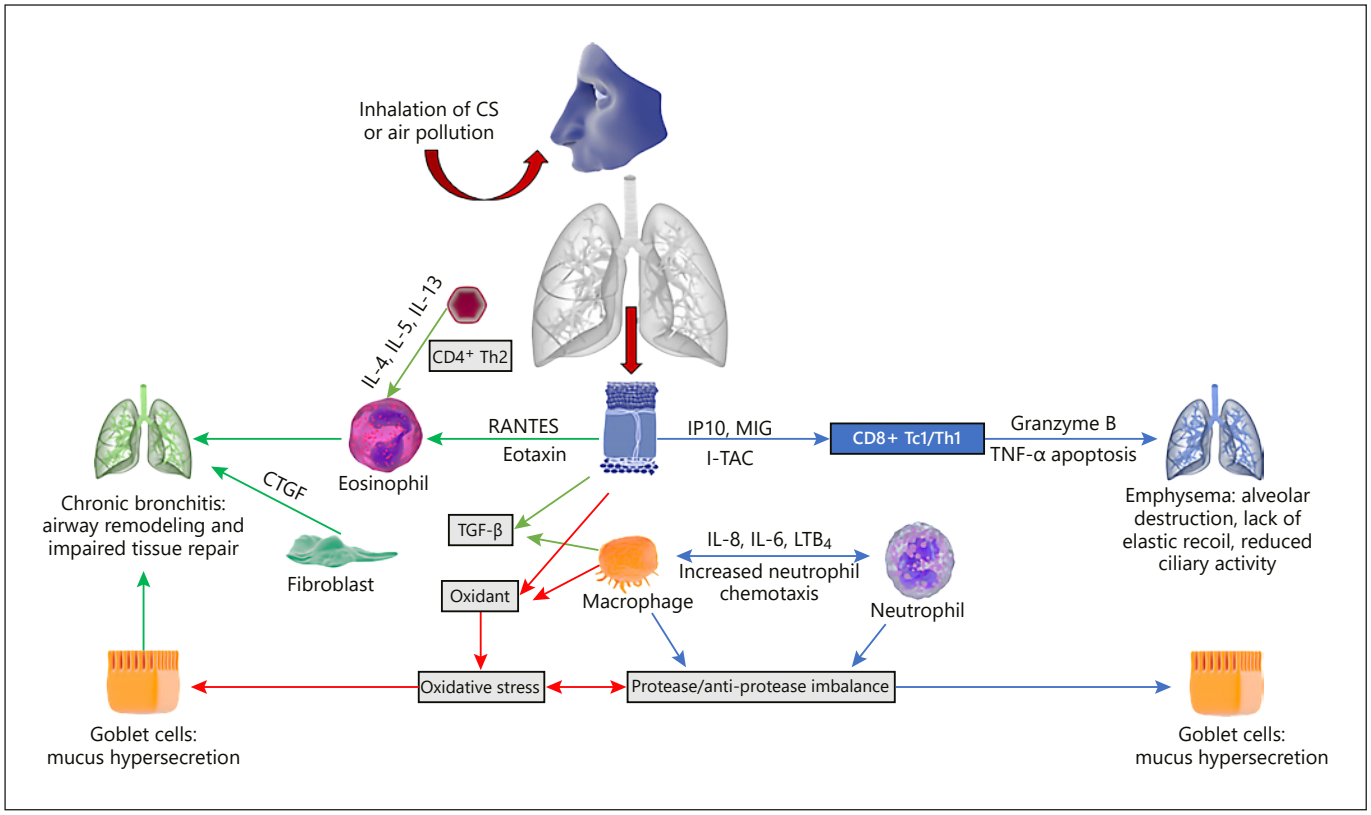

Fig. 2. Molecular mechanisms of COPD development (adapted from Linden et al. [105]). MIG, monokine induced by interferon-γ; LTB_4, leukotriene B_4; CTGF, connective tissue growth factor; IP10, interferon-γ-induced protein 10; I-TAC, IFN-inducible T cell α-chemoattractant.

stage intervention is required in order to significantly impact disease progression [4, 5, 66]. Minimizing exposure to both smoking and indoor air pollution decreases the rate of disease progression and is positively correlated with a decrease in mortality [67, 68]. Physical activity is exceptionally important in reducing exacerbation frequency and mortality rates in patients with COPD [69–72]. Other interventions include vaccinations against influenza and pneumococcal infections, as these may reduce the risk of exacerbation events and hospital admission [73, 74]. Pulmonary rehabilitation is an effective multidisciplinary approach to improving dyspnea and exercise tolerance [75, 76].

Pharmacotherapeutic approaches are mainly targeted at improving symptoms, increasing quality of life, and avoiding exacerbations [77]. The primary treatment for airflow limitation is inhalation of long-acting β_2-agonists (LABAs) and long-acting muscarinic antagonists (LAMAs) [29]. LAMA monotherapy is favored over LABA monotherapy in patients with a history of exacerbations [78, 79]. Fixed doses of LABA/LAMA are available to make drug administration more manageable [80]. Patients who continue to experience symptoms after LABA/LAMA treatment may benefit from administration of oral phosphodiesterase 4 inhibitors (e.g., roflumilast [81]) or macrolide antibiotics [82, 83]. However, both drug classes are subject to challenging side-effect profiles such as diarrhea and promotion of bacterial resistance, in the case of roflumilast and macrolides, respectively [29]. Considerable efforts have been made in the last decade to develop compounds that control inflammation, with little success thus far [84]. This is largely due to underlying heterogeneity in patient responses to treatment [85–89]. There have been several trials comparing dual bronchodilators with LABA/LAMA monotherapy or triple combination therapies comprising LABA, LAMA, and an inhaled corticosteroid [90–92]. However, few late-phase clinical trials are currently being conducted with many compounds still in the early phases [21].

Treatment for COPD is therefore comparably underdeveloped, with fewer compounds being produced in the last 40 years than for the majority of other disease areas

[22]. With complex underlying disease heterogeneity and a funding gap, the unmet needs for COPD and other respiratory diseases continue to grow [22].

Molecular Mechanisms of Disease Progression

COPD is a complex disease involving various inflammatory pathways that have proved difficult to understand in the context of disease progression [36, 38, 93–95]. One theory proposes that protease/anti-protease imbalances and oxidative stress are the main mechanisms contributing to COPD pathogenesis (Fig. 2). Air pollutants such as cigarette smoke (CS) inhaled through the nose and mouth induce airway epithelium and macrophages to release chemotactic factors, including interleukin (IL)-4, 5, 6, 8, and 13, interferon-γ-induced protein 10 (IP10), monokine induced by interferon-γ, and leukotriene B_4, which attract and activate key inflammatory cells lining the airway mucosa [96, 97]. Sustained activation results in airway accumulation of neutrophils, CD8 T cells, and/or CD4 helper T cells [98, 99]. These cause sustained and abrogated immune responses causing the degradation of elastin, particularly in the alveoli, ultimately resulting in emphysema and lack of elastic lung recoil. The accumulation and hyperactivation of neutrophils result in mucus hypersecretion from goblet cells. High blood eosinophil counts represent an independent risk factor for future exacerbations in patients with COPD [100]. Along with chemotactic factors, macrophages and epithelial cells secrete transforming growth factor-β, which causes fibroblast proliferation [101]. Additionally, fibroblasts secrete connective tissue growth factor, a chemokine that increases lung epithelial senescence, decreasing cellular regeneration and leading to emphysema [102]. Similarly, granzyme B excreted by CD8+ cells, has been implicated in extracellular matrix degradation and subsequent tissue remodeling associated with emphysema [103, 104].

Other driving factors of COPD include age-associated cellular senescence, as evidenced by the high prevalence among elderly patients with COPD [105]. Evidence suggests emphysema is caused by accelerated ageing of lung tissues and is associated with flawed endogenous anti-ageing (sirtuins) and autophagy mechanisms [106–108]. This process leads to eventual telomere shortening, cellular senescence, and the development of COPD [109, 110]. Oxidative stress is another key driver of COPD pathogenesis. Reactive oxygen species (ROS) are produced by several cells, including macrophages and neutrophils [111]. ROS activate nuclear factor-kappa B and p38 mitogen-activated protein kinase, thereby increasing protease activity and activating inflammatory genes, whilst decreasing anti-protease activity and promoting elastolysis [112, 113].

Establishment of Disease and Exacerbation Biomarkers

Knowledge in the field of COPD research continues to grow, with an array of animal models currently in use. However, assessing their utility is increasingly difficult in the absence of clinically validated biomarkers that are predictive of both disease onset and progression [114]. Spirometry assessment is currently the most trusted diagnostic tool; however, it does not account for the heterogeneity of symptoms seen amongst patients. Patients with stable spirometry readings are still subject to cardiac arrest and muscle weakening, the most common aspects of COPD mortality and morbidity, respectively [115].

Despite the critical need for novel biomarkers, very few clinical studies have linked disease outcome (morbidity/mortality) with any reliable biomarkers [116–118]. Patient studies have shown correlations between generalized inflammatory markers such as increased white blood cell count [119, 120], TNF-α [120, 121], IL-6 [121, 122], C-reactive protein [123], and IL-8 [120, 124], but few show significant correlations with the prediction of exacerbation onset [125, 126]. Few biomarkers have been approved for use in clinical trials, with only plasma fibrinogen and soluble receptor for advanced glycation end products (sRAGE) approved by the US Food and Drug Administration (FDA) [127–129]. Correlations in animal models have also been investigated, revealing associations between disease state and plasma fibrinogen and sRAGE levels [130–133]. Novel studies investigating volatile organic compounds as breath-based biomarkers using gas chromatography have provided new insight in the field [134]. The establishment of proteomic, genomic, and metabolomic platforms continues to deliver possible biomarker alternatives [135], although these cannot be implemented without proper validation in clinical trials.

In vitro Models

In vitro models facilitate the study of selected physiological processes within a controlled environment before confirming the hypothesis in a more complex in vivo environment [136, 137]. The human lung features a range

Table 1. Summary of advantages and disadvantages of using various animal models to understand COPD

Animal model	Advantages	Disadvantages	COPD studies (Ref. No.)
Mouse	Genetic heterogeneity Diverse responses to lung injury Low comparative costs Abundance of species-specific reagents	Replication of severe COPD not possible Low number of submucosal glands Monopodial airway branching Obligate nose-breather Size makes pulmonary parameter measurement difficult	190, 228, 310, 311
Rat	Higher lung surface area compared to other rodents Greater size makes procedures easier Fibrotic depositions following smoke exposure	Lower genetic similarity to humans Monopodial airway structure Higher mucociliary clearance No goblet cells Fewer mucosal glands	312–316
Guinea pig	Similar inflammatory response to humans Uniform airway epithelial arrangement Docile Anthropomorphic response to smoke inhalation	Axon reflex controls inflammation Low genetic variation Few immunological markers Difficult blood collection (absence of tail/thick skin) Soft palate makes intratracheal procedures challenging	250, 317–321
Hamster	Higher nitric oxide production More important ciliated epithelium Moderate goblet cell numbers	Low availability of species-specific reagents Low genetic diversity Aggressive behavior (handling more challenging)	263, 322–324
Rabbit	Similar response to histamines and antigens More similar lung structure compared to rodents Larger size (procedures easier) Fairly docile Longer lifespan (longer studies)	Susceptibility to non-experimental disease No cough reflex Monopodial lung structure Different mucus composition	325–328
Dog	One lung can be used at a time Larger mouth (easier intratracheal procedure) Greater alveoli number More representative epithelial microstructure than rodents Cough reflex	Few species-specific reagents High housing costs	329–333
Pig	Large number of diverse breeds Organ-to-body weight similar to humans Similar lung structure to humans	Narrow mouth (intratracheal procedures challenging) High housing costs	334, 335
Sheep	Docile Dichotomous lung structure Similar mucus content to humans Similar response to smoke inhalation	Disease progression poorly understood Few species-specific reagents High risk of vomiting during procedure Need for human breathing devices	286, 288, 336, 337
Non-human primate	Dichotomous airway Genetic similarity Human reagents can be used	Skilled handling required Specialized equipment Higher costs Higher ethical implications	102, 338–343

of different cell types including epithelial cells, macrophages, fibroblasts, smooth muscle cells, and pathogenic cells that exist in a milieu of microenvironments [138, 139]. Many COPD mechanisms have been tested in immortalized cell lines, such as BEAS2B and A549, derived from human bronchial epithelium and human lung alveolar epithelium, respectively [140]. Although these cells do not offer direct representations of the human lung environment, they offer less variability and are easier to maintain and manipulate in culture, whilst still maintaining lung-derived primary cell features [141]. COPD involves the interaction of the respiratory, immune, and cardiovascular systems. Understanding disease pathology and progression requires the use of co-cultured cells in either monolayers or spheroids from each system to more accurately represent the in vivo environment [142, 143].

Primary cells obtained directly from patients through lung resections, bronchoalveolar lavage fluid extraction, and biopsies offer a more patient-specific phenotype compared to immortalized cell lines [142, 143]. The utility of these cell lines is increased when used as structurally representative models such as air-liquid interface (ALI) cultures, which facilitate cell differentiation and the development of structural features such as cilia [144–146]. A noteworthy drawback of using in vitro cultured cells is their removal from the extracellular matrix and milieu of inflammatory cells, potentially skewing readouts and resulting in poor translational value for clinical application [143]. This may be minimized by culturing cells on biorelevant matrices [147], limiting passage number, and ensuring cell morphology consistency [143].

Alternatives to this approach include ex vivo lung slices that retain lung structural and organizational properties [148], and other perfusion models which allow for extracellular matrix and tissue factor involvement [149, 150]. Three-dimensional (3D) organoid models have also been proposed for possible inclusion in biomedical research efforts [151–153]. Several models involving undifferentiated bronchial epithelial cells have been established and are commercially available, including EpiAirway® (MatTek Corporation, Ashland, MA, USA) and MucilAir™ (Epithelix Sarl, Geneva, Switzerland). These systems offer 3D human lung organoid cultures that are co-cultured or stimulated with smoke or alternative stimulants to induce inflammatory or fibrotic phenotypes similar to those seen in COPD, asthma, and pulmonary fibrosis. These cells can be cultured for a significantly longer time than ex vivo lung and perfusion models [147]. Benam et al. [154] demonstrated an innovative microfluidic system incorporating endothelial and epithelial cells exposed to airflow and culture medium, respectively. This system provided a means to study immune cell recruitment to the airways [154]. This model was further expanded to the lung-on-a-chip model by incorporating a flexible membrane, which mimics inspiratory and expiratory movements, and produces ALI-induced features [155, 156]. Despite several advantages offered by in vitro/ex vivo approaches, there are limitations to experimental reproducibility due to variations in human tissues/cells, obtaining tissues from individuals with underlying diseases, the limited availability of human tissues, and the interlaboratory differences in cell culture protocols, particularly involving 3D models [147]. With the incorporation of tissue printing technologies and the greater understanding of stem cell biology, the development of lab-grown, standardized human tissues may not be too far in the future [157, 158].

Animal Models

Although the 3 Rs (reduction, replacement, and refinement) should always be implemented where possible, it is important to confirm in vitro findings in a whole-lung environment in a pre-clinical animal model prior to clinical trials. It is vital to interrogate an organism at multiple levels including molecular, cellular, organ, and whole organism to fully comprehend the intricacies of a disease [159]. There are a number of small animal models that adequately recapitulate individual features of COPD and choosing the most representative model has become an integral factor in the development of novel COPD treatments [160]. Current models used to investigate COPD, which display a range of similar anatomical and physiological responses to COPD-inducing stimuli in humans, include rodents, dogs, guinea pigs, non-human primates, and sheep (as reviewed in [23, 161–163] and summarized within Table 1). Examples of COPD studies in animals range from the discovery of porcine pancreatic elastase-induced emphysema in murine and hamster lungs [164, 165], to the discovery of airspace enlargement induced by CS in mice, rats, and guinea pigs [166, 167].

Several experimental models of COPD are available, each with their own advantages and disadvantages with many models replicating different stages of disease progression [168]. Due to complex COPD pathophysiology, it is difficult to capture both chronic bronchitis-related or emphysematous changes in a single model. Multiple biologically relevant procedures may be used to recapitulate aspects of COPD (Fig. 3).

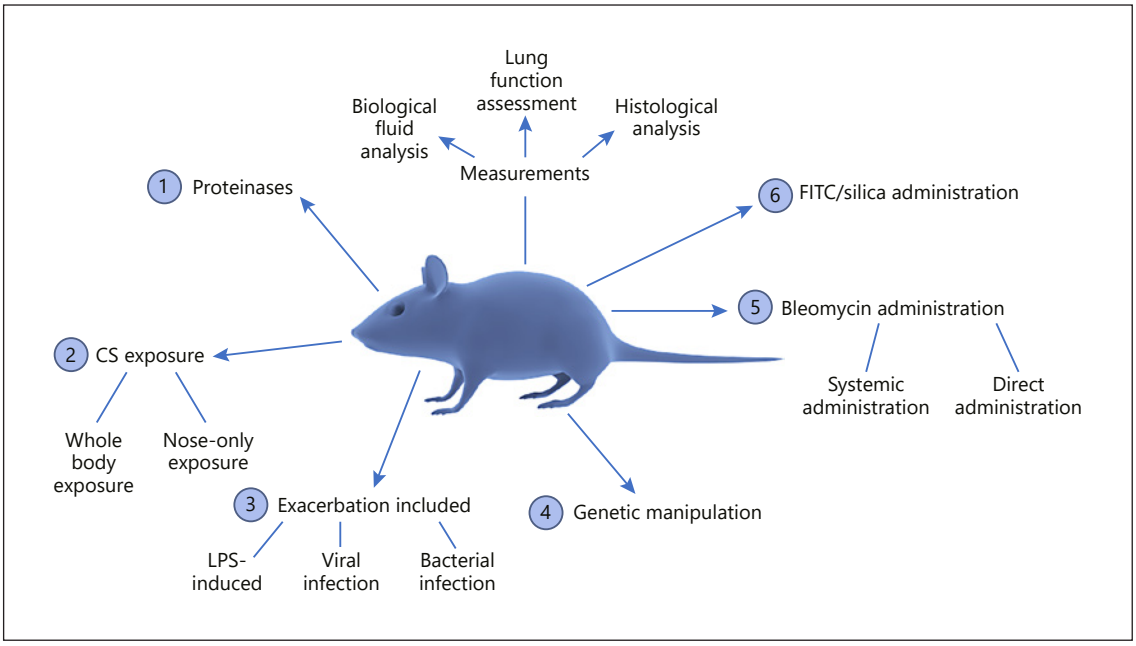

Fig. 3. Commonly utilized animal models of COPD/fibrosis.

Elastase Instillation

The tracheal installation of tissue-damaging agents was one of the first models to be adapted for studying lung damage [169]. In 1963, Laurell and Eriksson [170] described the development of emphysema in elderly patients deficient in α_1-antitrypsin, causing a buildup of elastase. This became the basis for the development of several elastase models, the earliest described by Gross et al. [171] who demonstrated that the plant elastase, papain, induced emphysematous changes in rats. Elastase is the most commonly used proteolytic enzyme (porcine pancreatic elastase or human neutrophilic elastase) to induce and perpetuate an inflammatory response in the mouse [172]. The attractiveness of the model is that a single dose of elastase results in immediate loss of alveolar wall structure. However, the narrow dosing window of elastase is restrictive, with concentrations below the recommended dose providing no change in lung structure and concentrations above a threshold level resulting in severe pulmonary hemorrhage and mortality in multiple species [173, 174]. Although elastase involvement in COPD has been shown, the direct instillation of elastases into an animal model offers little understanding of disease pathogenesis [175]. The model reduces the impact of immune cell involvement, a vital aspect in human COPD pathophysiology, and instead induces "artificial" tissue destruction [176, 177]. These models may be more relevant for the initial assessment of alveolar damage mechanisms. Recently, Gu et al. [178] demonstrated the use of a murine model of anti-elastin autoimmunity resulting in emphysema that could be used to examine the role of immune tolerance in diseases, such as COPD, and enhance the clinical relevance of elastase-based models.

Genetic Manipulation

Several studies have demonstrated the role of genetic factors in COPD development [179, 180], with naturally occurring mutant murine models [165, 181], conditional transgenic [182, 183], and transgenic animals [184–186] developing emphysema. Murine models are favored over other animal models due to their large litter size and fast reproductive cycle, which speeds up the selection of favorable genetic traits that can be used in disease models [187]. Prior to conducting research involving transgenic animal models, one should always consider the translational relevance of potential findings to the human disease context. An example of a successful approach can be seen in matrix metalloproteinase-12 (MMP-12), which is essential for the development of emphysema in mice exposed to CS, with MMP-12 associated with risk of COPD in adult smokers [188–191].

CS Exposure

CS exposure is one of the major risk factors for the development of COPD and is a model which many groups have adopted [162]. The protocols for smoke exposure in mice vary greatly with differences in length, frequency, and number of CS exposures, as well as the type of exposure and cigarette being used [26]. Additionally, the method of smoke production is a critical factor and is described as either mainstream smoke, produced by the act of puffing or inhalation, or sidestream smoke, which is generated passively from the burning of cigarettes [26]. These different methods of smoke generation influence smoke component concentrations but seemingly do not significantly influence the animal disease state [192].

The differences between the method of smoke exposure, either whole-body or nose-only systems, has also been highlighted as a potential source of variation in mouse disease phenotypes [25, 168]. Whole-body CS exposure involves animals being exposed to varying concentrations of CS in a chamber. A downside to this approach is that animals retain constituents of CS on their fur which is ingested and can influence results [168]. This is alleviated using the nose-only method, whereby animals are exposed to CS using a restraining cone. The use of CS nose-only models eliminates most fur exposure, allowing concentrations of CS to be regulated. However, nose-only exposure is more stressful for the animal, sometimes resulting in weight loss due to prolonged confinement in restraining devices [168]. It is suggested that nose-only exposure produces more pronounced mouse phenotypic changes that may be more similar to human emphysema [26, 168].

Interspecies differences influence the development time of emphysematous phenotypes following CS exposure, with many animals taking 4–6 months [193] to display signs of disease whilst rats produce a similar phenotype in 2 months [194] due to physiological differences discussed later in this review. The disadvantages to using CS exposure include the resistance of some species, such as mice, to CS-induced neutrophilic inflammation and damage, which ceases after CS exposure is discontinued [176, 195]. As such, many have commented that the murine model of CS exposure fails to capture clinical disease complexities [177].

Lipopolysaccharide Exposure

Lipopolysaccharide (LPS) is a Gram-negative bacterium cell wall component, present in air pollution and organic dusts [196, 197]. LPS in COPD patient bronchoalveolar lavage fluid has indicated a potential role for LPS in disease progression [198, 199]. A single LPS exposure induces pulmonary inflammation in many animals, characterized by neutrophil and macrophage influx [200–205]. Chronic exposure to LPS induces structural changes to the murine and guinea pig lung, which persist after LPS exposure has ceased, mediated primarily by increases in TNF-α, IFN-γ, and IL-18 [203, 206–210]. The advantages of using LPS-induced inflammation and fibrosis models over other models such as CS include the relatively short time frame to achieve pathological features of COPD [211]. The changes reported in animal LPS studies are equivalent to features seen in mild human COPD [168].

This distinction makes the LPS model more valuable for understanding exacerbation-related COPD mechanisms [212]. Bacteria isolated from the lungs of patients immediately after exacerbation events are predominantly Gram-negative [213, 214]. Several groups have attempted to replicate COPD exacerbations in animals by combining COPD-induced animals (using CS or other induction agents) with exposure to bacterial/viral infection or LPS, often increasing the severity of emphysematous changes [168, 215–217].

Bleomycin Administration

The most widely used method for fibrotic lung damage induction is via bleomycin administration, a chemotherapeutic agent, known to induce lung fibrosis in approximately 10% of humans [218]. In animal models, bleomycin may be administered using a number of different procedures, each leading to different disease phenotypes. C57BL/6 mice appear to be more susceptible to bleomycin-induced fibrosis, with BALB/c mice more resistant due to differential cytokine expression [219].

The most common method of bleomycin administration consists of a single intranasal or intratracheal dose of bleomycin, with analysis 3–4 weeks later. Bleomycin causes acute tissue damage in lung regions where the solution permeates, followed by localized inflammation which peaks after 7 days, and subsequent fibrosis [220]. Intratracheal/intranasal bleomycin-induced fibrosis is reproducible, only affects the lungs, and is utilized by many research groups globally, allowing the generation of comparative results [221, 222]. Inflammation-associated fatalities reported using this model are common, with bleomycin deposition occurring predominantly within the central lung, modelling an acute lung injury or acute

respiratory distress syndrome akin to the human phenotype [223].

Systemic bleomycin administered intravenously or subcutaneously results in more homogenous, distal patterns of lung fibrosis throughout the lung. Unfortunately, several bleomycin administrations are required over a longer time period to achieve this fibrotic effect [222]. The systemic administration of bleomycin results in less inflammation compared to direct intratracheal administration and is less predictive of localized fibrotic conditions such as idiopathic pulmonary fibrosis [220].

Fluorescein Isothiocyanate and Silica Administration

Fluorescein isothiocyanate (FITC) induces a similarly localized pattern of inflammation in mice compared to bleomycin, which persists for up to 5 months after administration, comparably longer than most other administered substances [220]. FITC is also fluorescent, allowing FITC-exposed lung regions to be easily imaged [222]. However, the FITC model does not produce robust results due to difficulties in FITC preparation, causing groups to utilize other models [220].

Silica particles induce similar responses in the lungs of humans and mice, with fibrotic remodeling persisting after silica administration ceases [220]. As with bleomycin, this phenotype appears more readily in C57BL/6 mice [220]. The model takes between 30 and 60 days to establish measurable fibrotic changes in mice, longer than bleomycin and several other models [222].

Animals Currently Included in COPD Studies

Mice

Mice offer several advantages over other species, including low housing costs, relatively short breeding time in comparison to higher species, extensive knowledge of the genome [224, 225], ease of genetic modifications [160, 226], and heterogeneity of reactions to exogenous stimuli amongst different murine strains [177, 224, 227]. CS is most commonly used to induce COPD in mice [25, 176, 195, 228–230]. Other models rely on the administration of LPS or proteases [195, 229], or the use of genetic alterations [187]. These models are able to replicate early-stage COPD symptoms including small airway and vascular remodeling, mucus hypersecretion, and increased lung inflammation [231]. However, modeling the more severe stages of COPD, including chronic bronchitis and debilitating lung function decline, have been more arduous as the mice either recover from the symptoms or succumb to disease before disease parameters can be adequately assessed [232].

It is important to note lung anatomical differences between humans and mice, with the latter possessing fewer submucosal glands and only six to eight pre-bronchioles before termination, with further anatomical differences reported elsewhere [177, 233]. Importantly for respiratory studies, humans inhale smoke through the mouth predominantly, whilst mice are obligate nose-breathers [234]. Arterial oxygen tension and mean arterial pressure are exceptionally difficult to measure considering the animal's small size, although full-body plethysmography allows for some of these challenges to be overcome [234, 235]. Considering these differences, it is important to incorporate multiple animal models to answer different biological questions, as an all-encompassing single model is still not available.

Rats

Rats have been used less frequently to model COPD than mice, partly because of their reported resistance to the development of COPD symptoms [25, 176, 236]. Although rats possess a less analogous genome to humans than mice [237], there are slight advantages to using the rat model in the study of COPD. Anatomically, the rats' larger body size facilitates biological sampling, with more straightforward surgical and lung function measurement procedures [238, 239]. Rats also harbor larger alveoli, with a mean linear intercept of 100 μm and an air-blood barrier size of 0.38 μm, although these are still not comparable to those of humans (210 and 0.62 μm, respectively) [240].

The bronchiolar branching pattern of both mice and rats is monopodial (single continuous bronchus with multiple emanating bronchioles) whilst it is dichotomous (the bronchus diverges into two bronchioles, which subsequently branch into two smaller airways) in humans, which may lead to differences in particulate matter distribution within the lungs [241]. Mucociliary clearance is markedly increased in rats with average particulate matter cleared from the lung within 8 h, although this process can take in excess of 24 h in humans [240]. Other disadvantages to using rat models include the fact that they are obligate nose breathers [235], possess no goblet cells [23], and possess significantly fewer mucosal glands than larger animals, rendering chronic bronchitis modeling difficult [242].

Guinea Pigs

Guinea pigs have been studied extensively in respiratory experimentation, particularly in the field of tuberculosis [243–245]. Guinea pigs mount inflammatory responses similar to those of humans, thus providing more accurate interspecies comparisons [246]. The development of emphysematous lesions, lack of elastic lung recoil, and inability to gain weight have also been recorded in this model [247]. Guinea pigs have comparably smaller lungs than other rodents as they possess a larger heart [248]. The lungs are divided into seven lobes with a human-like dichotomous bronchiolar structure [248, 249]. The tracheal epithelium of the guinea pig is more uniformly arranged than that seen in mice and comprises mucus glands and goblet cells in the larger airways [23]. Upon CS exposure, guinea pigs exhibit anthropomorphic responses such as acute neutrophilia, increased epithelial permeability, sustained monocyte recruitment, mucus secretion, and alveolar destruction [23, 250]. Viral infection also appears to elicit similar neutrophil recruitment in guinea pigs, highlighting the utility of this model for exacerbation [251]. The major disadvantage of this model is the axon reflex, which plays a limited role in human airways but controls the majority of responses to lung injury in the guinea pig [245]. The lack of available inbred strains also severely restricts the potential for variation in disease phenotype [245]. There are also fewer commercially available cytokine and immunological markers for guinea pigs [245, 252]. Blood collection and the administration of substances to guinea pigs is more challenging because of their lack of a tail and thick skin, whilst the presence of an elongated soft palate may hinder intratracheal procedures [166, 253, 254]. The guinea pig is a docile animal, making handling and procedures easier than that with other rodents [255].

Hamsters

Hamsters are the fifth most used laboratory animal (behind mice, rats, rabbits, and guinea pigs [23, 160]), and are more commonly used for vaccine production and therapeutic studies [256–258]. Hamsters produce significantly more nitric oxide than other rodents following lung injury [259] and have been used in several studies investigating both CS exposure [260–263] and diesel smoke exposure [264, 265]. In addition, hamsters have ten times more non-ciliated bronchiolar cells than other rodents and moderate numbers of goblet cells [266, 267].

As with other rodents, the hamster bronchiolar branching pattern is monopodial [266]. As the hamster is not commonly used, the issue of disease-specific reagents such as cytokine and immunological markers is a problem [268]. The hamster can be more aggressive than many other rodent species because of its nocturnal and territorial nature, making handling more challenging [269].

Rabbits

Rabbits are phylogenetically closer to humans than other rodents as reflected by closer anatomical, physiological, genetic, and biochemical similarities [270, 271]. Rabbits have been used to study a number of pulmonary diseases including obstructive airway disorders, cystic fibrosis, and embolic stroke [272–274]. Furthermore, airway inflammatory responses to stimuli such as histamines and antigens show comparable responses in rabbits and patients suffering with asthma [275]. The rabbit allergy model, which involves the sensitization of young rabbits to antigen, has shown sensitivity to similar asthmatic drugs such as B-adrenoreceptor agonists, corticosteroids, and phosphodiesterase inhibitors [275].

The disadvantages of using rabbits as experimental animals include their propensity to bacterial and viral infection during non-sterile procedures [276]. Furthermore, the inability of the rabbit to cough, its monopodial lung structure, and different mucus composition compared to that of humans make this a poor model for CS inhalation studies [271, 277–279]. However, the long lifespan and large body size make the rabbit attractive to long-term lung function studies, although this is accompanied by higher housing costs [275].

Dogs

Experimental dog models weigh between 10 and 20 kg and possess larger airways than rodent and lagomorph models, thus allowing targeted delivery of disease-causing reagents or treatments to one lung, whilst allowing the other lung to serve as a control to minimize animal usage and interanimal variability [280, 281]. Dogs also possess a large mouth, allowing for easier pulmonary delivery system studies [282, 283]. The lung structure of the dog is monopodial [284], with higher numbers of alveoli and more human-like epithelial microstructure than rodents [285]. The dog is one of few animal models that exhibits a cough reflex, an important physiological symptom of COPD [281]. Respiratory studies using dogs are hampered by increased costs and limited species-specific reagents [160].

Sheep

Sheep are fairly docile laboratory animals weighing 35–55 kg [160]. Irregular dichotomous branching of the bronchioles combined with similar mucus production

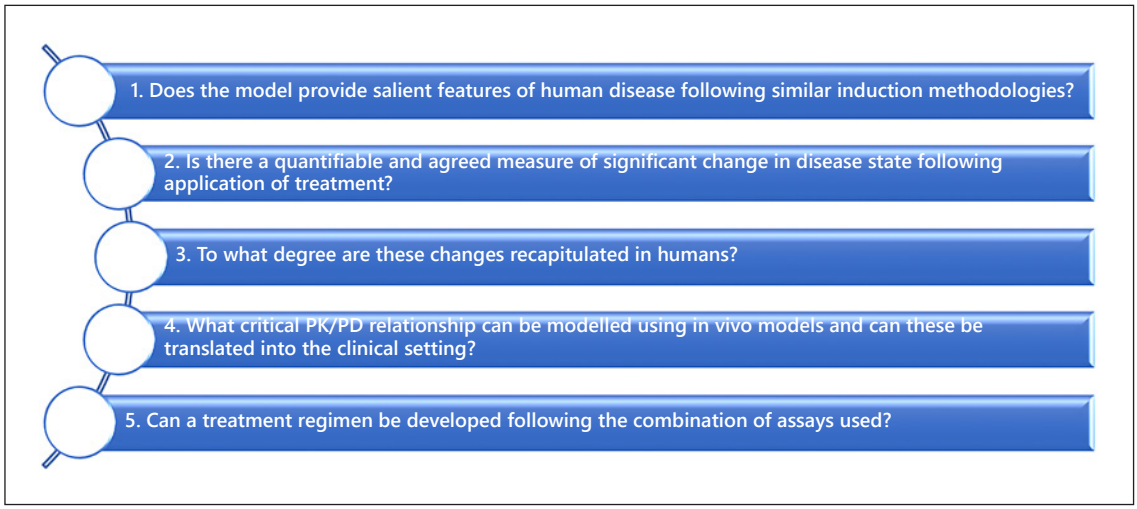

Fig. 4. Determining the clinical importance of assay inclusion in a drug discovery cascade. PK, pharmacokinetic; PD, pharmacodynamic.

and epithelial cell distribution in the airways lead to human-like responses following smoke exposure [286–288]. However, COPD progression in sheep is poorly understood due to the limited array of available reagents to study the specific inflammatory environment of the sheep respiratory tract [287]. Further drawbacks to the sheep model include an increased risk of vomiting and the need to use human devices because of the similarity in breathing parameters, which increases the costs of experimentation but may also allow human-like inhalation devices and techniques to be trialed [287–290].

Pigs

There are over 500 known breeds of pig, presenting a wide variety of experimental variables with which to work [291]. Large pigs (100–115 kg) have organ-to-body weight ratios similar to those of humans and very similar lung structures to humans, with only primate lungs showing more similarities [292]. The major downfall of the experimental pig model is the exceptionally narrow mouth opening, making intubation and intratracheal procedures very challenging, often requiring the use of modified human equipment [293, 294]. Furthermore, extensive housing requirements increase the cost of using pigs in respiratory studies [295].

Non-Human Primates

Non-human primates range in size from 130 g to 200 kg, with the closest physiological representation to humans being the chimpanzee. However, chimpanzees are endangered and therefore alternative animals such as macaque monkeys are more commonly used [296, 297]. In particular, the rhesus macaque (*Macaca mulatta*) possesses the most similar lung structure to humans, with regular dichotomous branching of the bronchioles and extensive alveoli distribution [298, 299]. Microstructures of the rhesus macaque also present striking similarities to humans, with ciliated, goblet, and basal cells lining the airways in analogous patterns [300, 301]. Genomic similarities present the added advantage of using human reagents in this model, allowing easier associations between species to be made [302].

The primate model requires a greater degree of skilled handling as the animals may anticipate procedures, increasing levels of undue stress. Specialized equipment and techniques are required to anaesthetize and perform procedures on the animals, which comes with increased housing costs and greater ethical implications.

Modelling Comorbidities of COPD

Patients with COPD are prone to co-morbidities such as lung cancer, osteoporosis, cardiovascular diseases, and cachexia in late-stage disease [303]. This highlights the multifactorial nature of COPD, which should be considered when designing models to fully understand disease interplay [116, 304]. Several groups have already modified CS smoke-induced animal models to mimic pulmonary hypertension [305–307], muscle atrophy [308], and

atherosclerosis [309]. These models, which incorporate co-morbidities, could offer a more holistic understanding of COPD.

Selecting a Model for COPD

The advantages and disadvantages of various COPD models have been highlighted throughout this review but selecting the most relevant model has not yet been addressed. The chosen model should answer key questions in the drug discovery process (Fig. 4) and fit into a high-throughput assay cascade that addresses various aspects of disease physiology.

The salient features of disease physiology should be predicted by each model. Models that fail to reproduce some aspect of human physiology do not offer any relevance in this treatment discovery algorithm and should be excluded. Similarly, the value of a model is greatly diminished if it does not show a measurable change following treatment. The translation of these findings in pre-clinical models to clinical trials is paramount to the process of novel treatment discovery. Crucially, following pre-clinical testing, the compound or treatment regimen should be safe and effective in patients as many hours and significant monetary investments are dependent upon this.

If the selected model does not fulfill these criteria, subsequent reassessments should take place to replace or refine the model in order to make it more predictive of the clinical environment. The testing cascade used to assess novel treatments should be optimized from the initial design of the molecule, pharmacochemical assessment, in vitro testing, testing in predictive animal models, and finally into human clinical trials. Refinement is key to developing a cost-effective treatment in the shortest period of time.

Conclusion

Significant progress has been made in the use of animal models since the initial introduction of animal experimentation. However, no single model of COPD captures all aspects of the human disease. The animal models discussed in this review mimic specific disease aspects without fully reproducing the broad spectrum of human COPD phenotypes. This points to a need for the careful selection of animal models, which sufficiently capture the most pertinent clinical features of disease, allowing for the performance of more predictive pharmacokinetic/pharmacodynamic analyses for clinical translation. This will strengthen our knowledge of current molecular mechanisms of COPD and allow for the provision of better treatments. With the inclusion of relevant animal models and newer technologies as outlined here, the treatment of COPD and other lung diseases could be closer than expected.

Acknowledgements

This work was supported by the Swedish Heart and Lung Foundation and the Alfred Österlund Foundation.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

L.T. and A.B.S. contributed to the conceptual design for this review. L.T. wrote the initial draft of the manuscript whilst A.B.S. edited and revised the manuscript.

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