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REVIEW

From preclinical to clinical models of acute respiratory distress syndrome

Ruoyang Zhai[1], Woodys Lenga Ma Bonda[1], Gustavo Matute-Bello[2,3], Matthieu Jabaudon[1,4,*]

[1]GReD, Université Clermont Auvergne, CNRS, INSERM, 63000 Clermont-Ferrand, France
[2]Center for Lung Biology, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of Washington, Seattle, WA 98105, USA
[3]Medical Research Service, VA Puget Sound Healthcare System, Seattle, WA 98105, USA
[4]Department of Perioperative Medicine, CHU Clermont-Ferrand, 63000 Clermont-Ferrand, France

***Correspondence**
mjabaudon@chu-clermontferrand.fr
(Matthieu Jabaudon)

Abstract

Various preclinical models that mimic the clinical causes of acute respiratory distress syndrome (ARDS) have been used to better understand the mechanisms of acute lung injury and its repair and to investigate novel therapies targeting such mechanisms. Despite important preclinical and clinical research efforts in recent decades, few candidate therapies with promising preclinical effects have been successfully translated into the clinical scenario, which could be attributable to the intrinsic limitations of the models as well as to the incorrect identification of appropriate phenotypes of patients to target with novel therapies that have proven beneficial in select preclinical models. However, current translational research strategies based on the use of multiple complementary preclinical and clinical models hold the promise of revolutionizing intensive care by using granular knowledge that should allow for a better diagnosis, greater predictability of the disease course, and the development of targeted therapies while ensuring patient safety through reduced adverse effects. Our goal was to summarize the strengths and limitations of the available models of ARDS, including animal, *in vitro*, and clinical models, and to discuss the current challenges and perspectives for research.

Keywords

Acute respiratory distress syndrome; Acute lung injury; Preclinical models; Translational research

1. Introduction

The acute respiratory distress syndrome (ARDS) is a severe form of acute lung injury characterized by the onset of hypoxemic respiratory failure associated with noncardiogenic pulmonary edema and dysregulated inflammatory responses [1–3]. The incidence of the syndrome is high, representing approximately 10% of patients admitted to the intensive care unit (ICU) and, despite important preclinical and clinical research efforts since its first description in 1967 [4], the syndrome is still associated with high mortality rates and long-term impacts on survivors [5]. Substantial progress has been made in improving supportive intensive care, such as the application of lung-protective mechanical ventilation, but specific pharmacological therapy is still lacking. Although this may be due to the incorrect identification of appropriate subsets of patients to target with novel therapies that have proven beneficial in select preclinical models, it may also be explained by the poor clinical translation of promising therapies from preclinical models, which can be attributable to the intrinsic limitations of the models [6, 7].

Research efforts have been held back in part by the difficulty of modeling human ARDS in animals, mainly due its heterogeneity, with many clinical or biological/functional variations among patients, in addition to its distinct causative factors, such as pulmonary or extrapulmonary sepsis, gastric fluid aspiration, transfusions, severe trauma, injurious mechanical ventilation, and/or reperfusion of ischemic tissues, among other causes [3, 8–11]. In this perspective, various preclinical models of "acute lung injury" that mimic the causes of clinical "ARDS" have been used to better understand the mechanisms of injury and its repair, and to develop novel therapies targeting these mechanisms [12].

Ideally, a comprehensive model of acute lung injury should be able to reproduce all the relevant features of ARDS pathophysiology, including all the physiological, functional, biological, and pathological symptoms related to injury and their consequences. However, such an ideal model mimicking the clinical scenario does not exist, and all the preclinical models have intrinsic limitations and strengths [13, 14]. Importantly, the "ideal" model may not always be the one that best reproduces human ARDS, but the one that is best suited to answer a specific scientific question. For example, despite all their limitations, mouse models remain key for mechanistic studies because of the ease of genetic manipulations, the ability to generate a large cohort in a short time, etc. Large animal models have more translational value but are less well suited to mechanistic studies. This leads to a proposed stepwise approach for animal studies, with reductionistic, targeted ro-

dent models as an initial step to identify potential mechanisms or therapeutic targets, and increasingly translational models (e.g., large animals) as recommended pre-clinical steps as the research gets closer to the bedside (e.g., testing of novel therapeutics). Although most preclinical studies on ARDS have been performed using animal models, other preclinical *in vitro* models are available and, more recently, the use of clinical models of ARDS has also broadened our ability to decipher injury or repair mechanisms and to identify novel targets for therapy development.

In this narrative review, our goal was to summarize the strengths and limitations of the available models of acute lung injury, including animal, *in vitro*, and clinical models, and to discuss the current challenges and prospects for research.

2. *In vivo* models of ARDS

Live animal models of ARDS play an important role as a bridge between clinical and laboratory studies in research translation. The consensus criteria of an *in vivo* model include acute onset of injury, altered alveolar-capillary membrane, alveolar inflammation, and lung histopathological changes that, together, lead to physiological impairment, such as arterial hypoxemia or impaired alveolar fluid clearance (Table 1, Ref. [14]) [14, 15]. Despite the many anatomical and physiological differences between animals and humans influencing the response of the lung to an acute injurious stimulus and affecting the evaluation of lung injury, *in vivo* models are frequently used as a reliable tool to test hypotheses with variably controlled parameters [14]. The latest updates on what constitutes an animal model of ARDS have focused on the clinical presentation, highlighted the importance of some degree of pre-existing lung injury, suggested the use of mechanical ventilation (to better coincide with the most frequent clinical scenario), and recommended the assessment of physiological outcomes to test potential therapeutic candidates [13, 14, 16]. Since no single animal model can fully replicate all the pathophysiological features of ARDS, multiple animal models have been developed, with the goal of replicating, sometimes in a very caricatural way, the clinical risk factors for ARDS, such as aspiration, pulmonary/extra-pulmonary infections, and mechanical ventilation-induced lung injury, among others [2, 3]. Schematically, preclinical ARDS can be caused *in vivo* through direct lung injury (such as after pneumonia or acid installation) or indirect lung injury (such as after peritonitis). "Double-hit" models have also been developed, which are intended to mimic clinical scenarios combining a specific risk factor (such as pneumonia) and a superimposed injury (such as hyperoxia or injurious ventilation) [17].

Different animal species have been used in the models, from large animals, such as non-human primates, pigs, dogs, cattle, sheep, and rabbits, to smaller animals, such as rats and mice. Larger animals are believed to better replicate human conditions, but these models are expensive and require specialized animal facilities. Models using smaller animals, such as mice, are more widely accessible and are a very powerful research tool, as the animals can be genetically modified in multiple ways to facilitate the detailed mechanistic study of complex pathways [13, 18–20].

2.1 Lipopolysaccharide-induced sepsis models

Lipopolysaccharide (LPS), often named endotoxin, is composed of a polar lipid head group (lipid A) and a chain of repeating disaccharides. It is present on the outer membrane of Gram-negative bacteria such as *escherichia coli* and *haemophilus influenzae*. The host response to LPS plays an important role as a mediator of bacterial sepsis via its binding with toll-like receptor 4 and the subsequent secretion of inflammatory mediators [21, 22]. LPS-induced lung injury caused by pulmonary or extra-pulmonary sepsis is one of the most commonly used ARDS models [13]. LPS can be administered into the lungs by intratracheal instillation or inhalation to produce direct lung injury in which the alveolar epithelium is the primary structure that is damaged. LPS can also be administered intraperitoneally or intravenously to reproduce peritonitis or blood infection, respectively, with marked systemic inflammatory response. Interestingly, repeated or continuous LPS exposure has been shown to exacerbate lung injury in models of extrapulmonary ARDS [23]. The LPS model ideally mimics a neutrophilic inflammatory response with increases in intrapulmonary cytokines and is, therefore, typically suitable for studies of inflammatory processes [13, 18]. However, it has significant disadvantages. First, the responses to LPS are highly variable among animal species, depending on the presence or absence of specific lung intravenous macrophages; for example, rodents are more tolerant to endotoxin exposure than pigs or sheep. Rodent models have been widely used to study LPS-induced lung injury due to their availability, easy accommodation, and relatively low cost. However, rodents are small animals with limited blood volume available for serial sampling [13, 24]. The endotoxin preparations used in animal studies may also vary in serotype and purity and can be contaminated with bacterial lipoproteins and other bacterial materials [25]. The duration of LPS exposure may also introduce some variability in the published results. In addition, the LPS model is often associated with mild changes in alveolar-capillary permeability and degrees of endothelial and epithelial injury, thus limiting clinical translation.

2.2 Live bacteria-induced sepsis models

Intrapulmonary or intravenous administration of live bacteria is another option to induce sepsis in animal models. Intratracheal instillation or inhalation of live bacteria, such as *streptococcus pneumoniae* or *pseudomonas aeruginosa*, can cause ARDS and, depending on the importance of the bacterial inoculum, systemic manifestations of sepsis [26–28]. The intravenous administration of live bacteria is followed within an hour by an initial phase of hypotension and leukopenia, which can progress to septic shock, intravascular coagulation, and death [13, 14]. Typically, live bacteria-induced sepsis models induce increased permeability, interstitial edema, and neutrophilic alveolitis. They are often used for studies of bacterial sepsis-induced lung injury. The intratracheal administration of live bacteria often results in localized pneumonia (rather than ARDS) in histological studies; however, the unilateral administration of bacteria can result in lung injury in the

TABLE 1. Features of experimental acute respiratory distress syndrome in animals and their main relevant measures in animals, as proposed by the official American Thoracic Society workshop report published in 2011 [14].

Features of experimental ARDS	Very relevant measures
Histological evidence of tissue injury	• Accumulation of neutrophils in the alveolus or the interstitium
	• Presence of hyaline membranes
	• Presence of proteinaceous debris in the alveolus
	• Thickening of the alveolar wall
	• Enhanced injury as measured by a standardized histology score
Alteration of the alveolar-capillary barrier	• Increase in extravascular lung water content
	• Accumulation of an exogenous tracer in the alveolar spaces or the extravascular compartment
	• Increase in total bronchoalveolar protein concentration
	• Increase in concentration of high molecular weight proteins in bronchoalveolar fluid (such as albumin, immunoglobulin M (IgM))
	• Increase in the microvascular filtration coefficient
Inflammatory response	• Increase in the absolute number of neutrophils in bronchoalveolar fluid
	• Increase in lung myeloperoxidase activity or protein concentration
	• Increase in the concentrations of proinflammatory cytokines in lung tissue or bronchoalveolar fluid
Physiological dysfunction	• Hypoxemia
	• Increased alveolar–arterial oxygen difference

It is recommended that at least three of the four "main" features are present in animal models of acute respiratory distress syndrome, and that at least one of the "very relevant" measures is performed for each feature of interest. This list of measures is indicative and may not be exhaustive, meaning that other measures may be relevant.

contralateral lung, depending on the bacterial dose. Therefore, the main technical issue with this model resides in the potential variability in the doses of live bacteria being administered.

Although viral infections are less frequent clinical causes of ARDS than bacterial infections outside of some specific pandemics (e.g., the coronavirus disease 2019 pandemic) [3, 5], animal models of viral pneumonia-induced lung injury are also being used to study the specific responses to or test the potential therapies for pathogens, such as influenza viruses or coronaviruses [29, 30].

2.3 Acid aspiration model

Gastric aspiration is one of the common causes leading to the development of ARDS in patients [31, 32]. This neutrophil-dependent form of lung injury is characterized by damage to the alveolar epithelium, alveolar hemorrhage, and intra-alveolar and interstitial edema. One of the most important features of this toxic process is the low pH of the gastric content, and hydrochloric acid (HCl) intratracheal instillation is the most used method to mimic gastric aspiration in animals, in particular in mice or larger animals such as pigs. This model induces the pathophysiological hallmarks of ARDS, with neutrophil recruitment and moderate effects on mortality [13]. The acid aspiration model is particularly useful for studying mechanisms of disruption of the alveolar-capillary barrier and of neutrophil recruitment. In addition, this reproducible model can be used to study the resolution phase of ARDS over multiple days after injury [33–35]. However, the narrow margin between injurious and noninjurious acid concentrations remains a limitation.

2.4 Abdominal sepsis models

Multiple models of peritonitis-induced lung injury have been described. In the cecal ligation and puncture (CLP) model, the cecum is ligated and punctured with a needle. The severity of the injury depends on the number of holes in the cecum and the size of the needle. In contrast to models using LPS and live bacteria, in which the effects are almost immediate, the effects of CLP develop over days, and the onset is less abrupt and consistent [13]. The main features of CLP-induced lung injury are mild hypoxemia, neutrophilic inflammation, and interstitial and alveolar edema, thus providing a complex representation of clinical extra-pulmonary sepsis [13]. Abdominal sepsis models can, therefore, be useful to study mechanisms of indirect lung injury due to sepsis. However, mortality is high, ranging from 25% at 18 h to 70–90% at 30 h, and it requires invasive surgery, although alternative surgical methods have been reported, such as colon ascendens stent peritonitis and laparoscopic cecal ligation [36, 37]. Other investigators have used intraperitoneal inoculation of fibrin clots containing controlled inoculum of bacteria, such as *escherichia coli*, to reproduce peritonitis-induced lung injury in mice and rabbits; in this model, lung injury more likely occurred at high doses, with overwhelmed host response, while lower doses only caused mild lung injury, such as in CLP model [38]. A more reliable and titratable model of peritonitis by the intraperitoneal injection of cecal slurry has been recently used to induce indirect ARDS [39, 40]. This model was first adapted from a neonatal necrotizing enterocolitis model [41]. Briefly, cecal contents are collected from euthanized donor mice, resuspended, and filtered before intraperitoneal injection.

2.5 Ventilator-induced lung injury

The use of ventilator-induced lung injury models has largely contributed to our understanding of the clinical benefits of lung-protective strategies of mechanical ventilation [42, 43]. Ventilation with high tidal volumes, especially without positive end-expiratory pressure, is associated with alveolar recruitment of inflammatory cells, changes in water and protein permeability, and histological injury, and, in general, severe hypoxemia develops within several hours [13, 14]. Although increased alveolar cytokine release has been reported in isolated lung preparations from mice and rats, it may not be present in all species. In addition, these models generally use very high tidal volumes (20–30 mL/kg body weight), which are not necessarily very relevant for clinical translation. However, they are very useful to study mechanical stretch and the activation of specific intracellular pathways involved in mechanotransduction. The effects of moderate increases in tidal volumes or of other changes in ventilator settings are best investigated in a "double-hit" model, following another primary insult.

2.6 Hyperoxia model

Prolonged exposure to hyperoxia may cause hyperoxia-induced lung injury in humans, with alveolar edema and endothelial and epithelial injury [44]. Animal models of hyperoxia have been used as direct lung injury models, sometimes as a secondary hit after peritonitis or LPS [45, 46]. The mechanism of hyperoxia-induced lung injury remains unclear and may be mediated by reactive oxygen species. The hyperoxia model provides an excellent model to study lung repair after lung injury. However, the major limitations are that this model requires specific equipment and prolonged exposure (for 72 h in many studies) [13].

2.7 Ischemia/Reperfusion model

Ischemia and reperfusion following lung transplantation or at other nonpulmonary sites can lead to a wide range of effects, including lung injury. This injury is associated with alveolar edema, epithelial and endothelial injury, inflammatory responses, massive production of free reactive oxygen species, and hypoxemia [13, 47]. Direct lung ischemia is generally induced by clamping the pulmonary artery, followed by reperfusion of the pulmonary and bronchial circulations. This model reproduces the development of acute lung injury after lung, intestinal or peripheral ischemia and reperfusion in humans and is probably more clinically relevant to transplantation studies than to ARDS. Of note, innovative approaches have been developed to allow non-invasive and repetitive in vivo microscopy of ectopic lung tissue using dorsal skinfold chambers in transplantation studies [48]. The main limitation of the ischemia/reperfusion model is that it requires specific surgical skills and equipment [13, 49, 50].

2.8 Models of viral infections

Live animal models of acute lung injury can be used to study the mechanisms of ARDS due to viruses, such as influenza viruses or, more recently, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [51–54]. Results from these models have emphasized the major role of the inflammatory host immune response to infection as a major contributor to lung injury. Although most airborne viruses initially affect the respiratory epithelium, the role of endothelial dysfunction has not been well established, and pathogen-specific pathways may contribute to diffuse alveolar damage [55]. Small animal models are widely used to study viral infections; however, translation may require genetic modifications (to the animal and/or the virus) to make the model susceptible, for example to SARS-CoV-2 [54]. Animal models can be rapidly mobilized to better understand the mechanisms of emerging viruses, such as during the recent coronavirus disease 2019 (COVID-19) pandemic, and to test new diagnostic, preventive, and/or therapeutic approaches [30].

2.9 Other models

The oleic acid model was first used to mimic clinical ARDS, although it is primarily based on the induction of fat embolism [56]. The intravenous administration of oleic acid leads to direct lung endothelial injury caused by necrosis and microvascular thrombosis. This model is rather reproducible, rapidly reproduces the most basic features of experimental ARDS in large animal models (pigs/piglets, sheep, dogs) [57–59], and is particularly useful to study lung mechanics, ventilatory strategies, and ventilation/perfusion distribution during acute lung injury. However, it is now seldom performed; its main limitations include a high mortality rate, a difficult application in smaller animals, and unclear effects on alveolar inflammation [13, 60].

Alveolar surfactant proteins regulate surface tension during breathing, and surfactant deficiency and dysfunction are frequent during ARDS [3], primarily due to decreased secretion by injured alveolar type II epithelial cells [61]. Surfactant depletion can be modeled by repeated saline lavage of the lungs and this model has good value to study surfactant functions and to assess the effects of ventilation strategies in animals. It induces rapid and reproducible, yet transient, hypoxemia and alveolar recruitment but only modest lung injury per se and very little neutrophil recruitment [13, 60, 62, 63].

Whether the bleomycin model is a good acute lung injury model is still discussed by many researchers, as it is one the few that leads to an acute inflammatory phase followed by a fibrotic phase that eventually resolves [13, 14].

3. *In vitro* models of ARDS

In vitro cell culture models can provide a direct link to lung cell responses in a simplified way and represent valuable methods to investigate basic biological and functional mechanisms and roles for specific cell types, receptors or pathways. They allow the manipulation of one or multiple variables through rigorously controlled, bias-free experiments to investigate the variation in quantitative protein markers, physiological functions, and/or gene expression in response to multiple conditions, including candidate therapies targeting precise mechanisms of injury or repair.

A monoculture of either alveolar epithelial, lung endothelial, or alveolar macrophages, among other cell types, can be performed to test mechanistic hypotheses or optimize the experimental parameters in subsequent *in vivo* or clinical research. *In vitro* monocultures can also be used to study important cellular functions, such as wound healing after a scratch assay [64] or transepithelial fluid transport by alveolar epithelial cells (often called “alveolar fluid clearance” *in vivo*), using transwell experiments [65, 66]. For example, monolayer cultures of commercialized, immortalized or primary isolated alveolar epithelial type I (AT I) or type II (AT II) cells have been used to mimic the alveolar epithelium and its barrier function [67]). Non-sterile inflammation was first studied in 2D monoculture or classical culture models exposed to LPS *in vitro*, mimicking the clinical infection with Gram-negative bacteria [68–72]. In contrast, the setting of sterile inflammation can be studied *in vitro* by exposing cultured cells to a mixture of cytokines, such as interleukin-1 beta, tumor necrosis factor alpha, and interferon gamma [65, 73, 74]. In addition, some biological mechanisms of mechanical ventilation-induced lung injury have been investigated *in vitro* through exposure to cyclic mechanical stretch, hypercapnia or hyperoxia [75–79]. Interestingly, alveolar epithelial cells or alveolar progenitor cells (such as induced pluripotent stem cells-derived AT2-like cells) can be differentiated at the air-liquid interface, inducing cell polarization, epithelial barrier formation through the establishment of intercellular junctions, and surfactant production.

However, monocultures are unable to reproduce the complexity of the alveolar-capillary environment, and, for example, a traditional submersion culture model does not reproduce the air-liquid environment of human alveoli. The main advances have, therefore, come from modeling the human airway at the air-liquid interface, building co-culture models (such as of epithelial cells and endothelial cells or macrophages), and developing 3D-engineered lung cellular environments. *In vitro* co-culture or multicellular models can better reproduce the *in vivo* environment, compared to 2D monocultures [80]. Unlike 2D cultures, co-culture or multicellular systems can model complex interactions between different cell types in a more relevant environment, such as a model of alveolar-capillary barrier using epithelial and endothelial cells [81, 82]. For example, a 3D multicellular model composed of an alveolar epithelial cell layer cultured in interaction with alveolar macrophages on one side and monocyte-derived dendritic cells on the other has been recently described [67, 81, 83]. *Ex vivo* organoid cultures have also been proposed to better model the multiple features of ARDS. These are 3D models assembled from cultured human alveolar stem cells to reproduce all the characteristics of a functional human alveolus *in vitro* [84]. These cultures are long-term, feeder-free, and chemically defined systems that represent a very powerful model to investigate complex mechanisms, such as those involved in severe acute respiratory syndrome coronavirus 2 infection [85–87]. Ultimately, the combination of microfluidic bioengineering and 3D cell culture has led

to the development of "lung-on-a-chip" models comprising a full alveolar-capillary interface that can be exposed to cyclic ventilation and perfusion [88–90]. This model requires long-term cultures of human cell lines, and it is only very recently that the use of primary human alveolar epithelial cells has been reported [91].

4. Human models of ARDS

4.1 Human in vivo models of ARDS

Because a recognized shortcoming in human ARDS research is the difficulty in translating the findings from bench to bedside, novel *in vivo* models have been successfully developed to investigate the mechanisms of lung injury or therapies for ARDS. These models are major breakthroughs in translational ARDS research and have clear advantages in allowing potentially effective therapies to be readily investigated *in vivo* in humans and to inform subsequent clinical trials in patients.

Seminal studies included intravenous administrations of LPS to human volunteers [92, 93]; however, in some studies, direct lung instillations of LPS [94] or other agents, such as leukotriene B4 (produced by human alveolar macrophages, with potent chemotactic activity for neutrophils) [95], were performed using bronchoscopy. More recently, the inhalation of low-dose LPS by healthy humans was successfully used to replicate alveolar epithelial cell activation, alveolar inflammation, and systemic inflammatory response without causing significant adverse effects [96–99]. In this recently developed human *in vivo* model, lung injury is only transient, and inflammation has mainly been investigated within a few hours after LPS exposure.

4.2 Human ex vivo models of ARDS

An *ex vivo* human lung preparation has recently been proposed to better reflect the *in vivo* settings of experimental ARDS [100]. In this model, donor human lungs that have been rejected for transplantation are ventilated and perfused *ex vivo* and used to study the mechanisms of lung injury, isolate multiple primary lung cell types, and test potential therapies before clinical translation into trials. The model allows for analyses of physiological indices, such as oxygenation and alveolar fluid clearance, and the sampling of multiple tissues and fluids up to 6–10 hours in most experiments [101]. Although the *ex vivo* human lung preparation is rather convenient, inexpensive, and the model closest to clinical conditions, ethical and practical issues in obtaining human lungs for research may exist depending on the country. The main limitation of *ex vivo* models resides in the heterogeneity in human lungs due to donor-specific and pre-procurement variables that limit baseline comparisons of measures among experimental lungs. Notably, in addition to its use in ARDS research, the *ex vivo* human lung preparation is being largely used in conditioning or therapeutic studies of donor lungs before transplantation [102].

5. Perspectives and challenges

Multiple models of acute lung injury induced by the main clinical risk factors for ARDS have been developed *in vitro* (from monocultures to more complex constructions), *in vivo* in animals, *ex vivo* (using human or animal lung preparations), and *in vivo* in human volunteers (Table 2). While no single model can truly reproduce the complexity and heterogeneity of clinical ARDS, combining multiple preclinical approaches with *in vivo* and clinical investigations is probably the most promising strategy for future mechanistic and therapeutic research (Fig. 1). Each experimental model has its limitations. For example, despite the recent developments of 3D cultures and lung organoids, *in vitro* models may probably never reproduce the clinical setting, but they are still very useful to inform mechanistic and drug discovery studies [6]. Recent animal studies are able to better reflect the multiple-hit hypothesis for ARDS pathogenesis, and they can be used to investigate the different phases of ARDS, from onset (such as in the hydrochloric acid and the oleic acid models) to recovery (such as in the hyperoxia acid and the bleomycin models), thus allowing studies of preventive ARDS therapies and the combined effects of pre-existing lung injury and exposure to high lung stress through mechanical ventilation use in the intensive care unit [14, 17, 103]. Animal studies typically use young and healthy animals, and older animals should also be investigated to better reflect the clinical picture of ARDS as a disease of aging. In addition, they do not reproduce the common clinical ARDS settings of patient comorbidities and multiorgan failure, with a prolonged need for intensive care over multiple days, if not weeks, and most studies do not take into consideration the impact of ventilatory settings (e.g., the level of positive end-expiratory pressure) on oxygenation. To distinguish models of acute lung injury from conditions that reflect more subacute or chronic lung injury, maximal lung injury should be evident within 24 hours of exposure to the inciting stimulus [14]. However, although some animal models allow studies of lung injury over multiple days and can capture the different phases of ARDS over time [33], most of them are limited to the first few hours after injury, thus limiting clinical translation and the ability to explore the nonlinearity of biological processes. Another limitation that is particularly relevant in mice is the profound impact of strain variability in murine models of injury; not only a particular observation may not be translatable to humans, but it may not even be translatable to other strains of mice. This highlights the need to restrict mice to mechanistic questions and use more translational models for preclinical studies.

Human models, *in vivo* or *ex vivo*, represent major progress toward the clinical translation of basic findings. In addition to the development of novel preclinical and clinical ARDS models, such as the lung-on-a-chip *in vitro* and the *ex vivo* human lung preparation, recent evolutions in the field of translational ARDS research have been made possible by the refinement of experimental techniques. In particular, the field of genome editing now offers a broad range of opportunities to develop investigational or therapeutic strategies by downregulating or upregulating the expression of a specific gene *in vitro* and *in vivo*, such as with the gene knockout or knockdown techniques based on ribonucleic acid interference or the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) system (capable of site-specific deoxyribonucleic acid

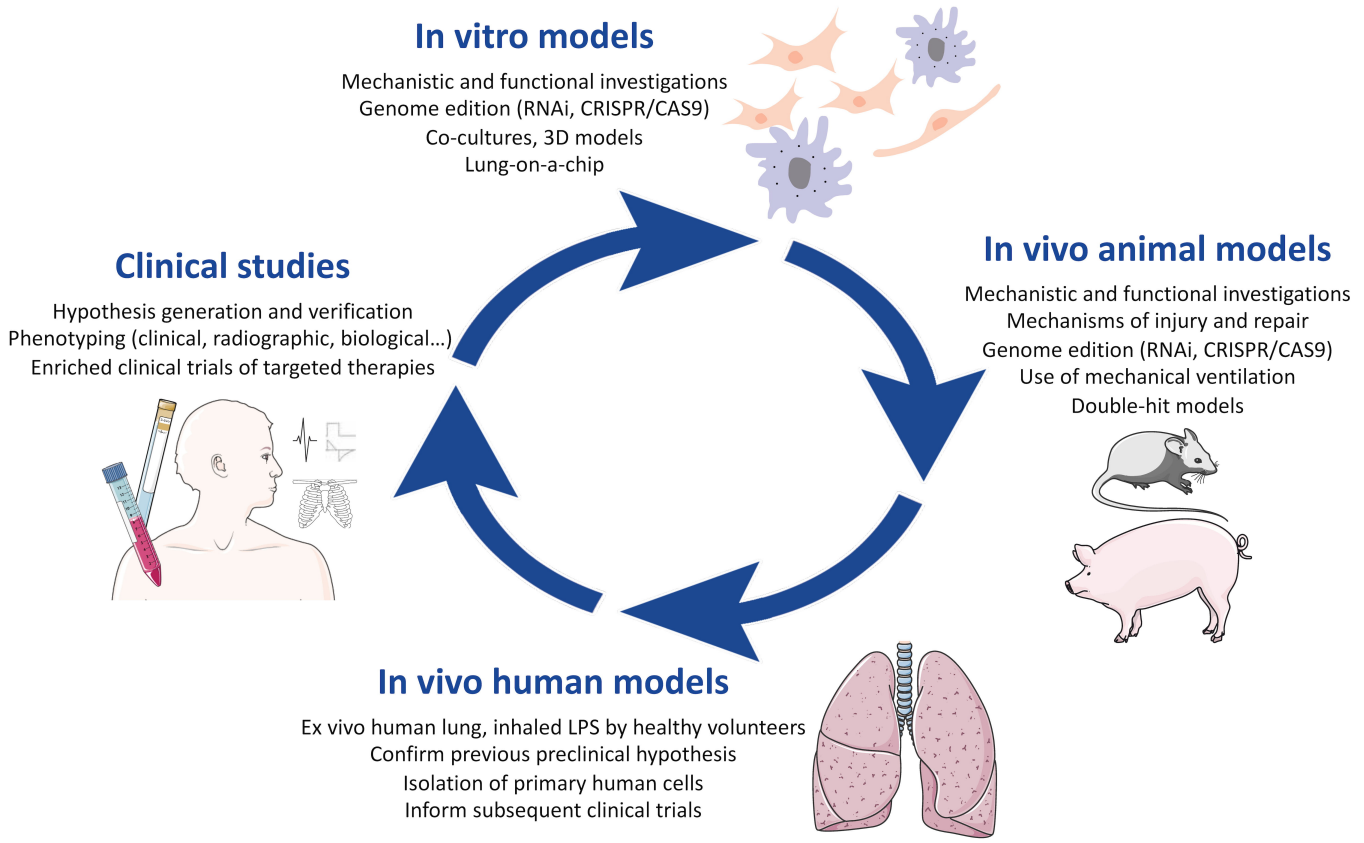

FIGURE 1. Schematic view of a translational research framework based on multiple experimental preclinical approaches and clinical studies. RNAi, interference ribonucleic acid; CRISPR, clustered regularly interspaced short palindromic repeats; CAS9, CRISPR-associated protein 9; LPS, lipopolysaccharide.

cleavage) [89, 104]. Novel methods have also improved our ability to understand the functional significance of polymorphisms or genes identified through genome-wide studies or transcriptomic screens in models and patients, to investigate the potential pathogenic causality for markers or mediators and the potential value of targeting treatment in specific genetic backgrounds. Such innovative approaches have been successfully deployed in COVID-19 research [30].

These traditional and novel experimental designs are important pieces, along with the acquisition of granular clinical, physiological, and biological data (e.g., from bronchoalveolar fluid and blood samples to study mechanisms of lung and systemic responses, respectively, to injury) within clinical studies, in identifying new drug targets, developing targeted therapies, and ultimately selecting patients most likely to benefit. Such strategies of trial enrichment, in which patients are selected who are more likely to develop an outcome, such as mortality (prognostic enrichment), and/or when they are more likely to respond to a targeted therapy (predictive enrichment), hold the promise of precision approaches for ARDS [105–107]. In such a translational framework, preclinical models may reveal that a biological/functional trait or marker has a causal role in the severity of ARDS and suggest that measuring this marker could have value for endotype identification [34, 105, 108, 109]. Ideally, such markers (e.g., biological or radiographic indices) could help in selecting patients to enroll in future precision trials and monitoring their individual responses to the intervention being tested [103, 110–112]. This would require markers that are rapidly available to inform potential trial eligibility. Of note, the first clinical study evaluating a point-of-care assay to prospectively identify hyper- and hypo-inflammatory phenotypes in patients with ARDS and hypoxemic acute respiratory failure is currently enrolling patients (ClinicalTrials.gov Identifier: NCT04009330), and the preliminary results in patients with COVID-19 have been published [113]. However, it remains uncertain to date whether available preclinical models may be representative of known clinical ARDS phenotypes/endotypes or may be helpful to identify phenotype-specific therapy responsive traits [114]. The performance of candidate markers over time during the natural course of ARDS and their kinetics under candidate therapies should also be rigorously evaluated [10]. In addition, even when a diagnostic or therapeutic precision approach is consistently supported by findings across combined preclinical and clinical ARDS models, it should not be associated with adverse effects that may preclude its application to improve patient outcomes.

In conclusion, multiple experimental models have been developed in the last few decades, with major recent developments in the fields of *in vitro*, *ex vivo*, and *in vivo* experimental ARDS. While some of these experiments failed, others succeeded in advancing our knowledge of the complex mechanisms of ARDS pathophysiology and the clinical translation of a few therapeutic interventions, such as lung-protective

TABLE 2. Non-exhaustive list of preclinical and clinical models of acute respiratory distress syndrome.

Setting	Model	Injury feature	Technical notes
Live animal models	Lipopolysaccharide-induced sepsis	Pulmonary or systemic sepsis	Variable response to injury across species
	Live bacteria-induced sepsis		Variability in the doses of live bacteria administered
	Acid aspiration	Direct lung injury	Mimics aspiration of gastric contents
			Narrow margin between injury and absence of injury
	Abdominal sepsis	Peritonitis-induced indirect lung injury	Invasive surgery required with high mortality
			Intraperitoneal injection of cecal slurry more reliable and titratable
	Ventilator-induced lung injury	Direct alveolar injury with severe hypoxemia	High tidal volumes not relevant for clinical translation
	Hyperoxia	Hyperoxia-induced injury	Requires prolonged exposure and specific equipment
	Ischemia-reperfusion	Induced by clamping the pulmonary artery lung or other nonpulmonary arteries	Requires specific surgical skills and equipment
	Viral infection	Acute lung injury of viral causes (mostly airborne viruses)	Can be rapidly mobilized to better understand the mechanisms of emerging viruses
			Translation may require genetic modifications to the animal and/or the virus
In vitro models	Submerged monoculture	Cell injury of sterile or non-sterile causes	Reproducible for testing and establishing experimental conditions
			Submerged cultures or cultures at the air-liquid interface
	Multicellular co-culture	Cell injury of sterile or non-sterile causes in a more relevant multicellular environment	Can model complex interactions between different cell types
	Organoid culture		
	Lung-on-a-chip	Cell injury of sterile or non-sterile causes in a human lung-like environment	Allows multicellular co-culture
			Reproduces all characteristics of a functional human alveolus unit
Human *in vivo* models	Lipopolysaccharide-induced sepsis in human volunteers	Intravenous administration or direct lung instillation of high-dose lipopolysaccharide	Seminal models
		Inhalation of low-dose lipopolysaccharide	Transient lung injury and rapid investigation of inflammation
Human *ex vivo* models	*Ex vivo* human lung preparation	Donor human lungs rejected for transplantation are ventilated and perfused *ex vivo* before exposure to sterile or non-sterile injuries	Most translatable model, allows for analyses of physiological indices
			Rather convenient and inexpensive but potential ethical and practical issues

mechanical ventilation, neuromuscular blockade, and corticoid therapy [115–117]. Therefore, the judicious and imaginative use of a broad range of experimental and analytical approaches is of paramount importance in developing translational discovery research, with the goal of developing prediction medicine strategies to ultimately improve patient outcomes.

AUTHOR CONTRIBUTIONS

RZ, WLMB, and GMB were involved in the conception and design of the review, in writing the paper, and in its revision prior to submission. MJ takes responsibility for the content of the paper and was involved in the conception and design of the review, in writing the paper, and in its revision prior to submission.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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