Acute Respiratory Distress Syndrome: Bench-to-Bedside Approaches to Improve Drug Development

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Despite 50 years of extensive research, no definite drug is currently available to treat acute respiratory distress syndrome (ARDS), and the supportive therapies remain the mainstay of treatment. To improve drug development for ARDS, researchers need to deeply analyze the “omics” approaches, reevaluate the suitable therapeutic targets, resolve the problems of inadequate animal modeling, develop the strategies to reduce the heterogeneity, and reconsider new therapeutic and analytical approaches for better designs of clinical trials.

In 1967, ARDS was described as a clinical syndrome1 that is characterized by the enhanced alveolar-capillary membrane permeability, interstitial and alveolar edema formation, neutrophil-derived inflammation, dysfunction of surfactant, impaired gas exchange, and respiratory failure due to progressive and refractory hypoxemia.2 According to the Berlin criteria, which has replaced the American-European Consensus Conference’s definition of ARDS,3 ARDS is generally diagnosed when following the criteria are fulfilled: 1) severe hypoxemia; 2) acute onset (<1 week); 3) bilateral radiographic abnormalities (not explained by atelectasis); 4) the lack of clinical heart failure; and 5) echocardiography demonstrating that the disorder is not caused by heart failure.4 ARDS can be classified as mild (200 mmHg < PaO2/FiO2 ≤ 300 mmHg), moderate (100 mmHg < PaO2/FiO2 ≤ 200 mmHg), or severe (PaO2/FiO2 ≤ 100 mmHg). Until the establishment of the Berlin criteria, all mild ARDS patients were termed as acute lung injury (ALI).

Since the late 1980s, clinical trials for ARDS have started but, unfortunately, no appropriate pharmacological therapies for ARDS management exist. Supportive therapies, such as lower tidal volume ventilation (6 ml/Kg of predicted body weight), a plateau airway pressure (<30 cm H2O), prone positioning, neuromuscular blockade, and fluid-conservative therapy remain the essential elements for good outcomes for ARDS patients.5 However, recent observational studies from all over the world revealed a high incidence and mortality rate, with 10% prevalence in intensive care units (ICU) and 40–44% mortality,6,7 while the mortality rate varies depending on age, etiology of lung injury, and the presence of nonpulmonary organ dysfunction. Moreover, patients who survive with ARDS are at high risk for depression, cognitive decline, persistent skeletal-muscle weakness, and post-traumatic stress disorder.8 Hence, new potential approaches are needed to enhance the drug development for ARDS in order to improve the quality of life of ARDS patients and to minimize the ARDS-associated mortalities.

In this review, we briefly discuss the pathophysiology and genomics of ARDS, the targets that have been scrutinized until now, and completed and ongoing clinical trials of these targets. Moreover, we also discuss our perspective regarding the reasons for failure, including the absence of authenticated preclinical data either due to poor representation of human conditions by animal models or enrollment of heterogeneous groups of patients into clinical trials, and arbitrary decisions regarding drug delivery or duration of therapy. We suggest some novel approaches to improve the probability of success, including the appropriate use of in vitro assays for screening of new compounds, implementation of new analytical approaches, and narrowing the subtypes of the target population to improve the clinical trial design. Finally, we summarize the therapies that warrant further testing, and future therapeutic strategies, including gene therapy, administration of mesenchymal stem cells, combination of therapies, targeting inflammasomes, and the ubiquitin-proteasome system.

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The lung's initial response to injury, referred to as the exudative/initial phase of ARDS, is characterized by increased permeability, rapid interstitial and alveolar edema, alveolar flooding by a protein-rich fluid, and gradual refractory hypoxemia. Type II cells of the alveolar epithelium are also injured, which eventually leads to disruption of epithelium integrity, attenuation of surfactant production, and inhibition of the epithelial repair process. Moreover, neutrophils activation and microthrombi formation in the lung potentiate the inflammatory response. A fibroproliferative phase, driven by the proinflammatory cytokine, is characterized by more refractory hypoxemia and architectural changes. In this phase, alveolar edema subsides, alveolar spaces are filled with neutrophils and macrophages, and the alveolar epithelium is repopulated by type II cells. Finally, chronic inflammation, neovascularization, and a fibroproliferative process take place, as acknowledged by the deposition of extracellular matrix.9 The repair processes initiated during the fibroproliferative phase of ARDS are essential for host survival. Once epithelial integrity has been reestablished, reabsorption of alveolar edema and the provisional matrix restores alveolar architecture and function. Neutrophil-mediated inflammation is also reversed, most probably due to apoptosis. The final or fibrotic phase of ARDS does not occur in all patients but has been linked to prolonged mechanical ventilation and increased mortality.

**GENOMICS OF ARDS**

Unpredictable consequences of ARDS are most frustrating to the pediatric intensivist because one or two ARDS patients, with the same age and identical triggers, may die and others may survive. Recent advances in genomics suggest that these unpredictable consequences might be due to the genetic background. Genomics is an emerging field, and a multicenter study is investigating the association between gene polymorphism and ARDS (NCT02644798). To date, numerous genomics studies have highlighted the association of ANGPT210 with trauma-associated ALI, IL1RN,11 and PPFIA12 with ARDS risk, ADIPOQ13 and rs78142040,14 but has been linked to prolonged mechanical ventilation and increased mortality.

**CLINICAL DRUG DEVELOPMENT EFFORTS FOR ARDS**

Hundreds of randomized controlled trials (RCTs) of pharmacological compounds have been accomplished for the adjuvant therapy of ARDS. To date, available therapeutic strategies are intended for early recognition and rectification of the underlying cause of ARDS. Treatments of ARDS have been difficult because the underlying disease process is incompletely understood and therapies to date (and under development) largely target individual components of this complex pathophysiology. Might the lack of a great therapeutic agent be that targeting only a portion of the perturbations may not be effective? Drugs/compounds studied in previous trials are outlined below.

**Corticosteroids therapy**

Therapeutically, both high-dose16 and moderate-dose corticosteroids15 have so far failed to exhibit efficacy in ARDS. Interestingly, prolonged low-dose corticosteroids effectively decreased the
ICU mortality in early adult ARDS patients and pediatric.\textsuperscript{18} Regardless of a meta-analysis and systematic review, the role of steroids in ARDS patients remains uncertain and unclear. Moreover, phase II (NCT017575899; PEDALI) and phase IV (NCT01731795; DEXA-ARDS) RCTs are ongoing to evaluate the safety and efficacy of methylprednisolone and dexamethasone, respectively. In addition, adverse effects associated with corticosteroids, including electrolyte imbalance, gastrointestinal bleeding, hyperglycemia, pancreatitis, fluid retention, neuromuscular weakness, and increased infection rate might be an important limiting factor of this therapy.

**Targeting lipopolysaccharide (LPS)**

Passive transfusion of antiserum, prepared from mutant strains of Gram-negative bacteria lacking sugar moieties responsible for conferring serotype specificity, showed protection against various strains of Gram-negative bacteria and LPS preparations. Prompted by these, a phase III study of HA-1A, a human monoclonal antitoxin antibody designed to neutralize the harmful effects of LPS, was conducted that showed improved survival and convincingly positive outcomes.\textsuperscript{20} In contrast, HA-1A did not show therapeutic benefits during multicenter RCTs to treat septic shock and sepsis-associated ARDS.\textsuperscript{21} Further research on HA-1A has been discarded.

**Statin therapy**

Preclinical and observational studies authenticate the potential role of statin in ARDS, whereas clinical trials of rosuvastatin (SAILS)\textsuperscript{22} and simvastatin\textsuperscript{23} failed to show a mortality benefit in ARDS. One-year follow-up of rosuvastatin vs. placebo in sepsis-associated ARDS demonstrated increased cumulative mortality, and survivors were experiencing physical and mental impairments.\textsuperscript{24} Phase II multicenter studies (NCT02895191, NCT03089957) are recruiting participants to evaluate the safety and dose–response relationship of ulinastatin, a serine protease inhibitor, for ARDS (Table 2).

**Targeting tumor necrosis factor alpha (TNF-\(\alpha\))**

Anti-TNF-\(\alpha\) therapy showed promising outcomes in preclinical studies. Afelimomab, an anti-TNF-\(\alpha\) monoclonal antibody, improved the survival rate of severe sepsis, a common cause of ARDS, but showed potentially confusing variables.\textsuperscript{25} A meta-analysis of anti-TNF-\(\alpha\) therapy also revealed improved survival in sepsis. Moreover, etanercept (anti-TNF-\(\alpha\) agent),\textsuperscript{26} and a combination of etanercept and corticosteroids improved survival in children with idiopathic pneumonia syndrome (IPS).\textsuperscript{27} IPS is an acute, noninfectious lung disorder associated with high morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Patients at the severe end of this spectrum may present with hypoxemic respiratory failure and pulmonary infiltrates, meeting the criteria for ARDS. Moreover, prompted by ARDS animal models study, an early-phase clinical trial using anti-TNF-receptors (anti-TNFFR1) monoclonal antibody (GSK1995057) was conducted that attenuated pulmonary inflammation via modulating the pulmonary microvascular endothelial function.\textsuperscript{28} However, further investigations are needed.

**Targeting neutrophils**

Neutrophils and/or neutrophils-derived products demonstrate a central role in the pathogenesis of ARDS. A multicenter, double-blind, STRIVE study of sivelestat, a neutrophil elastase inhibitor, did not show efficacious results in a broad spectrum of ALI/ARDS cases,\textsuperscript{29} whereas phase III and phase IV studies of prolonged use of sivelestat conducted in Japan demonstrated positive outcomes.\textsuperscript{30} Moreover, recent studies further support the therapeutic effectiveness of early administration of sivelestat to ARDS patients,\textsuperscript{31} as well as in nonrandomized postmarketing.\textsuperscript{32} Hence, the therapeutic effectiveness of sivelestat to treat ALI/ARDS is yet inconsistent and controversial.

**Modulation of coagulation cascade**

Tissue factors (TFs), the potent initiator of the extrinsic coagulation cascade, are released during ARDS in alveolar epithelial cells to mediate the procoagulant state via fibrin formation that subsequently results in vascular injury, microthrombi formation, and complement-mediated activation of platelets and leukocytes. Treatment of ARDS baboon models with site-inactivated FVIIa (FVIIai) attenuated ARDS, while a phase II study of FVIIai on human ARDS patients was discontinued prematurely due to increased bleeding complications.\textsuperscript{33} A phase II study of ALT-836 (also known as TNX-832; a recombinant antibody that binds to TF or TF-Factor VIII complex) in sepsis-induced ARDS has been completed (NCT00879606) and the results are awaited. Moreover, nebulized heparin was found to be associated with attenuation of mechanical ventilation duration in at-risk ARDS patients.\textsuperscript{34} A trial of nebulized heparin is ongoing (ACTRN12612000418875); hence, more trials are needed. In addition, prehospitalization aspirin therapy\textsuperscript{35} and a recent clinical study revealed the significant effect of aspirin.\textsuperscript{36} Other phase II RCTs (STAR; NCT02326350 and ARENA; NCT01659307) are enrolling participants in order to assess the oxygenation index of aspirin in ARDS patients (Table 2). Additionally, prompted by animal studies, activated protein C (APC) was tested in human models, but APC (Xigris) therapy revealed negative outcomes in sepsis and ARDS patients.\textsuperscript{37} Moreover, intravenous recombinant human-APC (rh-APC) did not ameliorate ARDS in critically ill patients.\textsuperscript{38}

**Growth factors**

Targeting the factors that endorse mitogenic and cytoprotective effects on lung epithelium is a recent paradigm in ARDS therapeutic strategies. Keratinocyte growth factor, KGF, stimulates the proliferation of type II alveolar cell to repair the injured alveoli. Previous data from clinically relevant human models of ARDS supported the potential therapeutic role of KGF (palifermin) in ARDS,\textsuperscript{39} but in contrast, a recent phase II clinical trial revealed that palifermin cannot be recommended to treat ARDS.\textsuperscript{40} Additionally, a phase II trial showed that granulocyte macrophage colony-stimulating factor (GM-CSF), a pleiotropic cytokine, did not change the ventilator-free days and mortality.\textsuperscript{41} Interestingly, promising results of phase I of inhaled molgramostim (rhGM-CSF) has motivated researchers to conduct phase II trials in pneumonia-associated ARDS patients (NCT02595060).
Table 2  In-progress clinical trials for ARDS

| Title of study                                                                 | NCT number       | Design  | Projected numbers | Interventions                                                                 | Primary outcomes                                                                 | Status/key finding                  |
|------------------------------------------------------------------------------|------------------|---------|-------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------|
| Efficacy study of dexamethasone to treat the ARDS (DEXA-ARDS)                | NCT01731795      | Phase IV| 314               | Dexamethasone, 20 mg/day for 5 days, then 10 mg/day for 5 days                  | Ventilator-free days and mortality                                               | Recruiting                          |
| Corticosteroid mediates ARDS via NLRP3 inflammasome signaling pathway        | NCT02819453      | Phase IV| 20                | Treating with dexamethasone for 3 to 5 days                                    | To check whether dexamethasone attenuates IL-18 level in plasma                | Recruiting                          |
| Effects and safety of infusion of low-doses of methylprednisolone in early ALI and ARDS in children (PEDALI) | NCT01757899      | Phase II| 30                | Methylprednisolone, Loading dose 1 mg/kg IV bolus mixed in 5 mL NS (30 min); Days 0 to 07, 1 mg/kg/day mixed in 24cc NS and infused at 1 cc/hr Days 08 to 10, 0.5 mg/kg/day mixed in 24cc NS and infused at 1 cc/hr Days 11 to 12, 0.25 mg/kg/day Days 13 to 14, 0.125 mg/kg/day | Ventilator-free days and pulmonary organ function                               | Recruiting                          |
| Efficacy and safety of Interferon-β (FP-1201-lyo) in ARDS (INTEREST)        | NCT02622724      | Phase III| 300               | FP-1201-lyo, I/V 10 μg daily for 6 days.                                       | Evaluation of Pharmacoconomics and mortality                                    | Recruiting                          |
| Aspirin as a treatment for ARDS (STAR)                                       | NCT02326350      | Phase II| 60                | Aspirin, 75 mg for up to 14 days                                               | Oxygenation index                                                               | Recruiting                          |
| Effect of aspirin on reducing inflammation in human in vivo model of acute lung injury (ARENA) | NCT01659307      | Phase II| 33                | Aspirin, 75 or 1200 mg for 7 days                                              | BALF IL-8 concentration and oxygenation index                                   | Recruiting                          |
| Repair of ARDS by stromal cell administration (REALIST)                      | NCT03042143      | Phase I/II| 75                | Single dose mesenchymal stem or stromal cells                                 | Oxygenation index or safety                                                     | Not yet recruiting                  |
| Iloprost in ARDS (THLLO)                                                     | NCT03111212      | Phase III| 900               | nebulized Iloprost vs. control (0.9% NaCl)                                    | 90-day mortality                                                               | Not yet Recruiting                  |
| Phase II Study of IC14 in ARDS                                               | NCT03017547      | Phase II| 160               | IC14 4 mg/kg IV on day 1, then IC14 2 mg/kg IV once daily for 2 to 4 days vs. placebo IV once daily for days 1-4 | Safety and ventilator-free days                                                 | Not yet Recruiting                  |
| Safety and dose-response relationship of Ulinastatin for ARDS               | NCT02895191      | Phase II| 60                | Ulinastatin vs. placebo for 7 to 14 days                                       | Oxygenation index                                                               | Enrolling by invitation             |
| Prevention of Ulinastatin on ARDS                                            | NCT03089957      | Not provided| 840               | Ulinastatin, 300,000 IU Ulinastatin dissolved in 50 mL of 0.9% normal saline by continuous intravenous infusion for 5h, 2 times per day for 5 days. | The incidence of ARDS                                                          | Not yet recruiting                  |
| Protective ventilation with veno-venous lung assist in respiratory failure (REST) | NCT02654327      | Phase III| 1,120             | VV-ECCO2R and lower tidal volume mechanical ventilation                        | 90-day mortality                                                               | Recruiting                          |
| Liberal oxygenation vs. conservative oxygenation in ARDS (LOC02)            | NCT02713451      | Phase III| 850               | Liberal vs. conservative oxygenation target in ARDS                            | 28-day mortality                                                               | Recruiting                          |
| Vitamin D to improve outcomes by leveraging early treatment (VIOLET)        | NCT03096314      | Phase III| 3,000             | Vitamin D vs. placebo in patients at high risk for ARDS and mortality          | 90-day mortality                                                               | Recruiting                          |

Table 2 Continued on next page
| Title of study                                                                 | NCT number       | Design  | Projected numbers | Interventions                                                                 | Primary outcomes                                      | Status/key finding          |
|------------------------------------------------------------------------------|------------------|---------|-------------------|-------------------------------------------------------------------------------|--------------------------------------------------------|-----------------------------|
| Re-evaluation of systemic early neuromuscular Blockade (ROSE)                | NCT02509078      | Phase III| 1,408             | Cisatracurium vs. placebo in moderate-to-severe ARDS                           | 90-day mortality                                       | Recruiting                  |
| Vitamin C infusion for treatment of sepsis-induced ALI (CITRIS-ALI)         | NCT02106975      | Phase II| 170               | Vitamin C vs. placebo in sepsis-induced ARDS                                  | Change in SOFA score at 96 hours                       | Recruiting                  |
| Study of ganciclovir/valganciclovir for prevention of cytomegalovirus reactivation in acute injury of the lung and respiratory failure (GRAIL) | NCT01335932      | Phase II | 160               | Intravenous ganciclovir vs. placebo in ARDS                                   | Change in serum IL-6 between baseline and study day 14 | Active, not recruiting      |
| Mesenchymal stems cells for ARDS (START)                                    | NCT01775774      | Phase II| 60                | Allogeneic mesenchymal stem cells, single intravenous dose, $10^{10}$ cells per kg | Safety                                                 | Active, not recruiting      |
| ECMO for ARDS (EOLIA)                                                       | NCT01470703      | Phase III| 331               | Extracorporeal membrane oxygenation                                            | Mortality                                              | Recruiting                  |
| Bone marrow-derived cells for ARDS (MUSTARDS)                                | NCT02611609      | Phase I/II| 36                | Escalation doses of cells per kg                                               | Safety                                                 | Recruiting                  |
| Mechanical ventilation adjusted by transpulmonary pressure (EP Vent2)        | NCT01681225      | Phase II| 200               | Mechanical ventilation directed by transpulmonary pressure                      | Mortality and days without mechanical ventilation       | Recruiting                  |
| Human umbilical-cord-derived MSCs therapy in ALI (UC-MSC)                   | NCT02444455      | Phase I/II| 20                | Human umbilical cord MSCs, intravenous infusion (5 × 105/kg) once a day, a total of three times. | Safety                                                 | Recruiting                  |
| MSCs for Treatment of ARD in Stem Cell Transplant Patients                  | NCT02804945      | Phase II| 50                | the maximum dose of 3 × 106 cells/kg by vein one time on Day 1                  | Infusional Toxicity                                    | Recruiting                  |
| Adipose-derived mesenchymal stem cells in ARDS                              | NCT01902082      | Phase 1  | 20                | one intravenous dose of $1 \times 10^6$ cells/kg of body weight               | Safety                                                 | Recruiting, status is known |
| Safety and efficacy of Multi-Stem therapy in ARDS subjects                  | NCT02611609      | Phase I/II| 36                | Low and high doses of Multi-Stem vs. placebo in ARDS                          | Safety                                                 | Recruiting                  |
| Mesenchymal stem cell in patients with acute severe respiratory failure ( STELLAR) | NCT02112500      | Phase II| 10                | Intravenous infusion of MSC                                                    | Oxygenation index                                       | Recruiting                  |
| Safety Study of inhaled carbon monoxide to treat ARDS                        | NCT02425579      | Phase I  | 48                | Inhalation of carbon monoxide                                                  | Measurement of inflammatory biomarkers                 | Recruiting                  |
| GM-CSF inhalation (molgramostim) to improve host defense and pulmonary barrier restoration (GH-POPE) | NCT02595060      | Phase II| 45                | Inhalation of molgramostim 150 mcg once a day for 3 days vs. inhaled placebo    | Oxygenation index                                       | Recruiting                  |
| Dexmedetomidine vs. standard clinical practice during noninvasive mechanical ventilation (DEX-PCH-VMMN) | NCT02958150      | Phase IV | 180               | Dexmedetomidine vs. standard clinical practice                                | Oxygenation index, ventilator-free days and Mortality  | Recruiting                  |
| Can Heparin Administration Reduce Lung Injury (CHARLI)                       | ACTRN12612000418875 | Phase II | 256               | Nebulized liquid heparin (25,000 IU in 5 ml) versus placebo (5 ml of nebulized liquid 0.9% sodium chloride) | Oxygenation index                                       | Not yet recruiting          |
Moreover, two clinical trials, including NCT00319631 and NCT01314066, were conducted to understand the role of vascular endothelial growth factor in ARDS, but both were stopped due to poor enrollment and lack of funding.

**Miscellaneous agents**

During early ARDS, immune activation leads to the intrapulmonary and systemic release of cytokines from alveolar macrophages and peripheral blood monocytes. Various antiinflammatory approaches have been performed to deactivate these cells. For instance, both vitamin C and vitamin D3 exhibit antiinflammatory properties, but the underlying molecular mechanisms are uncertain. Phase II/III trials are recruiting participants to evaluate the effect of high-dose vitamin C in established ARDS patients (NCT02106975) and vitamin D supplementation on ARDS development in high-risk patients (NCT03096314) (Table 2). Insulin exhibits antiinflammatory effects via inhibition of nuclear factor kappa B (NF-κB). A phase II trial of insulin therapy in preventing ARDS (NCT00605696) has been completed, and the results are awaited. Additionally, other ineffective to date pharmacological strategies include antioxidants, N-acetyl cysteine, exogenous surfactant, inhaled nitric oxide, prostaglandin E1, lisofylline, β2 agonist, procysteine, omega-3 supplementation, nebulized sodium nitroprusside, calfactant, and furosemide.

**STRATEGIES TO IMPROVE DRUG DEVELOPMENT**

In consideration of the disappointing RCT outcomes, what kind of strategies might be adopted to improve the possibility of drug development for ARDS? Actually, no single answer can justify this question, but numerous strategies warrant consideration. In this section, we provide our perspective regarding strategies to improve the drug development for ARDS.

**Cell-based in vitro assays**

In the case of ARDS, outstanding care and attention are required for cell-based assays. For instance, primary cell cultures might be advantageous rather than immortal cell cultures. Likewise, outcomes obtained from human cells will be more reliable as compared to murine cells. In addition, in the in vivo environment, cells feedback to proinflammatory stimuli is thought to be distorted by local cellular and humoral factors. For this reason, in vitro new advances are being adopted for better growth of the cells or combinations of different cell types in order to imitate the in vivo environment in tissues or organs that might facilitate the compound’s screening for the selection and further development of most promising candidates.

**Preclinical models of ARDS**

Unique challenges in ARDS models have limited the evaluation of appropriate results of clinical trials. First, young and healthy animals are mainly used in preclinical studies, while the majority of ARDS patients are of old age. The severity and character of ARDS in mice are age-dependent. In old-age mice, the inflammatory response is impaired, with decreased adaptive immunity that further leads to worsening of ARDS. Therefore, using aged animals rather than young could improve the clinical significance of animal models of ARDS. Second, rodents, particularly mice, are chronically cold-stressed when housed in a laboratory/animal center at 20–22°C, suggesting that appropriate physiological conditions for housing laboratory mice might help to get better preclinical findings. Third, compounds are mainly administered prior to the onset of ARDS in the experimental setup, while clinical diagnosis and treatments are delayed in the case of ARDS patients. Thereby, for proper justification of outcomes, the compound should be tested prior as well as after the onset of ARDS. Fourth, animals, such as rats, mice, and baboons, are surprisingly less sensitive to the toxic effects of LPS than humans. This obvious inconsistency in LPS sensitivity seems to be one of the key factors that may lead to inappropriate and inconsistent outcomes. Fifth, fundamental differences are exhibited in the physiology, anatomy, size, and species of the animals (both rodents and primates) and humans. Primates and pigs are more closely related to human as compared to mice because many aspects of immunological function in humans and pigs are alike. For instance, circulating white blood cells in humans and pigs are primarily polymorphonuclear leukocytes, but not in mice, and interleukin (IL)-8 has a direct ortholog in pigs but not in mice. Moreover, large quantities of NO is produced by murine macrophages after LPS stimulation, whereas neither porcine nor human macrophages counter the LPS in such a way.

Humanized mice are thought to be a potential way to improve the ARDS animal models. Humanized mice were developed by transplanting human CD34+ umbilical cord blood hematopoietic stem cells into gamma-irradiated neonatal NOD–SCID–IL-2Rγnull mice (nonobese diabetic, severe combined immunodeficient mice lacking the γ-chain of the IL-2 receptor). Developed humanized mice represent the absolute lineage of human cells, such as macrophages, monocytes, T cells, B cells, natural killer cells, plasmacytoid, and myeloid dendritic cells, but exhibit some limitations. The limitations include expensive, multifaceted and time-consuming generation, inconsistent adoption of the transplanted human cells, and the presence of murine epithelial and endothelial cells in the respiratory tract. Investigators are trying to overcome the current limitation to create improved humanized mice.

An ex vivo lung perfusion (EVLP) system can be a potential approach to solving the issue of scarcity of human lungs. Owing to poor oxygenation, poor lung compliance, or visible lung injury, almost 80% of evaluated lungs are thought to be inappropriate for transplantation. EVLP can ventilate and perfuse these lungs for several hours, for better in vivo stimulatory conditions, and allows observing various physiological measures. An EVLP system can also be implemented in a preclinical model by applying endotoxin or bacteria for hypothesis-testing for ARDS therapies, and screening of the mechanism of drug actions by using pharmacological agonists or antagonists. Clinical trials are being performed to check whether EVLP can improve the suitability of lungs for transplantation.

Lung-on-a-chip microdevices is another potential therapeutic screening strategy to create a clinically relevant human disease model. This system is suitable for those human cell lines that
can persist in long-term culture. Recently, alveolar epithelial cells derived from a lung cancer cell line have been used to study the toxic effects of the drugs on IL-2-induced pulmonary edema in a lung-on-a-chip microdevice.\textsuperscript{50,51} Most remarkably, this model also evaluated the therapeutic effectiveness of the coadministration of angiopoietin-1 and TRPV4 (a new inhibitor of transient receptor potential vanilloid 4) to suppress pulmonary vascular leakage.\textsuperscript{50} This method is thought to be more convenient for drug screening than EVLP. Hence, both EVLP and lung-on-a-chip microdevices might be helpful to test the compounds before proceeding to human trials.

Finally, genome editing by endonucleases, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) systems, has been revolutionized that induces the site-specific DNA cleavage to insert specific point mutations into the human genomes of tissue. Thus far, a coronavirus-induced ARDS mouse model has been developed by CRISPR/Cas9 editing.\textsuperscript{52} Future implementation of genome editing in ARDS will be helpful for polymorphisms or genes identification via genome-wide association study to provide various genomic evidence for the pathogenesis of ARDS and will be advantageous for researchers to develop a new drug to treat ARDS in different genetic backgrounds.

**Reducing heterogeneity in clinical studies**

The most controversial issue in clinical studies of ARDS is the heterogeneous group of patients that makes it indistinguishable from other lung pathologies, such as cryptogenic organizing pneumonia, diffuse alveolar damage, pulmonary hemorrhage, and allergic pneumonitis. Various preclinical and clinical outcomes have revealed that some pharmacological approaches are advantageous for some patients but detrimental to others, due to variations in ARDS etiology, pathology, and associated morbidities.\textsuperscript{17,53} Hence, the signal-to-noise ratio might be improved by tightening the enrollment criteria via recognizing the suitable subgroups and reducing the heterogeneity. The trials of neurovascular blockade\textsuperscript{53} and prone positioning\textsuperscript{54} can illustrate the worth of reducing heterogeneity for more severe ARDS.

Excluding patients with major comorbidities, such as advanced lung or liver disease, malignancy, and dementia, is an important approach to minimize the heterogeneity in clinical studies. Moreover, the presence of vasopressin-dependent shock,\textsuperscript{55} higher pulmonary dead space fractions,\textsuperscript{56} and response to PEEP, positive end-expiratory pressure, on a computerized tomography (CT) scan\textsuperscript{57} might be helpful to minimize the heterogeneity in clinical trials.

Identification of subphenotypes of patients who meet ARDS criteria is another effective approach to reduce the heterogeneity. ARDS have been subdivided into trauma vs. sepsis on the basis of clinical risk factors\textsuperscript{58} and diffuse vs. focal on account of radiographic changes.\textsuperscript{59} Accumulated evidence proposes that different clinical outcomes and treatment responses in direct and indirect lung injuries are caused by both clinical\textsuperscript{60} and biological\textsuperscript{61} differences. Measuring plasma levels of lung injury biomarkers is another complex approach to identify the ARDS patients with a hyperinflammatory subphenotype and higher mortality. For example, discrete ARDS subphenotypes have been recognized on the basis of biomarker profiles\textsuperscript{62} and responses to fluid management strategy.\textsuperscript{63} Meyer and Calfee\textsuperscript{11} discussed the implementation of these approaches in detail.

**Novel analytical approaches**

Novel analytical approaches are needed to exploit the insight gained and integrated with composite molecular and clinical data for drug development. Measuring biomarkers with the regression-based method is a common approach that led to understanding the advances in the biology of ARDS as well as to analyze genetic polymorphisms, RNA and DNA sequencing, proteomics, and metabolomics. An important limitation of this approach is that this does not facilitate the analyses of heterogeneity within ARDS. Hence, alternative analytical approaches are needed.

Over the past several decades, researchers have quantified biological complexity and have developed novel statistical methods to examine heterogeneity. These novel statistical methods, in the case of asthma, have resulted in significant advancement in understanding the disease endotypes and differential responses to therapy. Currently, some of these statistical methods, with a similar goal, are being extensively focused on translational studies of serious illness.

Cluster-based methods include different analytical techniques that identify clusters of observations with identical characteristics. For instance, $k$-means clustering and hierarchical clustering methods are normally used to identify the clusters of patients with similar genomic data. Accumulated cluster data are evaluated for the difference in clinical phenotypes, clinical outcomes, and other desired variables. Examples include the identification of subclasses of pediatric septic shock\textsuperscript{64} and identification of T-helper-2-high endotype in asthma studies.\textsuperscript{65} Clustering in ARDS exhibits an advantageous role in reducing heterogeneity, and it can be performed on baseline characteristics without considering results.

Classification and regression tree analysis/classification trees, similar to cluster analysis, is another advanced analytical approach. This approach identifies unexpected cutpoints in the data, and generates a branching tree-like structure of a given variable and ends in various terminal nodes that are frequently acclaimed by the characteristic of outcomes. Tree-based models have been used to recognize a prognosticator of clinical deterioration in hospital inpatients,\textsuperscript{66} to improve prognostic stratification, on the basis of plasma biomarkers, in septic shock patients,\textsuperscript{67} and to identify clinical features linked with poor outcomes in ARDS.\textsuperscript{68} These tree-like structures are established on the basis of the relationship between deliberated variables and explicit clinical outcome. Tree-based models also needed potentially for arbitrary decisions, concerning a number of branches and terminal nodes, the same as cluster-based models, while methods with resampling and crossvalidation have been developed to recognize these decisions.\textsuperscript{66}

The latent class analysis is another approach that identifies unobservable subgroups (so-called latent) within a larger group and helps the researchers to estimate movement between subgroups over time. It has been extensively used in psychiatric research and to study asthma endotypes.\textsuperscript{69} Further, two discrete ARDS subphenotypes have been recognized, on the basis of...
b biomarker profiles, responses to randomly assigned PEEP, and a fluid-conservative management strategy, by latent class analysis. The latent class analysis also identified subgroups of ARDS after major trauma that were mainly distinguished by plasma biomarker expression and clinical characteristics. Comparatively large datasets \( n > 300 \) are needed to fit this model, which is a drawback.

**FUTURE THERAPEUTIC STRATEGIES**

**Therapies that warrant further testing**

Some therapeutic agents with low-risk profiles, used for other indications, warrant further testing for ARDS. For instance, macrolides, particularly azithromycin, warrants further evaluation because it decreased mortality and improved outcomes in ALI/ARDS patients. Of note, paracetamol, a specific hemoprotein reductant, can decrease the capacity of oxidized cell-free hemoglobin to drive oxidant-mediated tissue injury and lipid peroxidation. A pilot study has demonstrated that enteral administration of paracetamol (1 g every 6 h for 3 days) to severe sepsis, a common cause of ARDS, patients exhibit the harmful and encouraging effects on biomarkers of lipid peroxidation and acute renal injury. Given that, larger trials of paracetamol are needed due to its well-recognized safety profile, low cost, and widespread availability.

CD73-mediated adenosine production exhibited a protective role in ARDS. A phase I/II study of ARDS has revealed that intravenous administration of FP-1201-lyo (recombinant human interferon-β (IFN-β) also known as Traumakine) strikingly reduced mortality, because synthesis of CD73 is stimulated by IFN-β in lung endothelial cells, and a phase III study (INTEREST) is currently recruiting participants (NCT02622724) (Table 2); hence, larger therapeutic trials of IFN-β are warranted. Additionally, prompted by preclinical outputs, a human trial involving anti-CD14 monoclonal antibodies was started in 2007 but was later terminated due to poor patient recruitment (NCT00233207). We expect further investigation in the future.

Adrenomedullin (AM), a vasoactive peptide hormone, reduced pulmonary vascular permeability and lung injury and the upstream signaling of NLRP3 inflammasome. While targeting Fbxo3 protein, Fbxo3-mediated adenosine production exhibited a protective role in ARDS. A phase I/II study of AM has revealed that intravenous administration of AM could decrease mortality, because synthesis of CD73 is stimulated by AM in lung endothelial cells, and a phase III study (INTEREST) is currently recruiting participants (NCT02622724) (Table 2); hence, larger therapeutic trials of AM are warranted. Additionally, prompted by preclinical outputs, a human trial involving anti-CD14 monoclonal antibodies was started in 2007 but was later terminated due to poor patient recruitment (NCT00233207). We expect further investigation in the future.

**Targeting the ubiquitin-proteasome system**

Ubiquitin is a small regulatory molecule found in eukaryotic tissues, and ubiquitination is a postranslational modification process, which takes place after the attachment of ubiquitin to a substrate protein that serves as a signal for ubiquitin degradation via lysosome or proteasome. ARDS is characterized by elevated expression of ubiquitin E3 ligase component and Fbxo3 within alveolar epithelial type II cells, the release of ubiquitin-proteasome components into bronchoalveolar lavage fluid, and activation of the ubiquitin-proteasome system. Targeting proteasomes induce antiinflammatory effects. The US Food and Drug Administration recently registered proteasome inhibitors including carfilzomib and bortezomib for multiple myeloma treatment. For ubiquitin-proteasomal degradation, hypoxia-inducible factor-1α (HIF-1α) is targeted. Pharmacologic stabilization of HIF-1α attenuated the ARDS severity in preclinical models, proposing that HIF-1α have a protective effect against ARDS. Moreover, the severity of ARDS, septic shock, viral pneumonia, and cytokine-driven systemic inflammation were effectively attenuated during preclinical models by targeting the Fbxo3 protein, emphasizing the potential therapy of ARDS via targeting ubiquitin-proteasome system.

**Targeting inflammasomes**

Inflammasomes, a large multiprotein complex, is made up of three constituents including NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3), ASC (apoptosis-associated speck-like protein), and pro-caspase-1. Hypoxic cellular injury or pore-forming toxins activate inflammasomes. Upon activation, inflammasomes cleave pro-IL-1β and pro-IL-18 into IL-1β and IL-18, respectively. Inflammasome-regulated cytokines are related to ARDS development. Numerous approaches have been performed to inhibit the upstream signaling of NLRP3 inflammasome. While targeting caspase-1 attenuated the IL-1β and IL-18 discharge in rat endotoxemia, inhibiting the downstream pathway in order to block inflammasome activation. Inflammasome activation can also be limited by anti-IL-1 therapy because new chemical entities directly targeting inflammasome (NLRs) are yet missing. Canakinumab (anti-IL-1 monoclonal Ab) is approved to treat cryopyrin-associated periodic syndrome (genetic disease), caused by autosomal-dominant mutations of the NLRP3 gene. Rilonacept (also known as IL-1 Trap; IL-1 inhibitor) and anakinra (IL-1 receptor antagonist) are registered to treat cryopyrin-associated periodic syndromes and rheumatoid arthritis, respectively. Nevertheless, the preferred roles of these agents have not yet
been properly evaluated in clinical settings of ARDS. A phase IV study to assess the role of corticosteroid to mediate ARDS via NLRP3 inflammasome signaling pathway is still recruiting participants (NCT02819453).

**Combination of therapies**
Treating ARDS via targeting a single pathogenic pathway might be deficient because the complex cascade of pathogenic events, such as acute injury to the alveolar–capillary membrane, activation of innate and adaptive immune cells, and alveolar edema clearance are involved in the pathogenesis of ARDS. For instance, therapies that could effectively treat the preliminary lung injury might not be sufficiently effective for established lung injury. In contrast, therapies that could improve the resolution phase might be ineffective in the case of a severely disrupted alveolar–capillary membrane. Hence, theoretically, a combination therapy of acute injury-reducing agents and resolution phase-enhancing agents might be more effective than alone therapy. For instance, a combination of β2-agonist (formoterol) and aerosolized corticosteroid (budesonide) improved the oxygenation of at-risk ARDS patients. As a secondary outcome, seven patients (23%) in the placebo group developed ARDS vs. no patients in the treated group. The aim of this therapy was to reduce lung inflammation and to enhance alveolar fluid clearance. Hence, this study shows how combination therapy might be more effective than therapy with either agent alone. Moreover, a four-arm trial, including inhaled placebo, inhaled budesonide, inhaled formoterol, and the combination of inhaled formoterol and budesonide, if feasible, would be more helpful.

**Stem cell-based therapy**
Stem cell-based therapy for ARDS is an emerging future pharmacological therapy. Numerous mechanisms supported the assumption role of stem cells in lung protection. First, stem cells secrete paracrine-soluble factors, including IL-1 receptor antagonist, prostaglandin E2, IL-10, antimicrobial peptide LL-37, keratinocyte growth factor, and angiopoietin-1 directly interact with injured cells, hence, promoting the tissue repair, alveolar edema clearance, and resolution of inflammation. Second, stem cells are potentially differentiated into lung endothelial or alveolar epithelial cells, and can directly reconstitute the capillary–alveolar barrier during cellular injury. Interestingly, bone marrow-derived mesenchymal stem cells (MSCs) are under intense clinical investigation because these can alter both local and systemic inflammatory responses, differentiate into cells that can reconstitute vascular and epithelial surfaces, and provide protection against LPS-induced lung injury. Exogenous administration of MSCs demonstrated positive outcomes in ARDS animal models. For instance, infusion of cryopreserved human MSCs repaired the ventilation-induced lung injury, attenuated the alveolar permeability, restored the alveolar fluid clearance, and minimized the inflammation in injured human lungs. Further, conditioned media obtained from MSCs might be therapeutic in the future, obviating the need for cell cryopreservation. Phase I trials demonstrated that infusion of bone marrow- or allogeneic adipose-derived MSCs is safe, and might attenuate circulating markers of alveolar epithelial injury in moderate to severe ARDS patients. Clinical studies recruiting participants for evaluation of phase I/II stem cell-based therapies for ARDS are depicted in Table 2.

**Gene therapy**
Gene therapy is a promising approach, but its use is limited to animal models. For instance, adeno-associated virus vectors containing the EC-SOD transgene reduced the severity of ARDS. Similarly, nanoparticles of β2-adrenergic receptors significantly attenuated the ARDS severity in established ARDS mice models. Interestingly, among various identified ARDS genes, only alveolar fluid clearance genes are being therapeutically focused on because ARDS is mainly characterized by abnormal accumulation of alveolar fluid in the alveolar spaces and interstitium. Thereby, Na+/ K+-ATPase, which regulates fluid transport across the cell membrane, is a potential preclinical target. In the ARDS model, gene therapy of Na+/K+-ATPase improved the developed lung injury via improving alveolar fluid clearance. Similarly, gene therapy of β1-Na+/K+-ATPase alone or in combination with epithelial sodium channel (ENaC) α1-subunit upregulated tight junctions to treat LPS-induced ARDS. Additionally, clinical investigations have revealed that aquaporin (AQP) acts as a candidate gene in lung injury and sepsis that regulates pulmonary vascular permeability, and further genetic studies are needed to link polymorphisms in selected genes with ARDS.

**CONCLUSION**
Taking together, ARDS has gained the status of a “Bermuda Triangle” in the field of drug development. Thereby, further studies on new developmental strategies in combination with increased knowledge in relevant areas such as genomics, immunology, appropriate animal modeling, apposite clinical-trial designing, prognostic and predictive enrichment strategies to reduce the heterogeneity and implementation of new analytical and pharmacological approaches would facilitate researchers to develop new drugs for ARDS.

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**CONFLICT OF INTEREST**
The authors declare no competing interests for this work.

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**AUTHOR CONTRIBUTIONS**
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