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 syndrome[1] that is characterized by the enhanced alveolar-capillary membrane permeability, interstitial and alveolar edema formation, neutrophils-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 $<$ PaO_2/FiO_2 $\leq$ 300 mmHg), moderate (100 mmHg $<$ PaO_2/FiO_2 $\leq$ 200 mmHg), or severe (PaO_2/FiO_2 $\leq$ 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 H_2O), 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,[4,6] 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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Table 1 Biomarkers involved in exudative and fibroproliferative phases of ARDS

Pathophysiological features of ARDS	Biomarker/source of biomarker
Exudative phase of ARDS (days 0–7)	
A. Lung injury	
1.Epithelium damage (i) Alveolar epithelial type 1 cells (ii) Alveolar epithelial type 2 cells (iii) Clara cells	RAGE, HTI56 Surfactant (SP-A, SP-B, SP-D), KL-6 CCl6
2. Endothelium damage	Ang-1, Ang-2, ICAM-1, selectins, VEGF, vWF
3. Lung extracellular matrix	Laminin, elastin, MMPs
B. Inflammation and inflammatory cascade	
1. Proinflammatory cytokines	TNF-α, IL-1β, IL-8/CXCL8, IL-6, CCL-2/MCP-1, IL-18
2. Antiinflammatory cytokines	IL-10, sIL-1RII, sTNF-RI/sTNF-II
3. Additional inflammatory markers	High mobility group box nuclear protein 1, lipopolysaccharide binding protein, nitric oxide, C-reactive protein
C. Coagulation and fibrinolysis	Plasminogen activator inhibitor-1, activated protein C, thrombomodulin, tissue factor, cell-free hemoglobin
D. Pulmonary microvascular permeability vs. EF/PL protein ratio	EF/PL ratio
Fibroproliferative phase of ARDS (since day 7)	
E. Endothelial proliferation	Vascular endothelial growth factor
F. Epithelial proliferation	Keratinocyte growth factor, hepatocyte growth factor
G. Apoptosis	Fas/FasL
H. Fibroblast proliferation	NT part of procollagen III (N-PCP-III)

RAGE, receptor for advanced glycation endproducts; HTI56, human type I cell-specific membrane protein; SP, surfactant protein; KL-6, Krebs von den Lungen-6; CC16, Clara cells; Ang, angiopoietin-1; ICAM-1, intercellular adhesion molecule-1; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor; MMPs, matrix metalloproteinases; TNF- α, tumor necrosis factor-α; IL, interleukin; sTNFR-1, soluble tumor necrosis factor receptor-1; sTNF-II, soluble TNF receptor II; sIL-1RII, soluble IL-1 receptor II; MCP, monocyte chemoattractant protein; EF/PL ratio, fluid-to-plasma protein ratio; Fas/FasL.

PATHOPHYSIOLOGY OF ARDS

Characteristics and severity of ARDS are perceived by an assortment of involved pathophysiological biomarkers, depicted in **Table 1** according to their origin or characteristics. 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 ANGPT2[10] with trauma-associated ALI, IL1RN,[11] and PPFIA[12] with ARDS risk, ADIPOQ[13] and rs78142040, rs9605146 and rs3848719[14] with severity and mortality of ALI/ARDS, and LRRC16A/CARMIL1[15] with outcome of ARDS, but knowledge of the genetic factors involved in ARDS susceptibility is in its infancy. Further studies in larger patient populations of different ethnicities are needed to identify genetic factors associated with ARDS to develop a personalized medicine approach.

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-dose[16] and moderate-dose corticosteroids[17] have so far failed to exhibit efficacy in ARDS. Interestingly, prolonged low-dose corticosteroids effectively decreased the

ICU mortality in early adult ARDS patients[18] and pediatric.[19] Regardless of a meta-analysis and systematic review, the role of steroids in ARDS patients remains uncertain and unclear. Moreover, phase II (NCT01757899; 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 antiendotoxin antibody designed to neutralize the harmful effects of LPS, was conducted that showed improved survival and convincingly positive outcomes.[20] In contrast, HA-1A did not show therapeutic benefits during multicenter RCTs to treat septic shock and sepsis-associated ARDS.[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)[22] and simvastatin[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.[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-α)

Anti-TNF-α therapy showed promising outcomes in preclinical studies. Afelimomab, an anti-TNF-α monoclonal antibody, improved the survival rate of severe sepsis, a common cause of ARDS, but showed potentially confusing variables.[25] A meta-analysis of anti-TNF-α therapy also revealed improved survival in sepsis. Moreover, etanercept (anti-TNF-α agent),[26] and a combination of etanercept and corticosteroids improved survival in children with idiopathic pneumonia syndrome (IPS).[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-TNFR1) monoclonal antibody (GSK1995057) was conducted that attenuated pulmonary inflammation via modulating the pulmonary microvascular endothelial function.[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,[29] whereas phase III and phase IV studies of prolonged use of sivelestat conducted in Japan demonstrated positive outcomes.[30] Moreover, recent studies further support the therapeutic effectiveness of early administration of sivelestat to ARDS patients,[31] as well as in nonrandomized postmarketing.[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.[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.[34] A trial of nebulized heparin is ongoing (ACTRN12612000418875); hence, more trials are needed. In addition, prehospitalization aspirin therapy[35] and a recent clinical study revealed the significant effect of aspirin.[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.[37] Moreover, intravenous recombinant human-APC (rh-APC) did not ameliorate ARDS in critically ill patients.[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,[39] but in contrast, a recent phase II clinical trial revealed that palifermin cannot be recommended to treat ARDS.[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.[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 Pharmacoeconomics 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 (LOCO2)	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

Table 2 Continued

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 NCT02097641	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 (5×10^5/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 x 10^6 cell/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 1×10^6 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 (GI-HOPE)	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-VMNI)	NCT02958150	Phase IV	180	Dexmedetomidine vs. standard clinical practice	Oxygenation index, ventilator-free days and Mortality	Recruiting
Can Heparin Administration Reduce Lung Injury (CHARLI)	ACTRN12612 000418875	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-acetylcysteine, 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[42] 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.[43] 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.[44] 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.[45] 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.[46]

Humanized mice are thought to be a potential way to improve the ARDS animal models.[47] Humanized mice were developed by transplanting human $CD34^+$ umbilical cord blood hematopoietic stem cells into gamma-irradiated neonatal NOD–SCID–IL-$2R\gamma^{null}$ 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.[48] 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.[49] Clinical trials are being performed to check whether EVLP can improve the suitability of lungs for transplantation.[48]

Lung-on-a-chip microdevices is another potential therapeutic screening strategy to create a clinically relevant human disease model.[50] 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.[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.[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.[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.[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 neuromuscular blockade[53] and prone positioning[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,[55] higher pulmonary dead space fractions,[56] and response to PEEP, positive end-expiratory pressure, on a computerized tomography (CT) scan[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[58] and diffuse vs. focal on account of radiographic changes.[59] Accumulated evidence proposes that different clinical outcomes and treatment responses in direct and indirect lung injuries are caused by both clinical[60] and biological[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[62] and responses to fluid management strategy.[63] Meyer and Calfee[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, κ-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[64] and identification of T-helper-2-high endotype in asthma studies.[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,[66] to improve prognostic stratification, on the basis of plasma biomarkers, in septic shock patients,[67] and to identify clinical features linked with poor outcomes in ARDS.[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.[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.[69] Further, two discrete ARDS subphenotypes have been recognized, on the basis of

biomarker profiles, responses to randomly assigned PEEP, and a fluid-conservative management strategy, by latent class analysis.[62,63] The latent class analysis also identified subgroups of ARDS after major trauma that were mainly distinguished by plasma biomarker expression and clinical characteristics.[70] 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.[71,72] 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 harmless and encouraging effects on biomarkers of lipid peroxidation and acute renal injury.[73] 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,[74] 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[75] of rodent models. In 2010, the European Medicines Agency (EMA) recommended AM as an orphan drug for ARDS treatment (EMA/COMP/104704/2010), while clinical trials with AM therapy are awaited. Interestingly, animal studies suggest that angiotensin-converting enzyme (ACE) is damaging and ACE-2 is protective in ARDS,[76] while human data are somewhat contradictory[77] due to genetic phenotype, but a proposed protective effect of ACE-2 therapy in selected populations. A human phase IIa clinical trial (NCT01597635) of the recombinant human ACE-2, GSK2586881, in early ARDS patients has been completed and the results are awaited; however, ACE-2 therapy warrants more testing.

Targeting complement cascade

During ARDS and sepsis, quick discharge of the complement peptides or anaphylatoxins such as C3a and C5a, and dysregulation of coagulation occur due to immune activation. Targeting C3/C3a and/or C5/C5a is limited due to the inherent redundancy of biological effects of complement peptides and lack of available therapeutics. Nevertheless, preclinical models revealed that complement cascade can be efficiently restricted by the protein C1-inhibitor (C1-INH; also known as a C1-esterase inhibitor), a constitutively released protease inhibitor belonging to the *serpin* superfamily. A multicenter phase II trial demonstrated that purified human C1-INH substantially attenuated the mortality (33% absolute reduction), and even improved the quality of life of sepsis-induced ARDS patients.[78] These fascinating outcomes yet are not being used in larger phase II/III trials.

Targeting the ubiquitin-proteasome system

Ubiquitin is a small regulatory molecule found in eukaryotic tissues, and ubiquitination is a posttranslational 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.[79] Targeting proteasomes induce antiinflammatory effects.[80] 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[81]; 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,[82] 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 procaspase-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.[83] 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,[84] 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-1β monoclonal Ab) is approved to treat cryopyrin-associated periodic syndrome (genetic disease),[85] caused by autosomal-dominant mutations of the NLRP3 gene. Rilonacept (also known as IL-1 Trap; IL-I inhibitor) and anakinra (IL-1 receptor antagonist) are registered to treat cryopyrin-associated periodic syndromes and rheumatoid arthritis, respectively.[86] Nevertheless, the pretended 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.[87] 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 support the assumed 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[88]; 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.[89] 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.[88] Exogenous administration of MSCs demonstrated positive outcomes in ARDS animal models. For instance, infusion of cryopreserved human MSCs repaired the ventilation-induced lung injury,[90] attenuated the alveolar permeability, restored the alveolar fluid clearance, and minimized the inflammation in injured human lungs.[91] Further, conditioned media obtained from MSCs might be therapeutic in the future, obviating the need for cell cryopreservation.[92] 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.[93] 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.[94] Similarly, nanoparticles of β2-adrenergic receptors significantly attenuated the ARDS severity in established ARDS mice models.[95] Interestingly, among various identified ARDS genes, only alveolar fluid clearance genes are being therapeutically focused on[96] 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.[97] 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.[98] Additionally, clinical investigations have revealed that aquaporin (AQP) acts as a candidate gene in lung injury and sepsis[99] 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

M.H., C.X., and M.A. wrote the article. L.T., and X.W. designed and supervised the article. M.L., and X.W. collected data for tables preparations. All authors read and approved the final article.

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1. Ashbaugh, D., Bigelow, D.B., Petty, T. & Levine, B. Acute respiratory distress in adults. *Lancet* **290**, 319–323 (1967).
2. Ferguson, N.D. *et al*. The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. *Intens. Care Med.* **38**, 1573–1582 (2012).
3. Bernard, G.R. *et al*. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am. J. Respir. Crit. Care. Med.* **149**, 818–824 (1994).
4. Force, A.D.T. Acute respiratory distress syndrome. *JAMA* **307**, 2526–2533 (2012).
5. Thompson, B.T., Chambers, R.C. & Liu, K.D. Acute respiratory distress syndrome. *N. Engl. J. Med.* **377**, 562–572 (2017).
6. Bellani, G. *et al*. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. *JAMA* **315**, 788–800 (2016).
7. Erickson, S.E., Martin, G.S., Davis, J.L., Matthay, M.A. & Eisner, M.D. Recent trends in acute lung injury mortality: 1996-2005. *Crit. Care Med.* **37**, 1574 (2009).
8. Herridge, M.S. *et al*. Functional disability 5 years after acute respiratory distress syndrome. *N. Engl. J. Med.* **364**, 1293–1304 (2011).
9. Ware, L.B. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. *Semin. Respir. Crit. Care Med.* (2006).
10. Meyer, N.J. *et al*. ANGPT2 genetic variant is associated with trauma-associated acute lung injury and altered plasma angiopoietin-2 isoform ratio. *Am. J. Respir. Crit. Care. Med.* **183**, 1344–1353 (2011).
11. Meyer, N.J. & Calfee, C.S. Novel translational approaches to the search for precision therapies for acute respiratory distress syndrome. *Lancet Respir. Med.* **5**, 512–523 (2017).
12. Christie, J.D. *et al*. Genome wide association identifies PPFIA1 as a candidate gene for acute lung injury risk following major trauma. *PLoS One* **7**, e28268 (2012).
13. Ahasic, A.M., Zhao, Y., Su, L., Sheu, C.C., Thompson, B.T. & Christiani, D.C. Adiponectin gene polymorphisms and acute respiratory distress syndrome susceptibility and mortality. *PLoS One* **9**, e89170 (2014).
14. Shortt, K. *et al*. Identification of novel single nucleotide polymorphisms associated with acute respiratory distress syndrome by exome-seq. *PLoS One* **9**, e111953 (2014).
15. Wei, Y. *et al*. A missense genetic variant in LRRC16A/CARMIL1 improves acute respiratory distress syndrome survival by attenuating platelet count decline. *Am. J. Respir. Crit. Care. Med.* **195**, 1353–1361 (2017).
16. Bernard, G.R. *et al*. High-dose corticosteroids in patients with the adult respiratory distress syndrome. *N. Engl. J. Med.* **317**, 1565–1570 (1987).
17. Steinberg, K.P. *et al*. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. *N. Engl. J. Med.* **354**, 1671–1684 (2006).
18. Meduri, G.U. *et al*. Methylprednisolone infusion in early severe ARDS: Results of a randomized controlled trial. *CHEST* **131**, 954–963 (2007).
19. Drago, B.B. *et al*. Double-blind, placebo-controlled pilot randomized trial of methylprednisolone infusion in pediatric acute respiratory distress syndrome. *Pediatr. Crit. Care. Med.* **16**, e74–e81 (2015).
20. Ziegler, E.J. *et al*. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant Escherichia coli. *N. Engl. J. Med.* **307**, 1225–1230 (1982).
21. Bigatello, L. *et al*. HA-1A in septic patients with ARDS: results from the pivotal trial. *Intens. Care Med.* **20**, 328–334 (1994).
22. Heart, T.N. Rosuvastatin for sepsis-associated acute respiratory distress syndrome. *N. Engl. J. Med.* **370**, 2191 (2014).
23. McAuley, D.F. *et al*. Simvastatin in the acute respiratory distress syndrome. *N. Engl. J. Med.* **371**, 1695–1703 (2014).
24. Dinglas, V.D. *et al*. One-year outcomes of rosuvastatin versus placebo in sepsis-associated acute respiratory distress syndrome: prospective follow-up of SAILS randomised trial. *Thorax* thoraxjnl-2015-208017 (2016).
25. Panacek, E.A. *et al*. Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F (ab') 2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Crit. Care Med.* **32**, 2173–2182 (2004).
26. Yanik, G.A. *et al*. The impact of soluble tumor necrosis factor receptor etanercept on the treatment of idiopathic pneumonia syndrome after allogeneic hematopoietic stem cell transplantation. *Blood* **112**, 3073–3081 (2008).
27. Yanik, G.A. *et al*. TNF-receptor inhibitor therapy for the treatment of children with idiopathic pneumonia syndrome. A joint Pediatric Blood and Marrow Transplant Consortium and Children's Oncology Group Study (ASCT0521). *Biol. Blood Marrow Transplant.* **21**, 67–73 (2015).
28. Proudfoot, A.G. *et al*. A novel TNFR1-targeting domain antibody attenuates pulmonary inflammation in a human model of lung injury, via actions on the lung micro-vascular endothelium. In: *A47 Critical Illness: Novel Molecules and Models* A6589-A (Am Thoracic Soc, 2014).
29. Zeiher, B.G. *et al*. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. *Crit. Care Med.* **32**, 1695–1702 (2004).
30. Aikawa, N. *et al*. Reevaluation of the efficacy and safety of the neutrophil elastase inhibitor, Sivelestat, for the treatment of acute lung injury associated with systemic inflammatory response syndrome; a phase IV study. *Pulm. Pharmacol. Ther.* **24**, 549–554 (2011).
31. Kido, T. *et al*. Efficacy of early sivelestat administration on acute lung injury and acute respiratory distress syndrome. *Respirology* **22**, 708–713 (2017).
32. Fukimbara, S., Niibe, K., Yamamoto, M. & Yamaguchi, T. Adjustment for propensity score in nonrandomized clinical studies: comparison of sivelestat versus conventional therapy for acute lung injury in acute respiratory distress syndrome. *Ther. Innov. Regul. Sci.* **51**, 89–99 (2017).
33. Vincent, J.L., Artigas, A., Petersen, L.C. & Meyer, C. A multicenter, randomized, double-blind, placebo-controlled, dose-escalation trial assessing safety and efficacy of active site inactivated recombinant factor VIIa in subjects with acute lung injury or acute respiratory distress syndrome. *Crit. Care Med.* **37**, 1874–1880 (2009).
34. Dixon, B., Schultz, M.J., Smith, R., Fink, J.B., Santamaria, J.D. & Campbell, D.J. Nebulized heparin is associated with fewer days of mechanical ventilation in critically ill patients: a randomized controlled trial. *Crit. Care* **14**, R180 (2010).
35. Chen, W. *et al*. Prehospital aspirin use is associated with reduced risk of acute respiratory distress syndrome in critically ill patients: a propensity-adjusted analysis. *Crit. Care Med.* **43**, 801 (2015).
36. Hamid, U. *et al*. Aspirin reduces lipopolysaccharide-induced pulmonary inflammation in human models of ARDS. *Thorax* thoraxjnl-2016-208571 (2017).
37. Liu, K.D. *et al*. Randomized clinical trial of activated protein C for the treatment of acute lung injury. *Am. J. Respir. Crit. Care. Med.* **178**, 618–623 (2008).
38. Cornet, A.D. *et al*. Recombinant human activated protein C in the treatment of acute respiratory distress syndrome: a randomized clinical trial. *PLoS One* **9**, e90983 (2014).
39. Shyamsundar, M. *et al*. Keratinocyte growth factor promotes epithelial survival and resolution in a human model of lung injury. *Am. J. Respir. Crit. Care. Med.* **189**, 1520–1529 (2014).
40. McAuley, D.F. *et al*. Keratinocyte growth factor for the treatment of the acute respiratory distress syndrome (KARE): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Respir. Med.* **5**, 484–491 (2017).
41. Robert Paine III, T.J.S. *et al*. A randomized trial of recombinant human GM-CSF for patients with acute lung injury. *Crit. Care Med.* **40**, 90 (2012).
42. Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y. & Ingber, D.E. Reconstituting organ-level lung functions on a chip. *Science* **328**, 1662–1668 (2010).
43. Brandenberger, C., Kling, K.M., Lopez-Rodriguez, E., Pfarrer, C. & Muhlfeld, C. Lipopolysaccharide-induced lung injury is more severe in old compared to young mice. In: C30 Acute Lung Injury: Cell Function, Signaling And Stretch A4815-A (Am Thoracic Soc, 2016).
44. Karp, C.L. Unstressing intemperate models: how cold stress undermines mouse modeling. *J. Exp. Med.* **209**, 1069–1074 (2012).
45. Warren, H.S. *et al*. Resilience to bacterial infection: difference between species could be due to proteins in serum. *J. Infect. Dis.* **201**, 223–232 (2010).
46. Meurens, F., Summerfield, A., Nauwynck, H., Saif, L. & Gerdts, V. The pig: a model for human infectious diseases. *Trends Microbiol.* **20**, 50–57 (2012).
47. Unsinger, J., McDonough, J.S., Shultz, L.D., Ferguson, T.A. & Hotchkiss, R.S. Sepsis-induced human lymphocyte apoptosis and cytokine production in "humanized" mice. *J. leukoc. Biol.* **86**, 219–227 (2009).
48. Cypel, M. *et al*. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N. Engl. J. Med.* **364**, 1431–1440 (2011).

49. McAuley, D.F. *et al*. Clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **306**, L809–L815 (2014).
50. Huh, D. *et al*. A human disease model of drug toxicity—induced pulmonary edema in a lung-on-a-chip microdevice. *Sci. Transl. Med.* **4**, 159ra47–ra47 (2012).
51. Gores, K.M. *et al*. Plasma angiopoietin-2 concentrations are related to impaired lung function, and organ failure in a clinical cohort receiving high dose interleukin-2 therapy. *Shock* **42**, 115 (2014).
52. Cockrell, A.S. *et al*. A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. *Nat. Microbiol.* **2**, 16226 (2016).
53. Papazian, L. *et al*. Neuromuscular blockers in early acute respiratory distress syndrome. *N. Engl. J. Med.* **363**, 1107–1116 (2010).
54. Guérin, C. *et al*. Prone positioning in severe acute respiratory distress syndrome. *N. Engl. J. Med.* **368**, 2159–2168 (2013).
55. Heart, T.N. Randomized, placebo-controlled clinical trial of an aerosolized β2-agonist for treatment of acute lung injury. *Am. J. Respir. Crit. Care Med.* **184**, 561 (2011).
56. Nuckton, T.J. *et al*. Pulmonary dead-space fraction as a risk factor for death in the acute respiratory distress syndrome. *N. Engl. J. Med.* **346**, 1281–1286 (2002).
57. Gattinoni, L. *et al*. Lung recruitment in patients with the acute respiratory distress syndrome. *N. Engl. J. Med.* **354**, 1775–1786 (2006).
58. Calfee, C.S. *et al*. Trauma-associated lung injury differs clinically and biologically from acute lung injury due to other clinical disorders. *Crit. Care Med.* **35**, 2243 (2007).
59. Mrozek, S. *et al*. Elevated plasma levels of sRAGE are associated with nonfocal CT-based lung imaging in patients with ARDS: a prospective multicenter study. *CHEST* **150**, 998–1007 (2016).
60. Luo, L. *et al*. Clinical predictors of hospital mortality differ between direct and indirect ARDS. *CHEST* **151**, 755–763 (2017).
61. Calfee, C.S. *et al*. Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies. *CHEST* **147**, 1539–1548 (2015).
62. Calfee, C.S. *et al*. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir. Med.* **2**, 611–620 (2014).
63. Famous, K.R. *et al*. Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. *Am. J. Respir. Crit. Care Med.* **195**, 331–338 (2017).
64. Wong, H.R. *et al*. Developing a clinically feasible personalized medicine approach to pediatric septic shock. *Am. J. Respir. Crit. Care Med.* **191**, 309–315 (2015).
65. Haldar, P. *et al*. Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* **178**, 218–224 (2008).
66. Churpek, M.M., Yuen, T.C., Winslow, C., Meltzer, D.O., Kattan, M.W. & Edelson, D.P. Multicenter comparison of machine learning methods and conventional regression for predicting clinical deterioration on the wards. *Crit. Care Med.* **44**, 368–374 (2016).
67. Wong, H.R. *et al*. A multibiomarker-based outcome risk stratification model for adult septic shock. *Crit. Care Med.* **42**, 781 (2014).
68. Brown, L.M., Calfee, C.S., Matthay, M.A., Brower, R.G., Thompson, B.T. & Checkley, W. A simple classification model for hospital mortality in patients with acute lung injury managed with lung protective ventilation. *Crit. Care Med*. **39**, 2645 (2011).
69. Wenzel, S.E. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat. Med.* **18**, 716 (2012).
70. Reilly, J.P. *et al*. Heterogeneous phenotypes of acute respiratory distress syndrome after major trauma. *Ann. Am. Thorac. Soc.* **11**, 728–736 (2014).
71. Walkey, A.J. & Wiener, R.S. Macrolide antibiotics and survival in patients with acute lung injury. *CHEST* **141**, 1153–1159 (2012).
72. Kawamura, K. *et al*. Efficacy of azithromycin in sepsis-associated acute respiratory distress syndrome: a retrospective study and propensity score analysis. *SpringerPlus* **5**, 1193 (2016).
73. Janz, D.R. *et al*. Randomized, placebo-controlled trial of acetaminophen for the reduction of oxidative injury in severe sepsis: The ACROSS trial. *Crit. Care Med.* **43**, 534 (2015).
74. Bellingan, G. *et al*. The effect of intravenous interferon-beta-1a (FP-1201) on lung CD73 expression and on acute respiratory distress syndrome mortality: an open-label study. *Lancet Respir. Med.* **2**, 98–107 (2014).
75. Müller, H.C. *et al*. Adrenomedullin attenuates ventilator-induced lung injury in mice. *Thorax* **65**, 1077–1084 (2010).
76. Imai, Y. *et al*. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436**, 112–116 (2005).
77. Watkins, T.R., Lemos-Filho, L.B., Dabbagh, O., Chang, S.Y., Park, P.K. & Gong, M.N. Use Of angiotensin converting enzyme inhibitors or angiotensin receptor blockers and clinical outcomes among patients at-risk for acute lung injury. In: D16 Epidemiology Of Acute Lung Injury A5598-A (Am Thoracic Soc, 2011).
78. Igonin, A.A. *et al*. C1-esterase inhibitor infusion increases survival rates for patients with sepsis. *Crit. Care Med.* **40**, 770–777 (2012).
79. Vadasz, I., Weiss, C.H. & Sznajder, J.I. Ubiquitination and proteolysis in acute lung injury. *CHEST* **141**, 763–771 (2012).
80. Orlicky, S. *et al*. An allosteric inhibitor of substrate recognition by the SCFCdc4 ubiquitin ligase. *Nat. Biotechnol.* **28**, 733–737 (2010).
81. Eckle, T. *et al*. HIF1A reduces acute lung injury by optimizing carbohydrate metabolism in the alveolar epithelium. *PLoS Biol* **11**, e1001665 (2013).
82. Mallampalli, R.K. *et al*. Targeting F box protein Fbxo3 to control cytokine-driven inflammation. *J. Immunol.* **191**, 5247–5255 (2013).
83. Dolinay, T. *et al*. Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am. J. Respir. Crit. Care Med.* **185**, 1225–1234 (2012).
84. Boost, K.A. *et al*. Targeting caspase-1 by inhalation-therapy: effects of Ac-YVAD-CHO on IL-1β, IL-18 and downstream proinflammatory parameters as detected in rat endotoxaemia. *Intens. Care Med.* **33**, 863–871 (2007).
85. Feist, E. & Burmester, G.R. Canakinumab for treatment of cryopyrin-associated periodic syndrome. *Expert Opin. Biol. Ther.* **10**, 1631–1636 (2010).
86. Moltó, A. & Olivé, A. Anti-IL-1 molecules: new comers and new indications. *Joint Bone Spine* **77**, 102–107 (2010).
87. Festic, E. *et al*. Randomized clinical trial of a combination of an inhaled corticosteroid and beta agonist in patients at risk of developing the acute respiratory distress syndrome. *Crit. Care Med.* **45**, 798–805 (2017).
88. Islam, M.N. *et al*. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat. Med.* **18**, 759–765 (2012).
89. Maron-Gutierrez, T., Laffey, J.G., Pelosi, P. & Rocco, P.R. Cell-based therapies for the acute respiratory distress syndrome. *Curr. Opin. Crit. Care* **20**, 122–131 (2014).
90. Curley, G.F. *et al*. Effects of intratracheal mesenchymal stromal cell therapy during recovery and resolution after ventilator-induced lung injury. *Anesthesiology* **118**, 924–932 (2013).
91. Curley, G.F., Scott, J.A, & Laffey, J.G. Therapeutic potential and mechanisms of action of mesenchymal stromal cells for acute respiratory distress syndrome. *Curr. Stem Cell Res. Ther*. **9**, 319–329 (2014).
92. Matthay, M.A., Pati, S. & Lee, J.W. Concise review: mesenchymal stem (stromal) cells: biology and preclinical evidence for therapeutic potential for organ dysfunction following trauma or sepsis. *Stem Cells* **35**, 316–324 (2017).
93. Wilson, J.G. *et al*. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir. Med.* **3**, 24–32 (2015).
94. Hassett, P. *et al*. Overexpression of pulmonary extracellular superoxide dismutase attenuates endotoxin-induced acute lung injury. *Intens. Care Med.* **37**, 1680 (2011).
95. Lin, E.H., Chang, H.Y., Yeh, S.D., Yang, K.Y., Hu, H.S. & Wu, C.W. Polyethyleneimine and DNA nanoparticles-based gene therapy for acute lung injury. *Nanomed. Nanotech. Biol. Med.* **9**, 1293–1303 (2013).
96. Lin, X. & Dean, D.A. Gene therapy for ALI/ARDS. *Crit. Care Clin.* **27**, 705–718 (2011).
97. Mutlu, G.k.M. *et al*. Electroporation-mediated gene transfer of the Na+, K+-ATPase rescues endotoxin-induced lung injury. *Am. J. Respir. Crit. Care Med.* **176**, 582–590 (2007).
98. Lin, X., Barravecchia, M., Kothari, P., Young, J. & Dean, D. β1-Na+, K+-ATPase gene therapy upregulates tight junctions to rescue lipopolysaccharide-induced acute lung injury. *Gene Ther.* **23**, 489–499 (2016).
99. King, L. *et al*. Aquaporin-1: a candidate gene in sepsis and lung injury. *Am. J. Respir. Crit. Care Med.* **167**, A662 (2003).