REVIEW

Macrophage-Targeted Nanomedicines for ARDS/ALI: Promise and Potential

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Abstract——Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are characterized by progressive lung impairment typically triggered by inflammatory processes. The mortality toll for ARDS/ALI yet remains high because of the poor prognosis, lack of disease-specific inflammation management therapies, and prolonged hospitalizations. The urgency for the development of new effective therapeutic strategies has become acutely evident for patients with coronavirus disease 2019 (COVID-19) who are highly susceptible to ARDS/ALI. We propose that the lack of target specificity in ARDS/ALI of current treatments is one of the reasons for poor patient outcomes. Unlike traditional therapeutics, nanomedicine offers precise drug targeting to inflamed tissues, the capacity to surmount pulmonary barriers, enhanced interactions with lung epithelium, and the potential to reduce off-target and systemic adverse effects. In this article, we focus on the key cellular drivers of inflammation in ARDS/ALI: macrophages. We propose that as macrophages are involved in the etiology of ARDS/ALI and regulate inflammatory cascades, they are a promising target for new therapeutic development. In this review, we offer a survey of multiple nanomedicines that are currently being investigated with promising macrophage targeting potential and strategies for pulmonary delivery. Specifically, we will focus on nanomedicines that have shown engagement with proinflammatory macrophage targets and have the potential to reduce inflammation and reverse tissue damage in ARDS/ALI.

KEY WORDS: nanomedicine; nanoparticles; macrophages; ARDS; ALI; COVID-19; targeting; drug delivery; inflammation.

INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are life-threatening conditions with characteristic clinical manifestations such as non-cardiogenic pulmonary edema, hypoxemic respiratory failure, decreased functional residual capacity, reduced pulmonary compliance, non-hydrostatic bilateral lung infiltration, and increased vascular permeability due to the presence of protein-rich exudates and neutrophil

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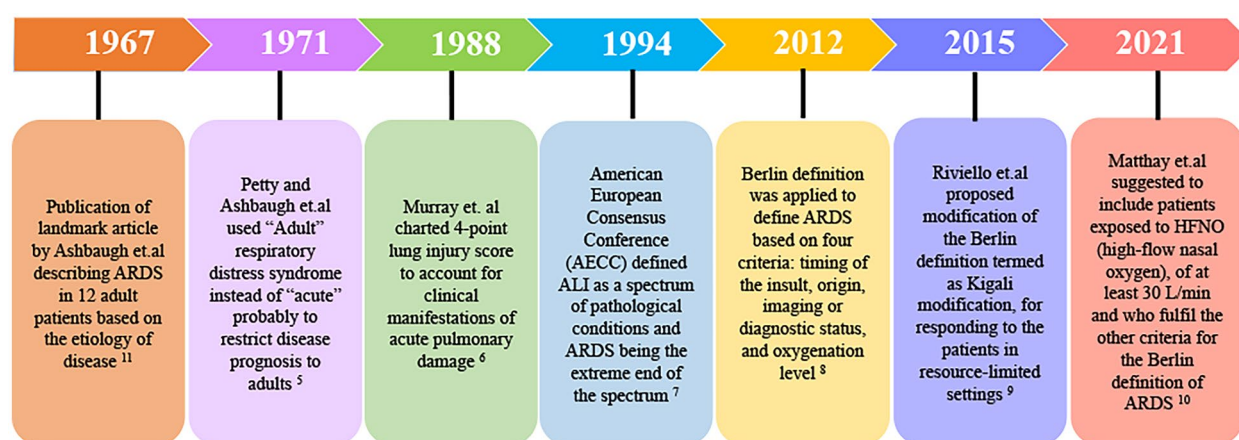
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infiltrates in the alveolar spaces [1–3]. The ALI and ARDS can be triggered either due to direct (pulmonary) or indirect (extrapulmonary) trauma [4]. The clinical definition of ARDS has been refined multiple times [5–10], since its first inception in 1967 by Ashbaugh *et al*. [11]. The overview of these refinements is summarized in Fig. 1. The currently established Berlin definition suggests four diagnostic criteria: timing of the insult, origin, imaging/diagnostic status, and oxygenation [8]. Several comorbidities and environmental factors including alcohol abuse, smoking, air pollution, and low blood albumin levels have been associated with increased susceptibility to ARDS/ALI [12–14]. Recently, a global literature survey reported one-third of positive COVID-19 patients contracted ARDS, and the mortality rate in those patients surged to 45% [15]. Furthermore, across all demographic groups, non-Hispanic blacks, the elderly, and men reported a higher death rate due to ARDS/ALI [16].

The patients that succumb to ARDS/ALI often require mechanical ventilation; however, if performed injudiciously, this can further damage the lungs leading to the development of ventilator-associated lung injury (VALI), a more severe form of ARDS [17]. Formerly, clinical trials were focused to explore the effects of systemic glucocorticoids [18] and inhaled nitric oxide [19], and with support from the National Heart, Lung, and Blood Institute (NHLBI), special impetus was on minimizing the magnitude of lung injury and optimizing supportive care, which contributed to more ventilator-free days [20]. However, the first three trials assessing lung protective strategies by curbing the peak inspiratory pressure and tidal volume reported no benefits in the experimental groups [21]. The diminishing effect of mechanical ventilation is evident as the global prevalence of ARDS in ICUs was roughly 10% but increased to 23% in ventilated patients [22]. Although a recent NIH-sponsored trial did observe a reduction in mortality (22%) with the adoption of controlled mechanical ventilation strategies [23], there is a growing body of evidence suggesting this life-saving intervention carries a risk of developing episodes of hyperoxia and cardiac remodeling changes outweigh the benefits of the procedure [24, 25].

We posit that alternative therapeutic options for ARDS/ALI rather than protocolized care are needed with a specific focus to target the underlying immunological mechanisms. However, this is challenging owing to the heterogenicity of ARDS/ALI histopathology and the presence of subphenotypes governing the treatment responses [26, 27]. Nanotechnology enables engineering of structural scaffolds of nanomedicines enabling cell-specific targeting [28]. Recently, ARDS-associated clinical trials are pivoted to study alveolar programming, thereby deciphering the role of macrophages as shown in Fig. 2 [29–36]. The extensive inflammatory responses in the compromised lungs are driven by the recruitment of resident and circulatory alveolar macrophages [37]. A marked increase in the macrophage pool was observed within 36 h of ALI, which may persist up to 28 days in a non-resolving lung injury [38]. Analysis of total bronchoalveolar lavage fluid (BALF) revealed that more than 90% of the cell population during ARDS/ALI consists of macrophages and neutrophils, further emphasizing their pivotal role in initiation, progression, and resolution of inflammation in ARDS/ALI [39]. In COVID-19 patients,

1967	1971	1988	1994	2012	2015	2021
Publication of landmark article by Ashbaugh et.al describing ARDS in 12 adult patients based on the etiology of disease [11]	Petty and Ashbaugh et.al used "Adult" respiratory distress syndrome instead of "acute" probably to restrict disease prognosis to adults [5]	Murray et. al charted 4-point lung injury score to account for clinical manifestations of acute pulmonary damage [6]	American European Consensus Conference (AECC) defined ALI as a spectrum of pathological conditions and ARDS being the extreme end of the spectrum [7]	Berlin definition was applied to define ARDS based on four criteria: timing of the insult, origin, imaging or diagnostic status, and oxygenation level [8]	Riviello et.al proposed modification of the Berlin definition termed as Kigali modification, for responding to the patients in resource-limited settings [9]	Matthay et.al suggested to include patients exposed to HFNO (high-flow nasal oxygen), of at least 30 L/min and who fulfil the other criteria for the Berlin definition of ARDS [10]

Fig. 1 Overview of proposed refinements in ARDS definition (data collected from references [5–11]).
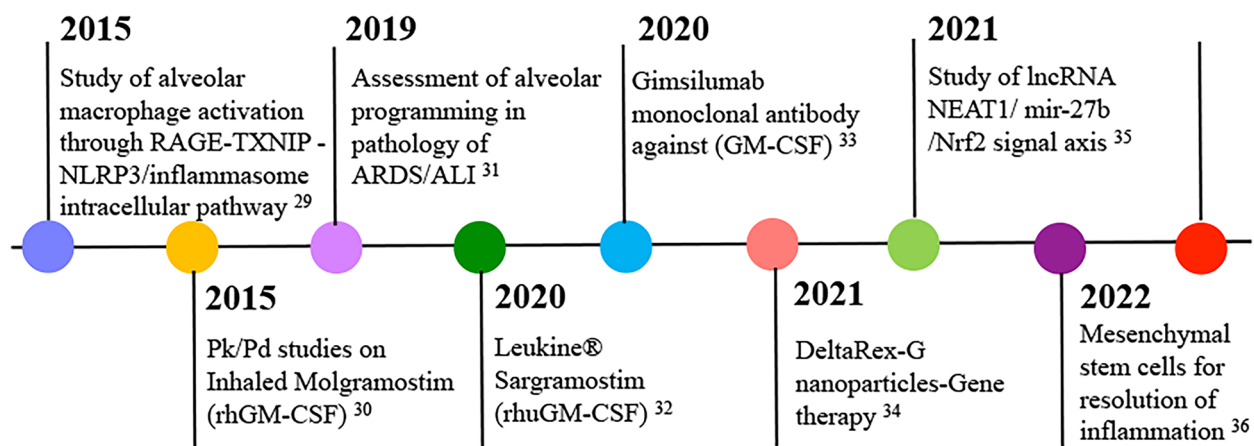
Fig. 2 Timeline of clinical trials oriented to explore role of macrophages in ARDS/ALI pathology (data collected from the following resources [29–36]). Abbreviations: RAGE, receptor for advanced glycation end-products; TXNIP, thioredoxin-interacting protein; GM-CSF, granulocyte–macrophage colony-stimulating factor; miR-27b, microRNA-27b; lncRNA, long non-coding RNA; Nrf2, nuclear factor-erythroid 2-related factor 2.

peripheral blood monocyte-derived macrophages made up the predominant macrophage subset as per single-cell RNA sequencing [40]. The purpose of this review is to address the potential of targeted nanomedicine to inhibit the molecular pathways behind excessive macrophage recruitment and inflammatory response, as well as the limitations associated with their clinical translation.

PATHOLOGY OF ARDS

Normal lung physiology comprises of a single layer of endothelial cells across the distal alveolar capillaries which facilitates carbon dioxide and oxygen exchange. The alveolar epithelium consists of flat alveolar type 1 cells (AT I) and cuboidal shaped alveolar type 2 cells (AT II) which contribute to the gas exchange and the formation of alveolar tight junctions [41]. Additionally, AT II cells secrete pulmonary surfactants necessary for alveolar compliance to prevent alveolar collapse due to increased surface tension. In conjugation with the lung endothelial injury, damage to these epithelium cells can lead to increased fluid accumulation in the alveoli triggering alveolar edema in ARDS/ALI. In normal physiology, the excess fluid from the alveolar airspaces is absorbed by the sodium channels and Na+/K+-ATPase pumps located on the AT I and AT II cells and transported into the lung interstitium. Then the fluid is cleared with the aid of the

lymphatic system and lung microcirculation. However, in ARDS pathology, the presence of edema in the lung interstitium and disruption of the tight junction barrier results in translocation and accumulation of fluid into the alveoli [42–44].

ARDS is determined by an injury to the alveolar-capillary unit characterized by three overlapping phases: exudative, proliferative, and fibrotic phases. Within 48 h, the exudative phase starts and lasts typically for over a week. This phase is characterized by the presence of pathological indications such as capillary congestion, the production of fibrin-rich microthrombus, interstitial and alveolar edema, intra-alveolar bleeding, necrotic death, and irregular endothelial alterations. At the end of the first week, the proliferative phase begins, resulting in production of exudates and proliferation of AT II cells into AT I cells and fibroblasts, ultimately leading to the fibrotic phase, marked by an increased collagen deposition and fibrosis in the lungs [45, 46].

In response to rapid acute inflammation, histological changes in the lungs revealed recruitment of blood leukocytes, activation of tissue macrophages, influx of neutrophils, and production of various mediators. Another major consequence is the disruption of endothelial linings, leading to increased alveolar-capillary permeability. Due to lung injury, alveolar macrophages (AM) and toll-like receptors (TLR) present on AT II cells are activated resulting in a chemokine storm into the airspaces inevitably leading to a second wave of

inflammation. The released chemokines recruit circulating immune cells into the airspace [47]. The versatile function of macrophages including both modulation of inflammatory responses and repair of damaged tissue is due to their highly plastic nature and different phenotypes [48]. Alveolar macrophages also produce interleukin 8 (IL-8) and epithelial neutrophil-activating protein (ENA-78) resulting in an increased neutrophil influx in alveolar airspace. Neutrophils present in edema fluid not only initiate the inflammatory response but also release protease, reactive oxygen species, and toxic mediators. Neutrophils along with platelets synergistically increase the vascular permeability of the proteins [49]. On the other hand, monocytes cause epithelial cell apoptosis due to TNF-related apoptosis-inducing ligand (TRAIL). Overall, these events result in disrupted endothelial and epithelial permeability resulting in airspaces filled with edematous fluids. Also, in patients suffering from ARDS, the mismatch of ventilation-to-perfusion rate results in atrial hypoxemia accompanied by dysregulation of right to left intrapulmonary shunting [50].

ORIGIN, POLARIZATION, AND FUNCTION OF MACROPHAGES

Macrophages are ubiquitously present in mammalian organs due to their phenotypic specialization and heterogeneity regulated in a tissue-specific manner [51]. During prenatal development, the first wave originates from the yolk sac precursors (embryonic F4 macrophages) which during embryonic development distributes rapidly throughout the lung interstitium. The second wave (embryonic Mac2 macrophages) initiates in the fetal liver, channeling fetal monocytes to egress towards the developing lungs, and shortly thereafter, hematopoiesis begins. The migrated cell eventually invades alveoli and sustains as alveolar macrophages (AMs). The third wave of macrophages is derived from hematopoietic stem cells in bone marrow where they are maintained as circulating progenitors for developing adult monocytes/macrophages during postnatal development [52]. Consequently, lung macrophages can be (1) alveolar macrophages that are primarily involved in phagocytosis and populated near the type 1 and type 2 epithelial cells of alveoli and (2) interstitial macrophages that participate in tissue remodeling and reside in the parenchymal tissue present between the alveolar epithelium and microvascular endothelium [53].

AMs are subjected to stimulus-specific reprogramming allowing them to initiate and resolve lung inflammation. However, AMs are not present as homogenous populations but rather exist in two subsets: resident and recruited macrophages [54]. During lung embryogenesis, the resident macrophages populate the lungs and have a slow turnover kinetic in the absence of inflammation. Their primary function is to maintain alveolar homeostasis and persist through the inflammatory cycle. Conversely, recruited macrophages accumulate in the alveolar spaces augmenting the inflammation and undergo apoptosis during resolution of the inflammation [38]. The decline in the recruited macrophages post inflammation is a result of Fas-mediated cell death programming mechanisms [55, 56]. This suggests macrophage kinetics is static for resident macrophages but dynamic for recruited macrophages. Once recruited, depending on the surrounding stimulus and pathophysiological conditions, they can be polarized into two distinct phenotypes: classically activated (M1) and alternatively activated (M2) depending on the microenvironment.

Proinflammatory macrophages (M1) polarization can be induced by molecules like lipopolysaccharide (LPS), Th1 proinflammatory cytokines like interferon γ (IFN-γ), and tumor necrosis factor-α (TNF-α). The M1 macrophages also stimulate the production of monocyte chemotactic protein 1 (MCP-1), interleukins (IL-1β, IL-6), reactive oxygen species (ROS), nitric oxide (NO), and reactive nitrogen species (RNS). This aspect of M1 is due to their induction by nitric oxide synthase (iNOS) expression. The M1 macrophages also differ from M2 in terms of their iron metabolism. M1 expresses high levels of ferritin, an iron storage protein, contrary to an iron exporter ferroprotein [57]. M1 macrophages play a role in tissue damage, initiation of inflammatory responses, radical formation, and antitumoral activities [58, 59]. The anti-inflammatory M2 phenotype is polarized by the Th2 cytokines like IL-4, IL-13, and IL-10. They are further divided into four subcategories, consisting of M2a, M2b, M2c, and M2d. In contrast to the M1 macrophages, M2 resolves inflammation by producing molecules like IL-10, transforming growth factor β (TGF-β), and chemokines like CCL17 and CCL18. M2 macrophages exhibit high levels of Arg-1 driving functions like tissue remodeling and cell proliferation [60]. The iron metabolism differs from M1 as the M2 expresses high levels of ferroprotein as compared to the ferritin molecule [57]. These are the key mediators in wound healing, resolving inflammation, phagocytosing debris, parasite clearance, and

facilitating tumor development. The remarkable plasticity of the macrophage is contingent on the damage/pathogen-associated molecular patterns, presence of cytokines, and other mediators in the lung microenvironment [53]. The specific differences between M1 and M2 macrophages are summarized and depicted in Fig. 3.

SIGNIFICANCE OF MACROPHAGES IN PATHOPHYSIOLOGICAL MECHANISMS OF ARDS/ALI

ARDS pathological processes can be divided into three phases–exudative, proliferative, and fibrotic phases–which involve damage to epithelial and endothelial linings resulting in increased vascular permeability and inflammatory responses. Here, we discuss each phase to scrutinize macrophage behavior and comprehend potential of targeted nanomedicines.

Exudative Phase

After the onset of respiratory failure, the exudative phase initiates within the first few hours or days. Pathologically, the following are observed: hemorrhage detected on the parenchymal surface, dilated alveolar ducts, increase in the lung weight (> 2000 g), presence of protein-rich edema, and damage to endothelial and epithelial barriers [61]. The distinctive histological changes of the exudative phase are disruption of the alveolar endothelial-epithelial barrier which allows the plasma proteins of the hyaline membranes to leak in the alveolar spaces commingling with the cell debris, presence of diffuse alveolar damage (DAD), neutrophilic alveolitis, reduced alveolar volume, and progression of microthrombi [62]. With regard to the proteomic differences, a lysosomal cysteine proteinase (cathepsin B) and heat shock protein 27 (HSP27) are upregulated during the exudative phase in response to the reduced
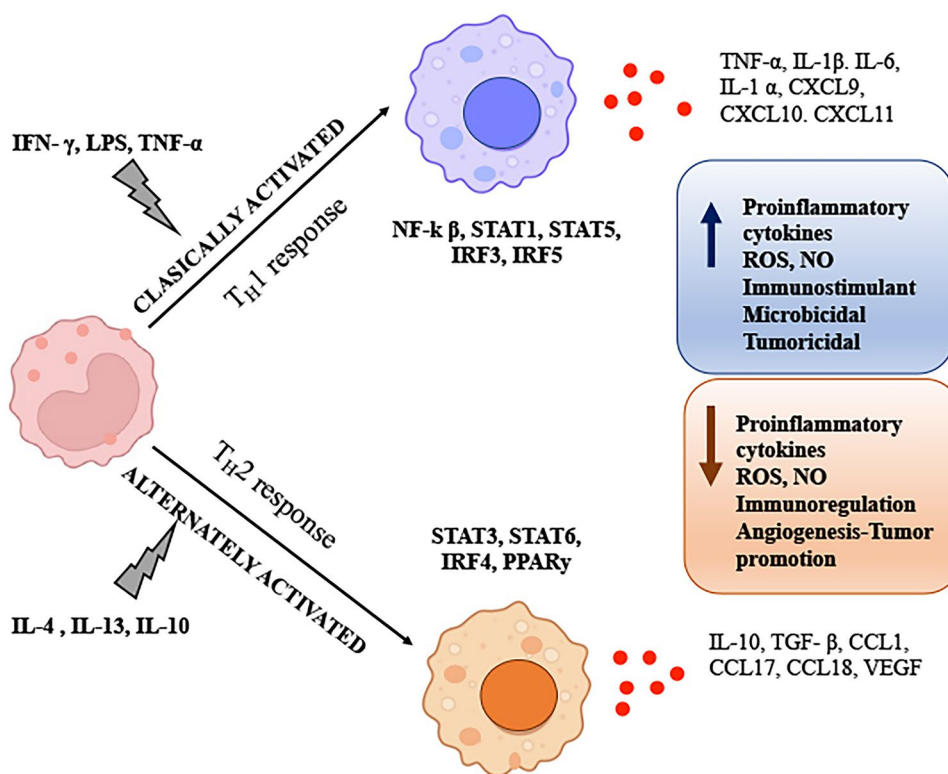
Fig. 3 Summarizes the different stimuli, released cytokines, and biological functions between M1 and M2 macrophages (image created using Pro-Create 5.2 software). Abbreviations: TNFα, tumor necrosis factor-alpha; CXCL, chemokine (C-X-C motif) ligand; TGF-β, transforming growth factor-beta; VEGF, vascular endothelial growth factor; CCL, chemokine (CC motif) ligand.

stability of lysosomal membrane and increased cytokine levels respectively [63, 64]. The lungs are infiltrated with inflammatory cells and the resident macrophages immediately polarize to the predominant M1 phenotype. During ARDS/ALI, M1 phenotype polarization is due to the infection-induced activation of TLR. Importantly, these resident macrophages form the first line of defense releasing various proinflammatory interleukins (IL) like IL-1β, IL-6, and IL-18 [65]. This brings about neutrophil recruitment from the intravascular spaces into the alveolar space channeling tissue damage in inflammatory diseases. Briefly, the polarization towards M1 macrophages is due to the binding of IFN-γ ligand to the cell surface is receptors activating Janus kinase 1 (JAK1) and JAK2, the dimerizing signal transducers, and activators of transcription 1 (STAT1) in JAK/STAT pathway [66, 67]. This homodimer later binds to the promoter of target M1 signature genes at the IFN-γ-activated site causing M1 polarization. The JAK/STAT signaling pathway leading towards polarization of M1 phenotype is downregulated due to feedback inhibition from suppressor of cytokine signaling (SOCS1 and SOCS3) [68]. In another study, lower expression of transcription factor (IRF5) correlates with a decreased proportion of the M1 phenotype subset [69]. Overall, a decreased macrophage population with M1 phenotype could attenuate lung damage and protect against ARDS/ALI. On the contrary, the recent finding indicated the potential of M1 macrophages to upregulate amphiregulin, which demonstrates protective epithelial barrier properties [37].

Proliferative/Rehabilitation Phase

After the clinical onset of ARDS, the proliferative phase can begin as early as 3 days and is characterized by the elimination of pathogenic factors, organization of the intra-alveolar structure, and accumulation of proliferating alveolar type 2 cells, fibrin, and collagen in the alveolar air spaces. During the proliferative phase, the epithelial cell linings regenerate along the denuded alveolar walls marked by the presence of the keratin-rich cuboidal cell rows apparent in the histopathological sections. The levels of cathepsin B and HSP27 are significantly downregulated during this phase. However, the neutrophil elastase (NE) inhibitor is upregulated, resulting in the suppression of cytokines by lowering inflammatory mediators [70]. In this phase, the M2 phenotype predominates as

the resident and the recruited macrophages are polarized from the M1 to M2 phenotype. The anti-inflammatory effect of macrophages is exerted by clearing apoptotic neutrophils and cell debris, thereby alleviating lung inflammation. [4]. The phagocytic activity of M2 macrophages to clear necrotic waste is called efferocytosis, which further promotes anti-inflammatory signaling. The process of efferocytosis is also increased due to the upregulation of the mannose receptor in the presence of M2-derived cytokines [71]. As the M2 phenotype limits the levels of proinflammatory cytokines and inducible nitric oxide synthase, it also enhances the expression of TGF-β-induced matrix-associated proteins BIG-H3, arginase 1, fibronectin 1, and IL-10. The anti-inflammatory effect is also exerted due to the reduced nitric oxide production [72]. The M2 polarization is dictated by several pathways. For instance, regulatory T cells (Tregs) in monocytes produced minimal cytokine production and inhibited proinflammatory responses in a lipopolysaccharide challenge assay [73]. Another study highlighted decreased expression of IL-6 and nitric oxide with an increased expression of mannose receptor and arginase due to decreased expression of suppressor of cytokine signaling 3 (SOCS3). This in turn promotes M2 polarization. The LPS-induced ALI was attenuated due to the administration of glucocorticosteroid (methylprednisolone) as it increased the polarization of M1 towards the M2 phenotype [74]. Additionally, peak expression of activated M2 marker (transferrin receptor) was observed during the resolution of lung injury indicating an increased M2 macrophage subpopulation. Overall, based on the reported data, the M2 polarization mitigates inflammatory conditions evident during ARDS/ALI [4, 37].

Fibrotic Phase

Fibrosis is apparent as early as 10 days after the onset of ARDS. The lung pathology is completely remolded presenting irregular zones of scared tissue, diffused cellular collagenous tissue, dispersed areas of microcystic airspaces, fibrosis in the alveolar ducts, and an overall increase in the total lung collagen levels. This phase develops in prolonged ventilator-dependent patients and is characterized by fibroblast proliferation. During the fibrotic phase, the activated M2 macrophages release anti-inflammatory molecules like IL-10 and TGF-β which counter the Th1

cytokine-induced inflammatory process. The TGF-β molecule promotes the formation of extracellular matrix components (ECM) and thus excessive deposition of the ECM is a characteristic feature of pulmonary fibrosis. Furthermore, alternate activation of macrophages promotes secretion of fibronectin, an ECM component. Arginase (Arg-1), an M2 macrophage-associated molecule, metabolizes L-arginine into l-proline, l-ornithine, and polyamine. It can promote collagen formation by myofibroblasts ultimately leading to fibrosis [75]. A hallmark of fibrogenesis is due to the steady expression of IL-4 and IL-13 which causes the persistent presence of M2 macrophages in the fibrotic phase [8]. On the other hand, M1 macrophages are involved in the production of matrix metalloproteinases (MMPs) which are associated with the resolution of fibrosis by activating ECM matrix degradation [76]. Overall, the balance between M1 and M2 macrophages in the inflammatory microenvironment is involved in the progression and alleviation of fibrosis.

NANOPARTICLES TARGETING MACROPHAGES FOR ARDS/ALI

The unmet medical need of targeting signaling pathways responsible for the inflammatory conditions developed during ARDS/ALI pathology can be addressed by the wide range of nanomedicines. As macrophages are involved in all the stages of ARDS/ALI pathology, drug targeting through nanodelivery systems is a useful strategy (Fig. 4).

In a recent clinical study, Schwartz et al. [77] found that patients without lung injury had significantly lower ($p < .02$) activation of nuclear factor-kappa B (NF-kappa B) in alveolar macrophages as compared to patients with established ARDS. This increased expression of NF-kappa B can be triggered by oxygen radical-dependent mechanisms. The oxidative stress could result due to hemorrhage, prolonged exposure to hyperoxia treatment, and other conditions associated with ischemia–reperfusion in ARDS patients [78]. Other mechanisms for activation of
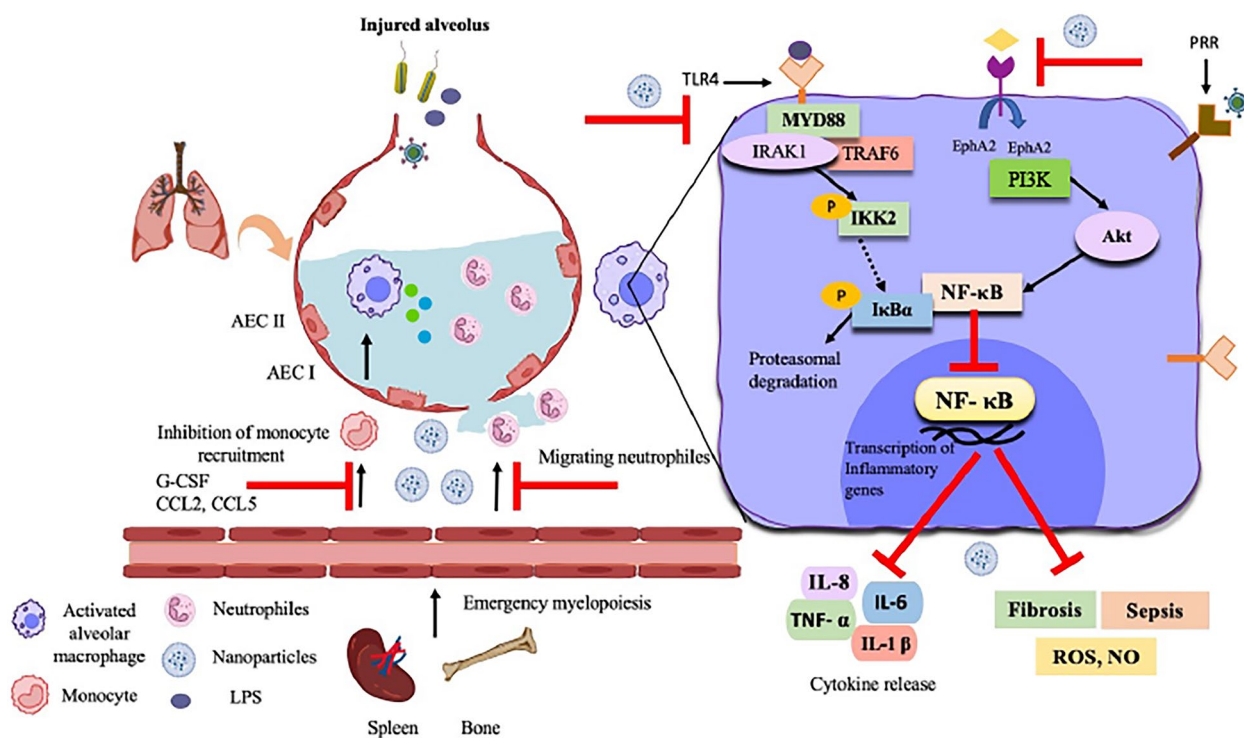
Fig. 4 Outlines the general mechanism of ARDS/ALI pathology, the role of macrophages, and approaches for ARDS treatment (image created using ProCreate 5.2 software). Abbreviations: MYD88, myeloid differentiation primary response 88; IRAK1, interleukin 1 receptor-associated kinase 1; TRAF6, TNF receptor-associated factor 6; PI3K, phosphoinositide 3-kinases; AKT, protein kinase B; PRR, pattern recognition receptor.

NF-kappa B could be LPS or another microbial ligand-stimulated toll-like receptor 4 (TLR4). Activated NF-kappa B critically upregulates gene expression of cytokine (IL-1β, IL-6, IL-8) and production of superoxide (SOD), hydrogen peroxide, and inflammatory genes including TNF-α [79] and cyclooxygenase 2 which are linked with proinflammatory M1 macrophage activation.

The inhibition of the NF-kappa B-activated inflammatory pathway in alveolar macrophages was studied by Niemiec *et al*. [80] by an intratracheal delivery of earth-based cerium oxide nanoparticles (CNP) conjugated with miR146a (anti-inflammatory miRNA). The CNP demonstrated protection against ARDS associated with coronavirus and ALI by further inhibiting transforming growth factor-beta (TGF-β), a key component leading to lung fibrosis. Additionally, cerium oxide (CeO$_2$) due to its multivalent oxidation state confers antioxidant potential and offers a stable delivery system to pharmacokinetically unstable biologics like microRNAs. Ma *et al*. [81] showed the application of simple polyethylene glycol-coated GNPs to LPS-induced RAW 264.7 macrophages not only down streamed NF-kappa B but also curbed the overproduction of nitric oxide (NO) by suppressing iNOS expression. Corroborating with the previous study, dos Santos Haupenthal *et al*. [82] observed lowered levels of proinflammatory cytokines (IFN-γ and IL-6), NO, and SOD and reversal of LPS-induced fibrosis on intraperitoneal administration of the GNPs. Wang *et al*. [83] developed gold nanoparticles (GNPs) coated with hexapeptides for inhibition of TLR4 receptor in LPS-induced ALI model. This bioactive nanoparticle can be easily phagocytosed by alveolar macrophages to attenuate TLR4 signaling cascades, thereby inhibiting NF-kappa B to reduce inflammation and promote M2 polarization. The size dependency of GNPs to inhibit TLR4 was studied by Gao *et al*. [84], who demonstrated potent inhibitory activity of GNP core of 20 nm (G20) as compared to smaller size GNPs (G13). In addition, G20 resulted in extended tolerance to endotoxins, reduced lethal effects due to LPS challenge, and decreased cytokine activation (CCL2, CCL4).

The lung targeting capability of NPs was explored by Li *et al*. [85], where surfactant protein A nanobodies (SPANbs) were functionalized on nano-sterically stabilized unilamellar liposomes loaded with methylprednisolone. This theranostic nanoparticle utilized a targeting moiety surfactant protein A which is rarely present on the extrapulmonary organs, but it is overexpressed on type II alveolar epithelial cells. The presence

of glucocorticoid-like methylprednisolone lowered the expression of NF-kappa B due to its anti-inflammatory properties in rats with bleomycin-induced ALI. Furthermore, this group also elucidated on the targetability of these NPs to specifically bind to human lung tissue but not to the human spleen, liver, and kidney tissues [86]. Wijagkanalan *et al*. [87] developed an intrathecally delivered dexamethasone palmitate (DP) encapsulated mannosylated cholesterol-based liposome which has the potential to target the mannose receptors expressed on the alveolar macrophages. This nanoparticle significantly suppressed the activation of NF-kappa B and mitogen-activated protein kinase (p38MAPK) signaling pathways resulting in decreased cytokine release and apoptosis as compared to the free drug and bare liposome. One of the extrapulmonary factors contributing for ARDS is the development is severe sepsis. Spence *et al*. [88] studied targeting Siglecs, sialic acid-binding immunoglobulin-like lectin-E receptors, which are expressed on macrophages and are capable of inhibiting TLR signaling. During ARDS, Siglec-E activation also regulates neutrophil infiltration. In this study, activation of Siglec receptors limited the activation of TLR and subsequently NF-kappa B by using a poly(lactic-co-glycolic acid) nanoparticle consisting of di(α2 → 8) N-acetylneuraminic acid. This sialic acid-decorated nanoparticle can abrogate inflammation and sepsis during pulmonary inflammatory conditions like ARDS. COVID-19-associated pneumonia can advance to ARDS due to exacerbated cytokine storm in the lungs. Ding *et al*. [89] maneuvered RBC hitchhiking to improvise lung targetability of chitosan nanoparticles loaded with methylprednisolone sodium succinate (MPSS). These NPs exhibited prolonged residence time after an intravenous administration, thereby decreasing pivotal cytokines like TNF-α and IL-6.

Recently, the effect of perfluorocarbons (PFCs) on the release of the primary inflammatory mediator (IL-6) has been studied on LPS-induced macrophages. It is proposed that PFCs resolve the inflammation by lowering IL-6 and thereby suppressing prostaglandins [90]. Hou *et al*. [91] developed a perfluorocarbon (PFC) containing emulsion which improved lung function by lowering cytokine release, polymorphonuclear neutrophils (PMN) activation, and improving increased arterial blood PaO$_2$. During ALI/ARDS pathology, overexpression of chemokine receptor type 4 (CXCR4) and upregulation of plasminogen activator inhibitor-1 (PAI-1) lead to migration of fibrocytes and macrophages, leading to chronic fibrosis and inflammation respectively. For combined inhibition, Wang *et al*.

[92] designed a PFC containing nanoemulsion as gene carriers to deliver fluorinated polymeric CXCR4 antagonist (siRNA) that silenced the expression of the plasminogen activator inhibitor-1 (PAI-1) to alleviate ALI induced by LPS. Another advantage of nanoemulsions is their capability to deliver bioactive oils. A nanoemulsion containing oil from Pequi (PE), a Brazilian fruit, was studied by de Sá Coutinho [93] for its anti-inflammatory properties. As compared to the free PE oil, nanoemulsion containing Pequi oil (PE-NE) reduced the influx of macrophages and leukocytes into the bronchoalveolar fluid. The stable PE-NE with an average particle size distribution around 220 nm also reduced myeloperoxidase (MPO), an indicator of polymorphonuclear-leukocyte (PMN) and cytokines like IL-1β and IL-6.

Jin *et al.* [94] synthesized sialic acid (SA)-modified lung-targeted microsphere (MS) loaded with antioxidant triphenylphosphonium cation (TPP)-modified curcumin (Cur-TPP). Curcumin, a natural radical scavenger, most likely affects the NF-kappa B pathway to mediate its anti-inflammatory properties. The TPP-based nanoparticle system easily penetrates the mitochondrial double-layer hydrophobic membranes. An increased ROS production during ARDS/ALI can inadvertently activate caspase 3 apoptotic factor leading to apoptosis of mitochondria in macrophages. The therapeutic potential of this microsphere system is to localize in mitochondria and to reduce ROS stress. Kim *et al.* [95] loaded hydrophobic curcumin in cholesterol-conjugated polyamidoamine (PamChol-Cur) complexed with heme oxygenase-1 (HO-1) pDNA. This combined drug and gene delivery nanomicelle complex exhibited higher gene transfection efficiency and pronounced anti-inflammatory effect as compared to the free drug and PamChol-Cur. The same micelle carrier system was utilized to encapsulate resveratrol (RSV), a polyphenol phytoalexin, exerting its action by inhibiting transcription factor NF-kappa B [96]. de Oliveira *et al.* [97] studied the anti-inflammatory action of RSV in LPS-induced ALI by orally administering lipid-core nanocapsules with an encapsulation efficiency of more than 99% for RSV. In contrast to the unloaded nanocapsules, RSV-loaded nanocapsules targeted TLR4-activated inflammatory molecular signaling pathways like lipid kinase phosphoinositide-3-kinase (PI3K/Akt) and MAPK.

In recent years, there has been an emphasis on developing nanosized lung-targeted drug delivery systems that exploit unique features of inflammatory microenvironments like low pH conditions, elevated temperatures, and specific enzyme-rich environments [98]. In ARDS/ALI, neutrophil recruitment at the site of inflammation is triggered as a response to the chemokines released by the activated macrophages [99]. Also, the preferential expression of intercellular adhesion molecule 1 (ICAM-1) on lung epithelial cells is studied as a lung targeting strategy. Neutrophil transmigration is promoted as the leukocyte-specific adhesion molecule (integrin β2) binds to the ICAM-1. Zang *et al.* [100] developed NPs composed of poly(β-amino esters) (PAE), a sharp acid-sensitive segment as the core and polyethylene glycol (PEG)-biotin for ease of bioconjugation. The NPs were further loaded with TPCA-1, a selective inhibitor of IκB kinase-2 (IKK-2) and for targeting conjugated with anti-ICAM-1 antibodies. This pH-responsive targeted nanotherapeutic increased the efficacy of TPCA-1 as it accumulated fivefold higher compared to the free drug. Another group developed simvastatin-loaded nanostructured lipid carriers (NLCs) using a similar lung targeting strategy to attenuate the proinflammatory mediators like TNF-α and IL-6 [101].

Nanoparticles can be fabricated to achieve a startling degree of complexity. Sadikot *et al.* [102, 103] used a micelle as part of a three-pronged approach to engage an anti-inflammatory response. Glucagon-like peptide-1 (GLP-1), triggering receptor expressed on myeloid cells 1 (TREM-1), and drugs such as 17-AAG, an inhibitor of heat shock protein 90 (Hsp90), were sterically stabilized micelles of around 15 nm. This system improved the short half-life of peptide drugs to exert actions like inhibition of NF-kappa B, TREM-1, and production of ROS. Bleomycin-induced lung injury upregulates ephrin type-A receptor (EphA2) [104], leading to increased vascular permeability and PI3K/Akt/NF-κB-dependent inflammatory processes. To downregulate EphA2 activation, Patil *et al.* [105] developed a PLGA polymeric NP functionalized with a YSA peptide which mimics the ephrin ligand. To facilitate future research on applications of nanomedicines in ARDS/ALI, we summarized recent examples in Table 1. Each example is presented with its specific molecular and cellular targets, specific model, and measured outcomes.

POTENTIAL CHALLENGES FOR CLINICAL TRANSLATION OF NANOPARTICLES

Though promising in pre-clinical studies for both their efficient and targeted delivery of therapeutic agents, nanoparticles also present with specific challenges needed to be addressed before their clinical translation (Fig. 5)

Table 1 Summary of Pre-clinical Studies on Nanoparticles, Drug/Gene Encapsulated, Mechanism of Action, Size (nm), Route of Administration, and the Potential Biological Effect on ARDS/ALI Pathology

	Nanosystem (type)	Drug/gene	Mechanism	Size (nm)	Route	Cell type/animal model	Therapeutically outcome measures	Ref
1	Cerium oxide NPs	miR146a, anti-inflammatory miRNA	Inhibits TRAF6, IRAK1, promoters of NF-κB	~190 nm	Intratracheal delivery	Bleomycin-induced ALI	↓NFκB, IL-6, IL-8, TNFα, ↓TGFβ	[80]
2	PEG-coated Gold NPs		Inhibition of iNOS gene Inhibition of PI3K/Akt pathway	10–15 nm		LPS-stimulated RAW264.7 macrophages	↓ NO, ROS, cytokines, ↓COX-2	[81]
3	Peptide-coated GNPs	Hexapeptide coated CLPFFD	Inhibits LPS-induced TLR4 activation	13.0 ± 0.4 nm	Intratracheal delivery	LPS-induced ALI	M2 polarization ↓IL-6, IL-8 ↑G-CSF, IL-4, IL-3	[83]
4	GNPs	Peptide coated CLPFFD	Inhibits LPS-induced TLR4	~20 nm	Intratracheal delivery	LPS-induced ALI	↓ IL-6, IL-8, ↓CCL2, CCL4 ↓Neutrophils ↑ LPS tolerance	[84]
5	Unilamellar liposomes	Methylprednisolone (MPS) Surfactant protein A antibody	Reduces the expression of NF-κB	106 nm	Intravenous delivery	Bleomycin-induced ALI in rats	↓NFκB, IL-6, IL-8, TNFα	[85]
6	Mannan-coated liposomes	Dexamethasone palmitate	Reduces the expression of NF-κB, p38 MAPK	~100 nm	Intratracheal delivery	LPS-induced ALI in rats	↓ IL-6, IL-8, ↓Neutrophils, ↑mannose mediated targeted uptake	[87]
7	PLGA-NPs	di(α2 → 8) N-acetylneuraminic acid	Regulates Siglec-E receptors	150 nm	Intraperitoneal delivery	LPS-induced ALI in rates	↓Sepsis, ↓Neutrophils, ↓TNFα	[88]
8	Chitosan NPs (medium molecular weight)	RBC hitchhiking Methylprednisolone sodium succinate	Reduces the expression of NF-κB	233.3 nm	Intravenous delivery	LPS-induced ALI in rates	↓IL-6 ↓TNFα ↓MPO	[89]
9	Nanoemulsion	Perfluorocarbon perfluorooctyl bromide; C8F17Br	Downregulated CD11b to reduce PMNs	180–160 nm	Intravenous delivery	LPS-induced ALI in rates	↓CD11b, ↓Neutrophils, ↑PaO2	[91]
10	Nanoemulsion	Fluorinated CXCR4 antagonist (F-PAMD)	Combined inhibition of CXCR4 and PAI-1	170 nm	Intratracheal delivery	Bleomycin-induced ALI in mice	↓MPO ↓Neutrophil infiltration	[92]
11	Nanoemulsion	Pequi oil	Curtails air hyperactivity	174–223 nm	Oral delivery	Intranasal LPS delivery	↓MPO, keratinocyte-derived chemokines, IL-6, IL-1β	[93]
12	Sialic acid-functionalized PEG-PLGA microspheres	TPP-Curcumin	Inactivation of caspase 3	250 ± 9.16 nm	Intravenous delivery	LPS-induced ALI in mice	Mitochondria-targeted ↓ROS, ↓ proinflammatory cytokines	[94]

Table 1 (continued)

	Nanosystem (type)	Drug/gene	Mechanism	Size (nm)	Route	Cell type/animal model	Therapeutically outcome measures	Ref
13	Cholesterol-conjugated polyamidoamine micelle	pDNA HO-1, Curcumin	Reduces the expression of NF-κB	120 nm	Intratracheal delivery	LPS-induced ALI in mice	↓COX-2, ↓Prostaglandins, ↓NO	[95]
14	Cholesterol-conjugated polyamidoamine micelle	pDNA HO-1, Resveratrol	Reduces the expression of NF-κB	120.4 ± 20.6 nm	Intratracheal delivery	LPS-induced ALI in mice	↓COX-2, ↓Prostaglandins, ↓NO	[96]
15	Lipid-core nanocapsules	Resveratrol	Blockage of the ERK and PI3K/Akt pathways	241 ± 7 nm	Oral delivery	LPS-induced ALI in mice	↓Leukocyte accumulation ↓IL-6, KC, MIP-1α, MIP-2	[97]
16	Poly (β-amino esters) polymeric NP	Anti-ICAM-1 antibody TPCA-1, selective inhibitor of IκB kinase-2	Targeted delivery to endothelia	100 nm	Intravenous delivery	LPS-induced ALI in mice	↓IL-6, KC, MIP-1α, MIP-2	[100]
17	Nano-based lipid carriers	Simvastatin, anti-ICAM-1 antibody	Downregulate MAPK signaling	337 nm	Intravenous delivery	LPS-induced ALI in mice	↓cytokines, ↓alveolar wall thickening	[101]
18	Micelle	GLP-1, TREM-1 peptides 17-AAG, an inhibitor of Hsp90	Inhibition of NF-κB, TREM-1	~15 nm	Subcutaneous injections	LPS-induced ALI in mice	↓NF-κB, IL-6, IL-8, TNFα	[103]
19	PLGA polymeric NP	Downregulate EphA2 activation	YSA peptide (YSAYPDSVPMMS)	219–279 nm	Tail vein injection	Bleomycin-induced ALI	↑vascular permeability	[105]

TRAF6 TNF receptor-associated factor 6, *IRAK1* interleukin 1 receptor-associated kinase 1, *NFκB* nuclear factor-kappa B, *TNFα* tumor necrosis factor α, *IL* interleukins, *ROS* reactive oxygen species, *TLR4* toll-like receptor 4, *G-CSF* granulocyte colony-stimulating factor, *p38 MAPK* p38 mitogen-activated protein kinases, *CD11b* type I transmembrane glycoprotein, *CXCR4* C-X-C motif chemokine receptor 4, *PAI-1* plasminogen activator inhibitor 1, *MPO* myeloperoxidase, *MIP-2* macrophage inflammatory protein 2, *TREM-1* triggering receptors expressed on myeloid cells-1
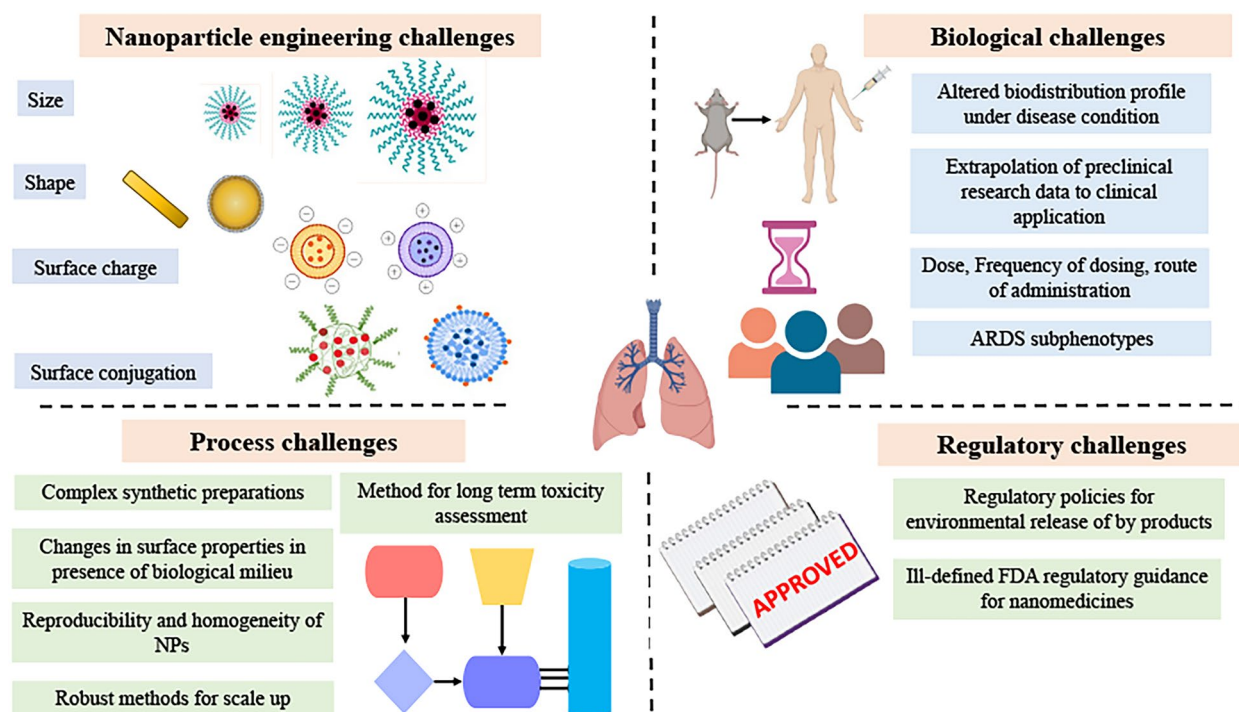
Fig. 5 Summary of the potential challenges for commercial translation of nanoparticles (image created using ProCreate 5.2 software).

[106]. Here, we summarize key considerations for the successful design of clinically viable nanoparticles as future nanomedicines.

Nanoparticle Size

The optimal size of nanoparticles (NPs) is determined by the location and type of targeted tissues [107] as it influences *in vivo* distribution, biological fate, toxicity, drug loading, drug release, stability, and targeting ability of the system [108]. Chen *et al*. [109] in their studies demonstrated that NP uptake is organ-specific and size-dependent in the presence of inflammatory conditions. In literature, ambiguity exists as to what should be the optimum size for prolonging the lung retention time. Although Huang *et al*. [110] and Kreyling *et al*. [111] reported positive correlation between the nanoparticle size and lung retention time, Anderson *et al*. [112] discovered an inverse relationship for silver nanoparticle size. Additionally, the size of alveolar diameter alters based on age and gender dictating the distribution of the NPs in the lungs [113, 114].

Macrophages play a crucial role in particle clearance, and while small-sized NPs induce potent macrophage influx required for macrophage targeting, they result in fewer ligand to receptor interactions as compared to large particles [112, 115]. It must be noted that NP size is also contingent on the route of administration. Lung delivery via inhalation is subject to different constraints in comparison to intravenous delivery. The major challenge during pulmonary targeting via intravenous delivery is the rapid bloodstream clearance facilitated by mononuclear phagocytes [116–118]. Typically, nanoparticles ranging from 50 to 500 nm deposit optimally in the alveolar region [119].

Another constraint while tuning nanoparticle size is the cell type targeted. For instance, the uptake of unmodified polystyrene NPs of 50 nm by type I alveolar epithelial cells was more effective as compared to 100 nm particles [120]. However, because macrophages can engulf larger particles (1 and 5 μm) due to their remarkable phagocytotic capacity, small-sized particles (< 100 nm) may remain unrecognized [121]. Accumulation in secondary organs depends on the size of NPs as larger particles (> 200 nm) tend to aggregate in the spleen and

liver resulting in toxicity [117], while NPs below 6 nm in diameter are quickly excreted by the body diminishing their therapeutic efficacy.

Nanoparticle Shape

For the design and performance of NPs, shape is a critical property as it influences the size, surface chemistry, and surface area. Additionally, it also influences cellular uptake as the highest degree of uptake was reported for rods, followed by spheres, cylinders, and cubes [122]. The shape of NPs alters their potential orientations and how they interact with cell surface receptors. It should be noted that while size and shape are different factors affecting NP behavior, they are interdependent [55]. The shape of NPs also influences circulation time in the body, with rod-shaped micelles having a circulation time ten times longer than their spherical counterparts. Rods may be an ideal shape due to their higher aspect ratio for some applications, but perhaps increased cellular uptake and longer circulation times may not be ideal due to delayed lung clearance [123, 124]. As a result of all these factors, shape selection is critical to the development of an effective ARDS/ALI lung-targeted nanomedicine system.

Nanoparticle Surface Properties

NP surface chemistry is influenced by the type of serum proteins adsorbed onto the surface and the strength of that interaction [125, 126]. The classical strategy to alter the surface charge is the functionalization of NPs by PEGylation, which evades immune detection, thereby reducing the phagocytotic ability of macrophages [127]. Previous studies have also underlined that the interactions and uptake profiles of nanoparticles are different for M1 and M2 macrophage subpopulations. For instance, the uptake of non-PEGylated nanoparticles was higher in classically activated M1 macrophages as compared to the M2 macrophages [128]. The surface topology not only allows for cell-specific targeting but also governs the nanoparticle clearance [129]. Cell specificity can be achieved by targeting receptors such as folate, opsonic CD16, and mannose receptors preferentially expressed on macrophages [130]. The receptor-mediated macrophage uptake can be tempered through surface modifications, for example, the folate ligand-conjugated nanoparticles preferentially accumulate in activated macrophages due to the upregulation of folate receptor (FR-β) on the surface [131]. Hattori *et al*. [132] developed a lipid-based folate NP conjugate carrying NF-kappa B decoy peptide that targeted

FR-β receptors on activated RAW 264.7 macrophages. Similarly, macrophage uptake is being evaluated by developing mannosylated NPs to target mannose receptors or by conjugation with surface ligands that target the peripheral benzodiazepine receptors [133, 134]. In contrast, certain surface modifications of NPs can reduce macrophage uptake. Qie *et al*. [135], in his study, demonstrated lower uptake resulting due to predominant phagocytosis inhibitory signals of the surface-conjugated recombinant CD47 protein NPs [136]. The surface charge must also be considered when designing a drug delivery system. Typically, positively charged NPs are taken up at a much faster rate as compared to those with neutral or negative charges [116], as this is driven by electrostatic interactions between the NPs and the cell membrane (negatively charged). However, positively charged NPs are also cleared quickly from the blood and lead to therapeutic inefficacy [137].

ARDS Phenotypes Impact on Therapeutic Response

ARDS/ALI occurs as a constellation of etiologies and pathologies that lead to complex biological and clinical heterogeneity [138]. As we lack the empirical data to untangle this heterogeneous disease, clinical translation of nanoparticles remains a challenge. Calfee *et al*. [27] after careful inspection identified two different phenotypes: phenotype 1 characterized by less severe inflammation and shock, and conversely, phenotype 2 resulting due to hyperinflammation and severe acidosis. Consequently, mounting evidence suggests that the treatment responses are predicted depending on these phenotypes [139–141]. For instance, secondary analysis of a multicenter HARP-2 simvastatin clinical trial revealed that patients with a hyperinflammatory profile exerted a statistically significant 28-day survival advantage [142]. Similarly, disparate clinical outcomes to systemic corticosteroids were observed based on phenotypes [143]. Taken together, one can anticipate comparable challenges while predicting responses to nanomedicines. Thus, the benefit of nanoparticles tailored to predict the distinct cellular milieu and precisely deliver therapeutics remains challenging, but also represents the next revolution in ARDS research.

Timing of Nanoparticle Therapeutic Intervention

Another intriguing fact is that a patient developing ARDS due to H1N1 influenza will have different

underlying pathophysiology and may not present the same risk factors, onset, and duration of disease as a patient with transfusion-associated ARDS [144]. The timing of ARDS onset holds a prognostic value [144], as it determines the optimal period for nanomedicine administration. Studies have shown rapidly resolving ARDS has better outcomes and survival chances as compared to late resolving (> 48 h) conditions [145, 146]. These constraints should be accounted while developing nanomedicines targeting the early phases of ARDS pathology as the time window for targeting the influx of macrophages may be even narrower. A research comparing intranasal and intravenous delivery of simple polydopamine NPs reported that intranasal treatment resulted in higher accumulation in the lungs and a more favorable anti-inflammatory impact [147]. However, this may not always hold true for nanoparticle systems involving complex engineering like RBC hitchhiking and specified cell targeting processes, where intravenous administration may be preferable [89].

CONCLUSION AND FUTURE DIRECTIONS

The advent of nanotechnology has opened new avenues for the development of novel therapeutic strategies for the treatment of ARDS/ALI that can utilize targeting macrophage-specific inflammatory pathways. Nanoparticles can deliver therapeutic and/or diagnostic cargo to the activated macrophages, specifically targeting receptors preferentially expressed on macrophages, producing unique anti-inflammatory and immunomodulatory effects, and circumvent the pulmonary barriers for increased therapeutic efficiency. Currently, there is no nanomedicine-based therapeutic fully approved for ARDS/ALI treatment on the market. However, patents on utilization of theranostic nanoparticles composed of inorganic materials such as iron oxide (WO2016007194A1, 2016) and gold (KR101873840B1, 2018) for real-time assessment of inflammation and macrophage trafficking have been filed. In this review, we presented multiple macrophage-specific targets and nanomedicines applicable to macrophage drug delivery. Our aim was to highlight the underutilized potential of nanomedicine for ARDS/ALI therapeutic development. We hope that with the advancement of nanotechnology manufacturing and implementation of quality by design methodologies, ARDS/ALI nanomedicine can become a clinical reality in the very near future.

AUTHOR CONTRIBUTION

AVAILABILITY OF DATA AND MATERIALS

DECLARATIONS

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