Signal Transduction and Targeted Therapy www.nature.com/sigtrans

REVIEW ARTICLE OPEN

Precision nanomedicine for pulmonary diseases: from molecular targeting to clinical translation

Zicheng Deng[1]✉, Wen Gao[1], Jonathan Do[1], Shravya Aragam[1], Orshina Dawd[1], Meghana Belthur[1], Donglu Shi[2,3], Tanya V. Kalin[1,4] and Vladimir V. Kalinichenko[1,5]✉

The pulmonary system is a vital interface between the body and the external environment, making it highly vulnerable to environmental, infectious, and genetic insults. Precision nanomedicine offers a promising strategy to overcome the limitations of conventional gene and drug therapies, including safety concerns associated with viral vectors, instability of therapeutic agents, suboptimal cellular internalization, and a critical lack of tissue- and cell-specific targeting. Nanoparticle-based delivery platforms address these challenges by enhancing therapeutic stability and bioavailability, enabling controlled release, facilitating cellular uptake and endosomal escape, and achieving targeted delivery to specific lung compartments. While recent literature often focuses on specific nanoparticle types or isolated pathologies, this work provides a comprehensive overview of the current state of respiratory nanomedicine, bridging fundamental nanoparticle bioengineering with a wide range of pulmonary pathologies and the obstacles to clinical translation. We discuss the key physicochemical properties of nanoparticles for pulmonary biomedical applications, along with advanced design strategies for targeted delivery. Given the unique architecture and physiology of the lung, we compare the advantages and limitations of pulmonary versus systemic administration routes, emphasizing context-specific delivery strategies. Nanoparticle design and therapeutic applications are explored across a broad spectrum of diseases, including pulmonary fibrosis, chronic obstructive pulmonary disease, infections, pulmonary vascular disease, cystic fibrosis, asthma, lung cancers, and neonatal pulmonary disorders. Finally, we evaluate the current status of clinical trials, highlighting translational challenges such as biological barriers, long-term safety, and manufacturing. Future perspectives and interdisciplinary strategies are proposed to advance the clinical translation of nanocarriers for respiratory diseases.

Signal Transduction and Targeted Therapy (2026)11:358 ; https://doi.org/10.1038/s41392-026-02982-0

INTRODUCTION

The respiratory system is a unique interface between the human body and the external environment. Characterized by extraordinary structural complexity and functional diversity, it exhibits a distinctive pattern of disease susceptibility that distinguishes respiratory pathology from other organ system pathologies. This system comprises a diverse array of specialized cell types organized across distinct anatomical compartments, including the trachea, conducting airways, pulmonary vasculature, and the highly vascularized peripheral alveoli where gas exchange occurs.[1] This complex organizational structure, while essential for efficient exchange of gases between the air and peripheral blood, creates multiple points of vulnerability where pathological processes can emerge. The respiratory tract's direct and continuous exposure to the external environment makes it uniquely susceptible to injury from airborne pathogens, environmental toxins, and physical trauma, distinguishing it from other organ systems that benefit from protective physical barriers.[2] The lung plays a central role in maintaining physiological homeostasis through oxygen delivery and carbon dioxide removal. However, this key function requires constant exposure to environmental agents, creating an inherent tension between functional necessity and disease susceptibility. The diversity of cells in the epithelial, endothelial, hematopoietic, and mesenchymal compartments means that damage to one type can lead to a pathological cascade that ultimately hampers respiratory functions or leads to death. Therefore, a better understanding of pulmonary physiology and pathology is essential for successful development of therapeutics.

Despite remarkable advances in therapeutic development over the past decade, gene and drug therapies for pulmonary diseases still face barriers that significantly limit their clinical translation and therapeutic efficacy. These challenges include fundamental issues related to the stability and safety of therapeutic agents, the mechanisms of cellular internalization, and the specificity of tissue and cell targeting.[3] Gene therapy approaches have demonstrated remarkable promise for treating inherited and acquired pulmonary diseases; however, viral-based delivery systems like adeno-associated viral (AAV) vectors present substantial safety concerns, including hepatotoxicity and severe inflammatory responses.[4] The increasing number of severe adverse reactions and deaths associated with viral gene therapy has prompted urgent calls for

[1]Phoenix Children's Research Institute, Department of Child Health, University of Arizona College of Medicine, Phoenix, AZ, USA; [2]The Materials Science and Engineering Program, College of Engineering and Applied Science, University of Cincinnati, Cincinnati, OH, USA; [3]Department of Biomedical Engineering, College of Engineering and Applied Science, University of Cincinnati, University of Cincinnati, Cincinnati, OH, USA; [4]Phoenix Children's Center for Cancer and Blood Disorders, Phoenix, AZ, USA and [5]Division of Neonatology, Phoenix Children's Hospital, Phoenix, AZ, USA
Correspondence: Zicheng Deng (dengz1@arizona.edu) or Vladimir V Kalinichenko (vkalin@arizona.edu)

Received: 30 September 2025 Revised: 23 July 2026 Accepted: 28 July 2026
Published online: 01 September 2026

alternative delivery strategies that maintain therapeutic efficacy while eliminating virus-related toxicity.[5]

One important barrier to the clinical translation of both gene and drug therapies for pulmonary diseases is the lack of tissue- and cell-specific targeting. Many current therapeutic strategies result in nonspecific systemic distribution, leading to subtherapeutic concentrations at the disease site and accumulation in healthy tissues, which often causes off-target side effects.[6] Pharmacokinetic models estimate that only 0.1% to 1% of intravenously administered monoclonal antibodies (mAb) actually reach their intended targets, with the majority accumulating in off-target tissues.[7] Additionally, the lung's intrinsic defense mechanisms, such as mucociliary clearance and innate cellular uptake pathways, actively eliminate therapeutics delivered through airways.[8] These targeting limitations are especially pronounced in chronic pulmonary diseases like pulmonary fibrosis (PF), where pathological alterations in lung architecture present further obstacles to effective therapeutic delivery.[9] Understanding these limitations is crucial for recognizing the translational potential of nanoparticle delivery systems in advancing precision medicine approaches for pulmonary disorders.[10]

This review provides a detailed overview of the current state and future directions in precision nanomedicine for respiratory diseases. We first discuss how physicochemical properties define the "biological identity" of nanoparticles and influence their behavior at the nano-bio interface to ensure successful targeted delivery. By comparing pulmonary and systemic administration routes, we evaluate how these nanoparticle properties can be adjusted to reach specific lung compartments while avoiding local clearance mechanisms. In addition to nanoparticle design and delivery mechanisms, we discuss the therapeutic applications of these systems across a wide range of conditions, including PF, respiratory infections, chronic obstructive pulmonary disease (COPD), pulmonary vascular disease (PVD), cystic fibrosis (CF), asthma, lung cancer, and various neonatal disorders. We highlight how nanoparticle designs can be modified to treat the unique pathophysiology of each disease. Finally, we address the current challenges of clinical translation, such as long-term safety validation, regulatory requirements, and large-scale manufacturing complexities. We also highlight future perspectives, including new technologies for nanoparticle production, advantages of novel therapeutics and personalized precision therapy, and the use of artificial intelligence (AI) and machine learning (ML) in nanoparticle design. By addressing these important issues, we aim to provide a clear path for bioengineers and clinicians to develop new nanoparticle-based therapies for respiratory disorders.

ETIOLOGICAL DIVERSITY AND PATHOPHYSIOLOGICAL DRIVERS OF PULMONARY DISORDERS

External environmental factors in pulmonary disease

The respiratory system can serve as a primary entry for infectious agents for bacterial, viral, and fungal pathogens. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the most devastating infectious diseases globally. This disease affects around 10.8 million people worldwide and has caused ~1.25 million deaths in 2023, according to the World Health Organization's latest Global Tuberculosis Report.[11] The persistent nature of the TB infection and its ability to maintain dormancy demonstrate the complex host-pathogen interactions that are often seen in respiratory infectious diseases.[12] Similarly, the recent COVID-19 pandemic has highlighted the vulnerability of the lung to airborne viral pathogens. COVID-19 infections cause pulmonary epithelial and vascular damage, defective tissue repair, and impaired fibrinolysis.[13] Post-mortem autopsy demonstrated that COVID-19-induced acute respiratory distress syndrome (ARDS) differs significantly from other causes of ARDS, with notable vasculopathy and severe alveolar-septal congestion.[14]

Beyond infectious agents, chronic exposure to environmental toxins could significantly contribute to the pathogenesis of pulmonary diseases. COPD represents an example of environmentally induced respiratory disease. Driven by long-term exposure to tobacco smoke and other noxious particles and gases, COPD results in chronic lung inflammation, progressive airflow obstruction, and destruction of alveoli.[15] COPD remains one of the leading causes of death globally and is expected to become even more prevalent in the coming years.[15] A recent meta-analysis estimated that COPD affects ~12.64% of individuals over age 40, with higher prevalence among males (15.47%) compared to females (8.79%).[16] The strong association between COPD and smoking, air pollution, and occupational exposures demonstrates the profound impact that external environmental factors can have on respiratory health.[17]

The mechanisms underlying environmentally induced pulmonary diseases involve simultaneous damage to multiple cellular compartments and aberrant inflammatory response. Inhaled toxins can directly injure respiratory epithelium and trigger pro-inflammatory cascades that affect the pulmonary vasculature, disrupting the balance of cellular repair mechanisms that maintain lung homeostasis.

Genetics in pulmonary disease

Genetic abnormalities contribute to the pathogenesis and etiology of pulmonary diseases, often disrupting specific cellular functions essential for normal lung physiology. CF is an example of a genetic disease, arising from mutations in the CF transmembrane conductance regulator (CFTR) gene that affect normal epithelial cell functions.[18] Mechanically, the CFTR allows for proper trafficking of chloride ions into the luminal space. Failure of this channel results in a buildup of chloride ions in the cell, creating an osmotic gradient desiccating the luminal mucus.[8] CFTR mutations affect ~89,000 individuals worldwide, demonstrating how mutations in homeostatic, physiologic channels can have profound impacts on pulmonary function.[19]

Pulmonary arterial hypertension (PAH) provides another compelling example of genetic influence on respiratory disease.[20] PAH is classified into five separate groups. Group 1 PAH is caused by inherited and associated genetic etiologies, with a global prevalence of ~2.8 cases per 100,000 and 22,000 deaths in 2021.[21] PAH can result from mutations affecting endothelial cell or smooth muscle cell functions, including mutations in BMPR2,[22] EIF2AK4 (GCN2),[23] and TBX4 genes.[24] These genetic defects influence microvascular and stromal networks in the lung, causing large vessel constriction or obliteration along with the loss of peripheral pulmonary capillaries and right ventricular hypertrophy.[25]

Alpha-1 Antitrypsin Deficiency (A1ATD) and primary ciliary dyskinesia (PCD) are genetic pulmonary diseases. A1ATD is an autosomal codominant disorder caused by mutations in the SERPINA1 gene on chromosome 14, which leads to a shortage of the alpha-1 antitrypsin protein.[26] The significant reduction in circulating alpha-1 antitrypsin protein levels in the bloodstream impairs the protection of lung tissue from proteolytic damage caused by enzymes like neutrophil elastase.[26] The SERPINA1 mutations result in an imbalance between proteases and anti-proteases, and leave the fragile alveolar tissue vulnerable to degradation, causing emphysema.[27] In contrast, PCD is a genetically heterogeneous, predominantly autosomal recessive motile ciliopathy which arises from a failure of mechanical airway defense.[28] There are currently 54 known genes that encode the structural components or assembly factors of motile cilia implicated in PCD.[28] These mutations lead to ineffective or uncoordinated ciliary movements, resulting in impaired mucociliary clearance, chronic pulmonary infections, and often organ laterality defects like situs inversus. Recent genomic data show that PCD affects ~1 in 7500 people. It commonly presents with

neonatal respiratory distress and can progress to severe respiratory failure, with up to 51% of adults with PCD ultimately requiring lung transplantation.[28,29] Currently, the management of PCD remains primarily supportive, focusing on clearing secretions and treating infections. However, pre-clinical studies involving mRNA for therapies are progressing. A phase 1 trial of an inhaled mRNA therapy is currently enrolling both people with PCD and healthy controls.[28,29]

Complex interactions: environmental and genetic factors

Many pulmonary diseases result from a complex interplay of both genetic and environmental factors. Asthma is a prototypical gene-environment interaction disorder. Genetic predisposition contributes to airway inflammation and hyperresponsiveness,[30] while environmental exposures such as allergens, pollution, and infections serve as triggers that precipitate clinical manifestations.[31,32]

Lung cancer, which is responsible for an estimated 2.2 million new cases and 1.79 million deaths annually, arises from both genetic predispositions and environmental risk factors.[33] Family history increases the risk of lung cancer by ~50%, with heritability estimated to be ~18% higher than that of many other common cancers.[34] In addition, tobacco smoke remains the dominant environmental risk factor for lung cancer. The multitude of carcinogenic compounds cause direct damage to lung epithelial cells and trigger inflammation.[35] This repetitive cycle of damage and repair from repeated insults results in carcinogenesis. The combination of genetic and environmental factors results in a particularly aggressive disease pattern that has made lung cancer the leading cause of cancer-related deaths globally.[35]

PF is another example of lung disease caused by a combination of external environmental factors and internal genetic factors. Recent studies have demonstrated that idiopathic pulmonary fibrosis (IPF), the most common form of progressive fibrotic lung disease, results from interactions between genetics, the environment, and aging.[36] The damage to type II alveolar epithelial cells from the environment is now recognized as a central driver of IPF. The dysregulated repair following injury results in irreversible fibrotic scarring of lung tissue, lung dysfunction, and progresses to respiratory failure.[37] In addition to epithelial type II cells, other cell types including hematopoietic cells, stromal cells, and endothelial cells contribute to the pathogenesis of IPF.[38,39]

Unique characteristics of neonatal respiratory diseases

Neonatal respiratory diseases significantly differ from adult diseases because of the ongoing developmental processes in the lung tissue. The immaturity of the neonatal pulmonary system creates unique developmental vulnerabilities.[40] This developmental context means that neonatal pulmonary diseases cannot simply be understood through the same lens as adult diseases but require specialized knowledge of lung development.

Neonatal disorders such as bronchopulmonary dysplasia (BPD) and alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) are notable iatrogenic or genetically derived pediatric ailments.[41,42] The unique nature of neonatal respiratory diseases extends beyond their immediate clinical impact, with lasting implications that even appear in adulthood. Pulmonary complications represent a leading cause of illness, impaired growth, and mortality in neonates, particularly those born prematurely.[43] The specialized nature of these conditions requires dedicated research efforts that account for the dynamic nature of the developing lung and the complex interactions between genetic predisposition, environmental exposures, and developmental timing.

Taken together, pulmonary diseases can be driven by infections, environmental exposures, genetic abnormalities, and neonatal lung immaturity. These diverse challenges highlight the need for innovative delivery strategies such as nanoparticle-based systems (Fig. 1).

ADVANTAGES OF NANOPARTICLE SYSTEMS IN BIOMEDICAL APPLICATIONS

Unique size-dependent features for nanoparticles

Nanomaterials have advanced significantly and are now widely applied across various biomedical fields, including cancer theranostics,[44] vaccine development,[45] and in vivo imaging.[46] These materials in the nanoscale size range demonstrate unique physicochemical properties that make them particularly valuable for medical applications. One key feature of nanomaterials is their exceptionally high surface-area-to-volume ratio, which enhances their interaction with biological molecules.[47] This property is critical for applications like drug or gene encapsulation, where a higher surface area allows for greater therapeutic loading capacity.[48] Moreover, the surface chemistry of nanomaterials can be precisely engineered through functionalization strategies such as ligand conjugation,[49] elemental doping,[50] or compositing with other nanomaterials.[51] The specific size also provides nanoparticles with distinctive physical properties. For instance, superparamagnetic nanoparticles and quantum dots are two highly promising classes of nanomaterials in biomedical applications, as both exhibit size-dependent behaviors.

When magnetic particles are below the critical size for maintaining multiple magnetic domains at the nanoscale, each nanoparticle becomes a single magnetic domain. These singular domains can rapidly flip their magnetization direction and eliminate magnetic hysteresis at room temperature.[52] Superparamagnetic nanoparticles offer unique advantages for biomedical applications due to their ability to respond to external magnetic fields while avoiding agglomeration in the absence of external magnetic fields.[53] This behavior enables magnetic resonance imaging,[54] magnetic guide targeting,[55] and magnetic hyperthermia[56] in biomedical applications. Similarly, quantum dots are semiconductor nanocrystals with dimensions comparable to or smaller than the Bohr exciton radius. When their size is confined within the 1–20 nm range, quantum confinement effects restrict the motion of charge carriers (electrons and holes) in all three spatial dimensions. This phenomenon results in unique optical and electronic properties, including high photostability, broad absorption spectra, narrow and size-tunable emission wavelengths, and excellent biocompatibility, all of which make quantum dots particularly attractive for biomedical imaging and sensing applications.[57]

Biocompatible liposomal formulations, such as amikacin liposome inhalation suspension (ALIS), consist of small liposomes with a size range of 200–300 nm. This specific size range allows for sterile filtration during processing and facilitates enhanced uptake by pulmonary macrophages to eliminate pathogens that persist within these cells. The size-dependent internalization of these liposomes enables the delivery of high antibiotic concentrations directly to the intracellular site of infection, overcoming the limitations associated with free drug delivery.[58]

Nanoparticle-based platforms offer comprehensive solutions to overcome the fundamental limitations that conventional delivery methods have struggled to overcome. These systems can protect therapeutic cargo from degradation or aggregation, enhance cellular uptake through size- dependent mechanisms, facilitate endosomal escape after entering the cells, and provide tissue or cell-specific targeting.[3,59]

Enhancing therapeutic stability and controlled release

In physiological conditions, hydrophobic drug molecules tend to interact with each other, leading to the formation of aggregates that reduce bioavailability and therapeutic efficacy. Many developed drug candidates are poorly soluble. Specifically, ~40% of

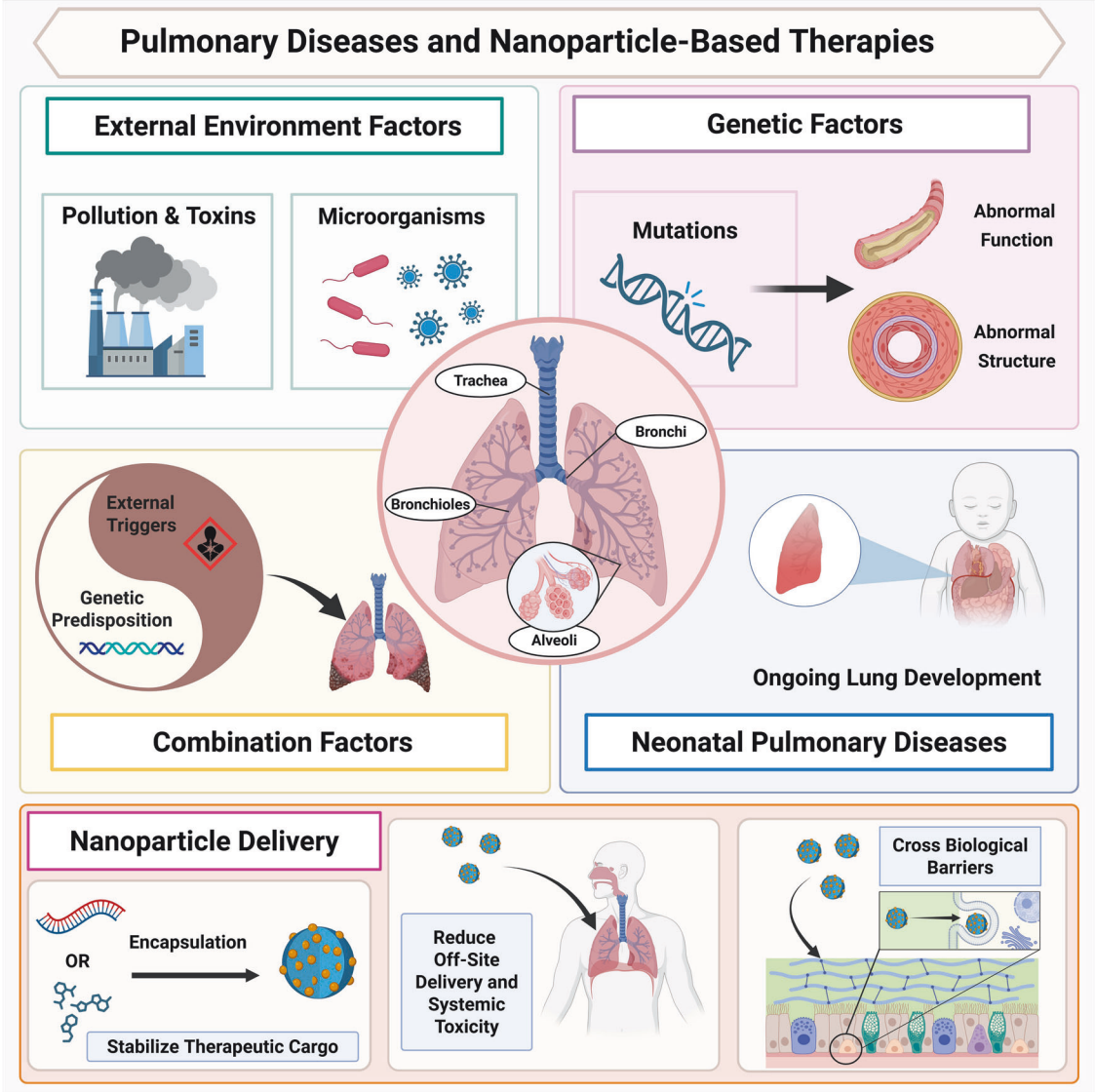

Fig. 1 Pulmonary diseases arise from diverse causes, including external insults and environmental factors, as well as genetic conditions that result in structural and functional abnormalities. Immature lungs of neonates are especially vulnerable to pulmonary diseases due to ongoing development. Nanoparticle-based delivery systems offer strategies to overcome therapeutic challenges by stabilizing therapeutic cargo, reducing off-target effects and systemic toxicity, and enabling transport across biological barriers. Created with Biorender.com

approved drugs and ~90% of those in the development pipeline are poorly soluble.[60] Some highly hydrophobic drugs are thermodynamically unstable, leading to colloidal instability, aggregation, and rapid crystallization in physiological environments. At the molecular level, the high interfacial tension (γ) between these hydrophobic drugs and water results in a positive Gibbs Free Energy ($\Delta G > 0$) for the dispersed state. To reach a lower energy state ($\Delta G < 0$), the system spontaneously minimizes the surface area (ΔA) of the drug molecules, leading to aggregation. This process can be described by the Gibbs Free Energy equation:

$$\Delta G = \Delta H - T\Delta S$$

$$\Delta H = \gamma \Delta A$$

Where ΔH is the change in enthalpy, ΔS is the change in entropy, γ is interfacial tension, and ΔA is the change in surface area. This behavior limits their formulation for injectable or inhalable delivery and restricts the achievable therapeutic concentrations in lung tissues. Nanoparticles address these limitations by providing a hydrophilic surface around the hydrophobic therapeutic agent, thereby lowering interfacial tension and moving the Gibbs Free Energy of the system toward zero to enhance physical stability. In addition, the nanoparticle structure functions as a molecular cage that induces steric hindrance, trapping the drug in an amorphous or small nanocrystalline state. This configuration prevents hydrophobic molecules from reorganizing into large, insoluble lattices. Therefore, nanoparticle encapsulation stabilizes the dispersed state by inhibiting crystallization and aggregation, facilitating clinically compatible aqueous administration. Beyond preventing aggregation, the encapsulation of hydrophobic drugs protects them from enzymatic degradation, thereby extending circulation time and improving therapeutic outcomes.[61] Recently, nanoparticles have been developed to enhance the stability of hydrophobic drugs through various encapsulation mechanisms. A common strategy involves amphiphilic molecules to create lipid-based or polymeric nanoparticles.[62] These nanoparticles are designed with hydrophobic cores to encapsulate drug molecules and hydrophilic surfaces to prevent aggregation in biological environments.

Another effective approach for stabilizing hydrophobic drugs is the use of inorganic nanoparticles, particularly those with mesoporous structures like mesoporous silica nanoparticles or iron oxide nanoclusters.[63] These materials possess high surface areas, large pore volumes, and tunable pore sizes, making them ideal platforms for drug encapsulation. Hydrophobic drugs can be loaded into the mesoporous nanoparticle structures using solvent-based approaches such as incipient wetness impregnation or solvent evaporation methods.[64]

Similarly, nucleic acid molecules face significant delivery challenges due to their susceptibility to nucleases in biological fluids.[65] Messenger RNA (mRNA) and plasmid DNA are typically vulnerable to degradation and poor uptake by target cells.[66] For example, stabilized mRNAs are widely used for gene therapies. However, mRNA molecules are highly susceptible to degradation by ubiquitous ribonucleases (RNases).[67] RNase enzymes can cleave the phosphodiester backbone of nucleic acids within minutes of administration, limiting their therapeutic potential.[67,68] Nanoparticle systems provide protection for nucleic acids through physical encapsulation and surface shielding that effectively guard from nuclease-rich environments.[69] Lipid-based nanoparticles, which achieved remarkable success with mRNA vaccines, form protective bilayers that encase nucleic acids within their interior, shielding them from enzymatic degradation.[70] On the other hand, polymeric nanocarriers often employ electrostatic interactions between cationic polymers and anionic nucleic acids to form condensed nanostructures that sterically hinder nucleases from reaching the phosphodiester bonds.[66]

Nanoparticle-based delivery systems also offer precise control over the release of therapeutic cargos. This controlled release capability addresses several fundamental challenges in drug delivery, including the maintenance of effective therapeutic concentrations, reduction of systemic toxicity, and overall enhancement of clinical outcomes.[71] Currently, nanoparticle-controlled release strategies are broadly categorized into two types: sustained release systems and stimuli-responsive systems. Sustained release systems are designed to provide a continuous and prolonged release of encapsulated therapeutics. Stimuli-responsive systems enable on-demand release of cargos triggered by specific stimuli.

Sustained release primarily relies on the degradation of the polymer matrix in the nanoparticles. For example, poly(lactic-co-glycolic acid) (PLGA) nanoparticles represent the most advanced sustained release platform. The biodegradable nature of PLGA enables controlled drug release through matrix degradation, with release profiles that can be engineered from hours to months.[72] A diffusion-controlled mechanism for a "steady state" release of drug involves the permeation of drug through polymeric networks at a steady rate. While PLGA is widely recognized for its high biocompatibility and biodegradability, the accumulation of acidic monomers (lactic and glycolic acids) during polymer hydrolysis can significantly lower the local tissue pH. This localized acidity may cause tissue irritation or adversely affect myofibroblast differentiation.[73] Furthermore, studies indicate that PLGA nanoparticles can interact with the lung surfactant film, where the hydrophobic nature of PLGA polymers is associated with higher particle retention at the interface, which intensifies the inhibition of surfactant function.[74] In contrast, stimuli-responsive nanoparticles, also known as "smart" nanoparticles, are designed to undergo conformational or chemical changes in response to specific triggers, enabling on-demand drug release.[75] Stimuli can be classified as internal (endogenous) and external (exogenous) triggers.[76] Internal stimuli involve biological cues specific to disease states.[77] For example, the tumor microenvironment presents disease-specific characteristics, which include lower pH levels (pH 5.6–6.8) compared to physiological pH (7.4). This feature can be used to trigger the pH-sensitive polymer degradation and therapeutic cargo release from the nanoparticles.[78] Similarly, the high concentration of glutathione (GSH) in cancer cells presents a highly translatable mechanism for triggering controlled drug release from redox stimuli-responsive nanoparticles.[79] Stimuli-responsive nanoparticles designed to respond to GSH often incorporate disulfide (S-S) bonds within their hydrophobic cores. Upon cellular uptake, the high GSH levels within tumor cells reduce and break these disulfide bonds, destabilizing nanoparticles and facilitating the release of therapeutics.[80] Conversely, external stimuli are applied outside the body to trigger a nanoparticle response, offering precise control of therapeutic release.[81] Among these, near-infrared (NIR) light, magnetic fields, and ultrasound are particularly promising due to their non-invasive nature and deep tissue penetration.[75,82] NIR-responsive nanoparticle systems typically contain photothermal agents that absorb NIR light, generating localized heat to disrupt the nanoparticle structure and trigger drug release.[83] Similarly, magnetic nanoparticles subjected to an alternating magnetic field produce heat through magnetic hyperthermia, which can activate drug release from thermosensitive nanoparticle carriers.[84] Ultrasound stimulation exerts both thermal and mechanical effects: acoustic energy can be converted into heat to induce drug release, while cavitation effects, particularly from ultrasound-induced microbubble collapse, can mechanically disrupt nanocarriers and promote cargo release.[85]

Enhancing cellular uptake and endosomal escape

The mammalian cell membrane is the primary barrier to drug or nucleic acid delivery due to the phospholipid bilayer, which selectively restricts the passage of therapeutic molecules based on their physicochemical properties.[86] Because of the hydrophobic nature of the bilayer, hydrophilic drugs struggle to pass through because of their polarity. The efficiency of cellular delivery varies significantly depending on molecular characteristics, creating distinct challenges for different classes of therapeutics used in pulmonary medicine. Small molecule drugs with molecular weights below 1 kDa primarily rely on passive diffusion for cellular entry, but their penetration efficiency is heavily influenced by their lipophilicity and compatibility with cellular membrane bilayers.[87] Patton et al. demonstrated that for small molecules between 100 and 1000 Da, absorption rates are primarily dictated by lipophilicity rather than size, as lipophilic drugs rapidly utilize transcellular pathways; however, their overall penetration efficiency remains modulated by dissolution kinetics within the lung's limited mucosal fluid.[88,89] Hydrophilic compounds experience difficulty crossing lipid bilayers, limiting their intracellular concentrations.[90] In contrast, biological macromolecules exceeding 1 kDa cannot diffuse across membranes and must be internalized through active processes like endocytosis.[91] However, due to their high molecular weight and lack of specific receptor interactions, these macromolecules often demonstrate low uptake efficiency.[87] Similarly, due to the large size and strong polyanionic nature, nucleic acids are inherently inefficient at traversing lipophilic cellular membranes via passive diffusion.[92,93]

To overcome these limitations, nanoparticle-based delivery systems use active transport mechanisms to facilitate cellular uptake.[94] The size of nanoparticles allows for recognition and internalization through various endocytic pathways, including clathrin-mediated endocytosis,[95] caveolin-mediated endocytosis,[96] and macropinocytosis.[97] These active uptake processes significantly enhance cellular internalization.[98] The physicochemical properties of nanoparticles, including size, shape, surface charge, and mechanical stiffness, play a critical role in determining their interaction with the plasma membrane and their preferred internalization pathway.[99–101] For example, nanoparticles between 100 and 150 nm tend to enter cells via clathrin-mediated endocytosis, while particles in the 60–80 nm range favor caveolae-mediated uptake.[102] Larger particles, ranging from 500 to 1500 nm, are typically internalized through macropinocytosis.[102]

Additionally, particle shapes influence cellular uptake: spherical nanoparticles are more likely to utilize clathrin or caveolin-mediated endocytosis, whereas rod-shaped or elongated particles can engage the macropinocytosis pathway.[103] Nanoparticles with phospholipids, such as lipid-based nanoparticles or cell membrane-coated nanoparticles, offer unique advantages due to their ability to fuse directly with cellular membranes. This property bypasses endocytic entrapment and facilitates efficient intracellular cargo delivery into the cytosol.[90,104]

The surface chemistry of nanoparticles can be precisely engineered to optimize cellular uptake. Coating nanoparticles with cationic polymers (such as chitosan or polyethyleneimine) can neutralize the negative charge of nucleic acids while enhancing electrostatic interactions with negatively charged cell membranes.[105] Cell-penetrating peptides (CPPs) represent one of the most effective strategies for overcoming membrane barriers. CPPs can be conjugated to nanoparticle surfaces to dramatically improve membrane translocation.[106] They typically comprise 4–40 amino acids that are rich in positively charged residues such as arginine and lysine to facilitate membrane and cellular internalization.[107] Recent studies have reported that CPP modification of nanoparticles can improve cellular uptake by 50-fold compared to unmodified counterparts.[108]

However, successful internalization is not sufficient to deliver functional cargos, endosomal escape is a critical challenge for intracellular delivery. After endocytosis, large molecules, including nucleic acids, frequently become entrapped in endosomes and are ultimately degraded in lysosomes, substantially reducing their intracellular bioavailability and therapeutic potential.[109] Recent studies show that >2% of internalized therapeutic small interfering RNAs (siRNAs) escape from endosomes and reach the cytoplasm.[110] Specialized nanoparticle formulations have been designed to address this challenge. The proton sponge effect relies on the buffering capacity of ionizable materials to disrupt endosomal membranes.[111] The protonation of amine groups on the nanoparticles in acidic endosomal and lysosomal environments leads to chloride ion influx, osmotic swelling, and membrane rupture.[112] Similarly, ionizable lipid nanoparticles facilitate endosomal escape by undergoing charge conversion in the acidic environment, allowing them to bind and fuse with the endosomal membrane, thereby releasing their cargo into the cytosol.[104]

Enhancing tissue-specificity and cell-specific targeting

Active targeting. The development of engineered nanoparticle delivery systems has introduced powerful strategies to overcome the longstanding obstacle of non-specific biodistribution of therapeutic drugs and nucleic acids. These technologies have revolutionized the field by allowing for enhanced precision in targeting pathological sites. Active targeting, also referred to as receptor-mediated targeting, is one of the most effective techniques for achieving cellular specificity. This method involves decorating the surface of nanoparticles with functional ligands that selectively bind to surface receptors unique to target cells.[113] By employing ligands with high affinity and molecular recognition specificity, nanoparticles are able to preferentially accumulate and are internalized by target cells, while minimizing uptake by off-target tissues.[114]

Antibodies, or immunoglobulins (Ig), are among the most extensively used ligands for nanoparticle surface modification due to their extraordinary specificity and affinity for antigens. Structurally, antibodies are Y-shaped molecules composed of two heavy chains and two light chains. They are classified into five major isotypes, IgA, IgD, IgE, IgG, and IgM, based on their structure and function.[115] Among these, IgG is the most abundant in human serum and is widely utilized in therapeutic applications and nanoparticle functionalization.[116] To conjugate antibodies onto the surface of nanoparticles, physical or ionic adsorption can be employed.[117] Physical adsorption relies on hydrophobic interactions, often resulting in conformational changes and random antibody orientations, which could compromise biological activity.[118] Ionic adsorption utilizes electrostatic interactions based on surface charge density of antibodies, offering better orientation but vulnerable to displacement by serum proteins.[116] Both methods require high antibody concentrations, increasing cost and limiting widespread application.[119] Moreover, adsorption methods depend on weak intermolecular forces, which result in reduced stability.[116] Compared to absorption strategies, covalent conjugation provides increased stability and properly oriented antibody attachment. One of the most widely used approaches is carbodiimide chemistry, where 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide (EDC) activates carboxyl groups on nanoparticles to form stable amide bonds with primary amines on antibodies. The addition of NHS (N-hydroxysuccinimide) enhances coupling efficiency.[120] Thiol-maleimide mediated conjugation is another approach for covalently binding antibodies to nanoparticles via coupling of disulfide bonds.[121] This approach involves reduction of antibody disulfide bonds to generate free thiol groups that react with maleimide-functionalized nanoparticle surfaces through Michael addition. The maleimide groups have high selectivity for the thiol side chain of cysteine on the antibody proteins.[122] Despite their effectiveness, these traditional covalent approaches could still result in random antibody orientations and partial masking of the antigen-binding (Fab) region. To address these limitations, click chemistry was introduced to enable site-specific bioconjugation.[123] Click chemistry encompasses a group of bioorthogonal reactions characterized by simplicity, high yield, and biocompatibility, producing minimal byproducts under mild conditions.[124] Common click chemistries include Copper(I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC), Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC), Diels-Alder and Inverse Electron Demand Diels-Alder (IEDDA) reactions, Staudinger ligation, Sulfur Fluoride Exchange (SuFEx), and photo-triggered reactions.[124–127] Alternatively, protein adaptors can be employed to immobilize antibodies onto nanoparticle surfaces in a consistent and functionally active orientation.[128] These adaptors bind specifically to the conserved Fc region of IgG antibodies, leaving the Fab domain accessible for antigen recognition. This approach allows for the exchange of antibodies to recognize different targets.[129]

In addition to antibodies, peptides are increasingly utilized as targeting ligands because of their smaller size, high synthetic accessibility, structural versatility, and reduced immunogenicity. Peptides also possess functional groups amenable to chemical conjugation using methods similar to conjugation of large proteins. Importantly, both the peptide's primary sequence, which dictates receptor binding, and its secondary/tertiary conformation, which determines structural stability and bioactivity, must be considered. For example, the Arg-Gly-Asp (RGD) peptide sequence is widely recognized for its high affinity toward αvβ3 integrins, which are abundant on lung cancer cells and tumor-associated endothelial cells.[130] Functionalization of nanoparticles with RGD peptides can significantly enhance targeted drug delivery through integrin-mediated internalization. However, linear RGD peptides are prone to rapid proteolytic degradation under physiological conditions. To enhance stability and binding efficiency, cyclic RGD (cRGD) peptides have been developed. The cyclic configuration offers a rigid structure which resists proteolytic cleavage and preserves receptor-binding conformations, thereby improving bioactivity and targeting performance.[131] Moreover, the mode of attachment of peptides onto nanoparticles significantly influences functionality.[132] For instance, it has been demonstrated that cRGD peptides must be linked to plasmonic nanoparticles using a long poly(ethylene glycol) (PEG) chain and a short oligolysine spacer to maintain optimal spatial presentation. Direct linkage of cRGD to PEG without a spacer can impair targeting capability.[133] Ligand

density should also be considered, as moderate densities (~0.2–0.5 ligands per nm^2) yield better targeting efficiency by avoiding steric hindrance and clustering effects.[134]

In addition to proteins and peptides, other small biomolecules can be used as targeting ligands and offer several advantages, such as simplicity of the conjugation chemistry, high manufacturability, and low cost.[135] However, because small molecules typically form weak non-covalent interactions with their targets, the associated receptors must possess deep binding pockets to ensure sufficient specificity and affinity.[136] A prominent example is folic acid (FA), a vitamin that binds strongly to folate receptors (FRs).[137] FRα receptor is overexpressed in a variety of cancer cells, whereas FRβ is found on activated macrophages implicated in autoimmune and inflammatory diseases. Both FRα and FRβ exhibit strong binding affinity ($K_d = 10^{-7}$ mM) to FA.[135] Published studies show that FA-functionalized nanoparticles accumulate in tumor lung tissues and myeloid cells adjacent to fibrotic lung regions due to their respective FRs, highlighting the potential translational use in lung cancers and PF.[138] Aptamers are another class of biomolecule used for nanoparticle functionalization. These synthetic single-stranded DNA or RNA fold into unique three-dimensional conformations that confer affinity and specificity. Typically, an aptamer contains 40–100 nucleotide bases, with the sequence itself dictating 3D structure and binding capabilities.[139] Their production is scalable and cost-effective using Systematic Evolution of Ligands by Exponential Enrichment (SELEX), a high-throughput process that iteratively selects high-affinity aptamer sequences from a large combinatorial library.[140] These unique features make aptamers particularly attractive for lung-targeted drug and gene delivery via nanoparticles.

Passive targeting. In contrast to active targeting strategies, passive targeting relies on the intrinsic physicochemical properties of nanoparticles. Unlike active targeting, which often requires ligand-receptor interactions at the cellular interface, passive targeting allows nanoparticles to achieve organ-selective deposition during circulation. This approach avoids the cost and complexity of ligand conjugation, which can be further compromised by protein corona formation in biological fluids. Protein coronas, formed by serum proteins adsorbing onto nanoparticle surfaces, can obscure targeting ligands and reduce binding efficacy.[141] The degree to which protein coronas impair targeting depends on the molecular size of the ligand. Small ligands (e.g., peptides or single domain antibodies) are easily masked compared to large antibodies.[142]

Passive targeting is largely influenced by formulation and structure of the nanoparticles, which leads to different physicochemical properties including size and surface charge. Hydrodynamic size can directly influence the fate of the nanoparticles in the organs/tissues that are targeted. Nanoparticles larger than 150 nm are typically cleared from the peripheral blood by Kupffer cells in the liver, while those larger than 200 nm are removed in the spleen.[143,144] In contrast, nanoparticles smaller than 6 nm (hard NPs) or 10 nm (soft NPs) are filtered by the kidneys and excreted via the urine.[144] Thus, for lung-targeted intravenous delivery, nanoparticles should be designed within an optimal size range (often between 60 and 120 nm) to avoid clearance by other organs and enhance accumulation in pulmonary tissues. This is particularly important because the lung possesses an extensive microvascular network with relatively low blood flow velocities, which favor entrapment of nanoparticles in this size range.[145,146] Cellular uptake mechanisms are also dependent on the size of the nanoparticles. Clathrin-mediated endocytosis typically favors particles in the 100–150 nm range, whereas caveolae-mediated endocytosis is more efficient for smaller nanoparticles between 60–80 nm.[102,147,148] Caveolin-1 protein (Cav-1) is critical for the formation and function of caveolae, regulating cargo uptake and trafficking, and it is highly expressed in lung tissues.[149] Published studies showed that Cav-1 is overexpressed in lung cancer.[150] Thus, nanoparticles engineered in the optimal size window are efficient for both tissue- and cell-level passive internalization.

In addition to size, surface charge of the nanoparticles plays a critical role in cell-specific targeting. The lungs contain a dense network of capillary endothelial cells, especially in the alveolar region in which gas exchange occurs.[151] The dysfunction of these endothelial cells contributes to a variety of pulmonary pathologies, thus lending itself as an attractive target for targeted, therapeutic intervention.[152,153] The apical surface of endothelial cells is coated with a negatively charged glycocalyx, composed of an anionic network of glycosaminoglycans, glycolipids, and glycoproteins.[154] Positively charged nanoparticles interact more readily with this negatively charged surface, enhancing cellular uptake. For example, PEI/PEG-based polyplex nanoparticles carrying nucleic acids were shown to selectively transfect pulmonary endothelial cells after intravenous administration without significant uptake of other lung cell types, underscoring the role of positive charge in endothelial cell delivery.[146] In contrast, negatively charged nanoparticles are more likely to be excluded by the glycocalyx and pass through endothelial junctions without being internalized.[146,155] Pathological changes in cell surface charge can be leveraged for passive targeting. Cancer cells, including those in the lung, typically exhibit negatively charged membranes due to altered glucose metabolism, a phenomenon known as the Warburg effect.[156] In 1920, Otto Warburg found that tumors used more glucose compared to the surrounding tissues.[157] This phenomenon is also called "aerobic glycolysis" because cancer cells ferment glucose, producing lactate in the presence of oxygen.[158] In normal cells, the presence of oxygen favors oxidative phosphorylation eliminating the shunt for lactate production. Compared to the complete aerobic respiration of glucose, glycolysis is inefficient in producing adenosine triphosphate (ATP). The same amount of ATP produced by aerobic respiration would require a higher amount of glucose using glycolysis. As a result, the lactate produced from aerobic glycolysis 10–100 times faster.[158] Thus, the behaviors of high glucose uptake level and lactate secretion are regarded as the most distinguishable metabolic patterns of cancer cells. Most importantly, the abnormal cross-membrane movement of lactate in the tumor cells can neutralize cationic ions like sodium. Thus, the net surface charge on cancer cells is usually negative, which can be explored for targeting cancer cells.[156] Nanoparticles with a positive surface charge can significantly enhance cancer cell targeting efficiency.[159] More importantly, the positive charge retains a strong electrostatic interaction with cancer cells in the presence of protein coronas.[160] Thus, charge-mediated targeting is a robust and scalable strategy for nanoparticle design considerations.

Other targeting. To further improve targeting efficiency, external physical forces can be applied to guide nanoparticle accumulation at desired sites. Among these, magnetic targeting has been recently explored. In this strategy, superparamagnetic iron oxide nanoparticles (SPIONs) are incorporated into delivery systems and guided to specific anatomical regions using externally applied magnetic fields. Computational models have shown that magnetic fields applied near bifurcating airway regions can dramatically enhance deposition efficiency in the pulmonary acinar regions, achieving ~100% localization of magnetized nanoparticles.[161] These predictions have been validated in experimental settings. For instance, inhalable nano-in-microparticles composed of SPIONs and solid lipid matrices have demonstrated enhanced lung tumor accumulation under magnetic guidance.[162] In vivo studies showed that magnetic activation increased lung deposition of SPIONs by more than tenfold in targeted lung lobes compared to lung lobes without magnetic activation.[163] Beyond pulmonary administration, magnetic targeting via intravenous

injection has also been demonstrated. For example, SPION-loaded mesenchymal stromal cells were intravenously administered into mice with experimental silicosis.[164] Application of an external magnetic field significantly improved lung retention of the magnetized mesenchymal stromal cells and associated nanoparticles.[164]

Ultrasound-guided targeting is another promising method that uses ultrasound-microbubble interactions to enhance vascular permeability and facilitate deeper nanoparticle penetration. Oscillating microbubbles generate mechanical forces such as acoustic microstreaming, shockwaves, and microjets, which transiently disrupt endothelial junctions.[165] A recent study employed Fe_3O_4 nanocarriers in combination with ultrasound to induce sonoporation and cavitation. This procedure enhanced nanoparticle accumulation in deep-seated lung tumors and improved the efficacy of non-small cell lung cancer (NSCLC) therapy.[166] In summary, the distinctive properties of nanoparticles, including high capacity for efficient drug encapsulation, protection and stabilization of therapeutic agents, promotion of cellular uptake, and the ability to achieve controlled release of therapeutic cargos, provide a versatile platform for overcoming major limitations of conventional therapies in pulmonary diseases (Fig. 2).

In summary, nanoparticle designs can be used to enhance targeting efficiency. Physicochemical properties of nanoparticles are fundamental to the targeted strategies. In passive targeting, these properties directly affect nanoparticle behavior by influencing interactions with cells or tissues. In contrast, physicochemical properties influence active targeting indirectly by determining the accessibility of surface-conjugated ligands. For example, if a nanoparticle size exceeds the critical thresholds required for lower respiratory tract deposition or mucus penetration, the surface-conjugated ligands will have minimal opportunity to interact with targeted cells, thereby significantly decreasing active targeting efficiency. Similarly, an inappropriate surface charge or high surface free energy can promote the rapid formation of a protein corona, which may mask surface-conjugated ligands, particularly smaller moieties like peptides, and prevent molecular recognition by target receptors. Furthermore, other targeting strategies like magnetic field guidance are inherently dependent on these properties because superparamagnetic behavior is a strictly size-dependent phenomenon. Therefore, the rational engineering of nanoparticles with optimized physicochemical properties is not merely a feature of passive distribution but is a key requirement for the precision and efficacy of active targeting systems in pulmonary nanomedicine.

NANOPARTICLE ADMINISTRATION FOR PULMONARY THERAPEUTIC DELIVERY

Nanoparticle-based delivery systems have emerged as a transformative platform for overcoming the intrinsic barriers that limit the efficacy of gene and drug therapies in the treatment of pulmonary diseases.[145,153] Owing to the unique anatomical and physiological characteristics of the lung, nanoparticles can be administered via two distinct routes: pulmonary and systemic (Fig. 3). The selection of the optimal administration route primarily depends on the specific cell types targeted and the therapeutic objectives, as each method presents fundamentally different biological barriers that must be carefully considered during the design of nanoparticle-based therapies.[145]

Pulmonary administration

Pulmonary administration (intratracheal or intranasal routes) represents one of the most promising approaches for nanoparticle delivery to lung tissues.[145] This route offers several critical advantages, with the most significant benefit of this route being the direct access to lung tissues, thereby increasing local concentration and enabling a rapid onset of therapeutic action.[167] By bypassing first-pass hepatic metabolism, pulmonary delivery can also reduce systemic toxicity and the required therapeutic dosage.[145,167] Liposomal ciprofloxacin is delivered by the pulmonary route, providing sustained drug release to improve therapeutic efficiency and tolerability. Nanoparticle formulations designed for pulmonary delivery enhance the penetration of bacterial biofilms and facilitate the treatment of infections in airways.[168] Furthermore, the pulmonary route provides a convenient method of therapeutic administration using appropriate inhalation devices. This feature is particularly crucial for chronic conditions requiring frequent dosing and long-term therapy in home settings.[169]

Despite these notable advantages, several significant barriers restrict the effectiveness of pulmonary nanoparticle delivery to lung cells, necessitating the careful design of the nanoparticle delivery system to overcome these obstacles. The hierarchical, branching architecture of the human respiratory tract results in distinct regional differences in airway diameter and airflow, which in turn critically influence nanoparticle deposition based on size.[145,170] Particles exceeding 5 μm in diameter primarily deposit in the upper airways because their inertia prevents them from following changing airflow patterns, leading to collisions with airway walls.[171] Conversely, published studies have reported that particle diameters between 1 and 5 μm are most effective for deposition in the lower respiratory tract of the lungs, given that particles smaller than 1 μm can be easily exhaled.[162,170] Although small particles have a greater potential to be exhaled, this is offset by their enhanced capability to be distributed throughout the entire lungs and reach the distal airways and alveoli with a high pulmonary diffusion efficiency.[172] In the alveolar region, where air velocity approaches zero, only particles with a diameter <500 nm can deposit effectively via Brownian diffusion and electrostatic attraction.[170] Numerous studies have demonstrated that nanoparticles smaller than 200 nm exhibit enhanced lung distribution and retention.[10,173,174] It is important to distinguish between pulmonary deposition and pulmonary cellular uptake, as they occur at different biological scales. Pulmonary deposition is an anatomical phenomenon where particles are distributed across the respiratory system. In contrast, pulmonary cellular uptake is a cellular-level process involving the interaction between a particle and the cell membrane. While deposition defines the anatomical location of the particles, cellular uptake describes the specific interaction of nanoparticles with targeted cells.

The airway mucus barrier is another critical challenge in pulmonary delivery, significantly impacting the efficacy of nanoparticle-based therapeutic systems designed to target lung cells. Secreted primarily by goblet cells, the airway mucus layer covers the epithelial surfaces in the trachea and conducting airways, acting as a line of defense to protect the epithelium from foreign substances such as pathogens and pollutants.[175] This complex viscoelastic gel can trap inhaled nanoparticles, leading to their rapid clearance from the lungs and setting a limited time window for pulmonary delivery, especially in chronic respiratory diseases where mucus is overproduced and increased mucus viscosity is prevalent.[176] To overcome mucociliary clearance, nanoparticles must penetrate the gel layer of mucus and rapidly enter the periciliary space.[8,177] Mucus possesses a mesh-like network with pore sizes ranging between 100 and 200 nm.[178] The mucus layer generally filters out larger nanoparticles, with only small particles, particularly those smaller than 100 nm, being capable of penetrating the mucus efficiently.[179] Thus, the mucus-penetrating ability of nanoparticles is size-dependent, with smaller nanoparticles having a higher chance to overcome this barrier. On the other hand, mucociliary clearance typically removes nanoparticles that directly interact with mucus molecules. The mucus layer is composed of ~98% water, 1% salts, and 0.3% mucin glycoproteins.[180] Mucins contain negatively charged domains

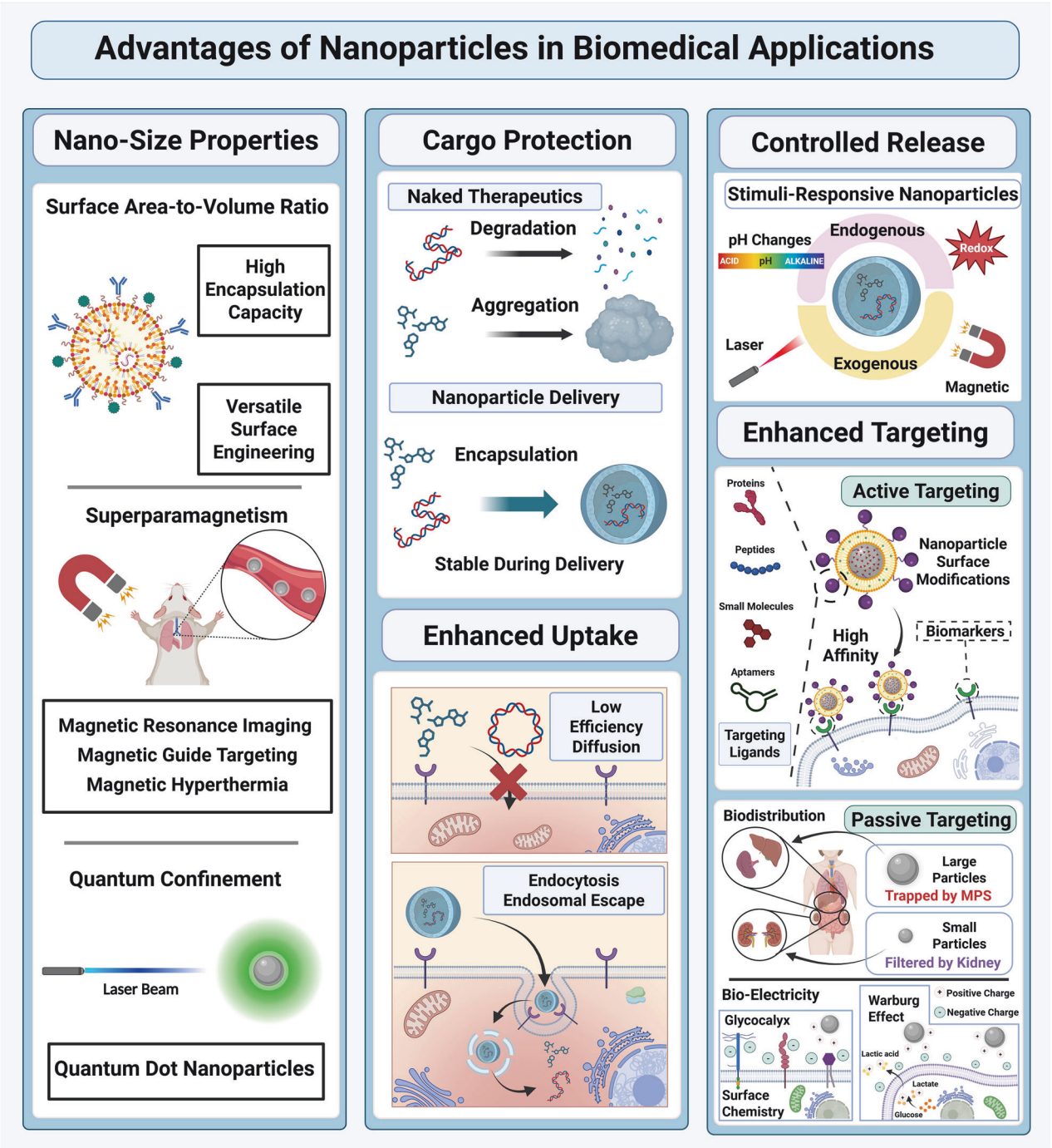

Fig. 2 Nanoparticles display unique nanoscale properties such as a high surface area-to-volume ratio, superparamagnetism, and quantum confinement, which enable efficient drug encapsulation, versatile surface engineering, magnetic applications, and advanced imaging. These nanocarriers can prevent degradation and aggregation of therapeutic cargo, thereby maintaining the stability of therapeutic agents during delivery. Nanoparticles can provide controlled and stimuli-responsive release in response to pH, redox conditions, magnetic fields, or light. Nanoparticle delivery systems can also improve targeting efficiency to specific tissues and cells through active targeting or passive targeting. Created with Biorender.com

that can interact with positively charged nanoparticles. Published studies have shown that amine- and carboxyl-modified nanoparticles are trapped in mucus due to electrostatic interactions and hydrogen bonding with mucins.[181] Additionally, mucins possess periodic hydrophobic naked protein globular domains, causing nanoparticles with hydrophobic surfaces to strongly adhere to mucus components, thereby becoming immobilized within the mucus network.[182] To minimize these adhesive interactions, nanoparticles must be engineered to exhibit hydrophilic, neutrally charged surfaces. PEGylation represents the most widely studied approach for creating mucus-penetrating nanoparticles.[181] Dense PEG coatings shield nanoparticles from adhesive interactions with mucus constituents, allowing rapid diffusion through the mucus layer.[182] Notably, the efficacy of PEGylation depends more on the surface density of PEG than its molecular weight.[183] A high density of PEG chains on the nanoparticle surface creates a brush-like

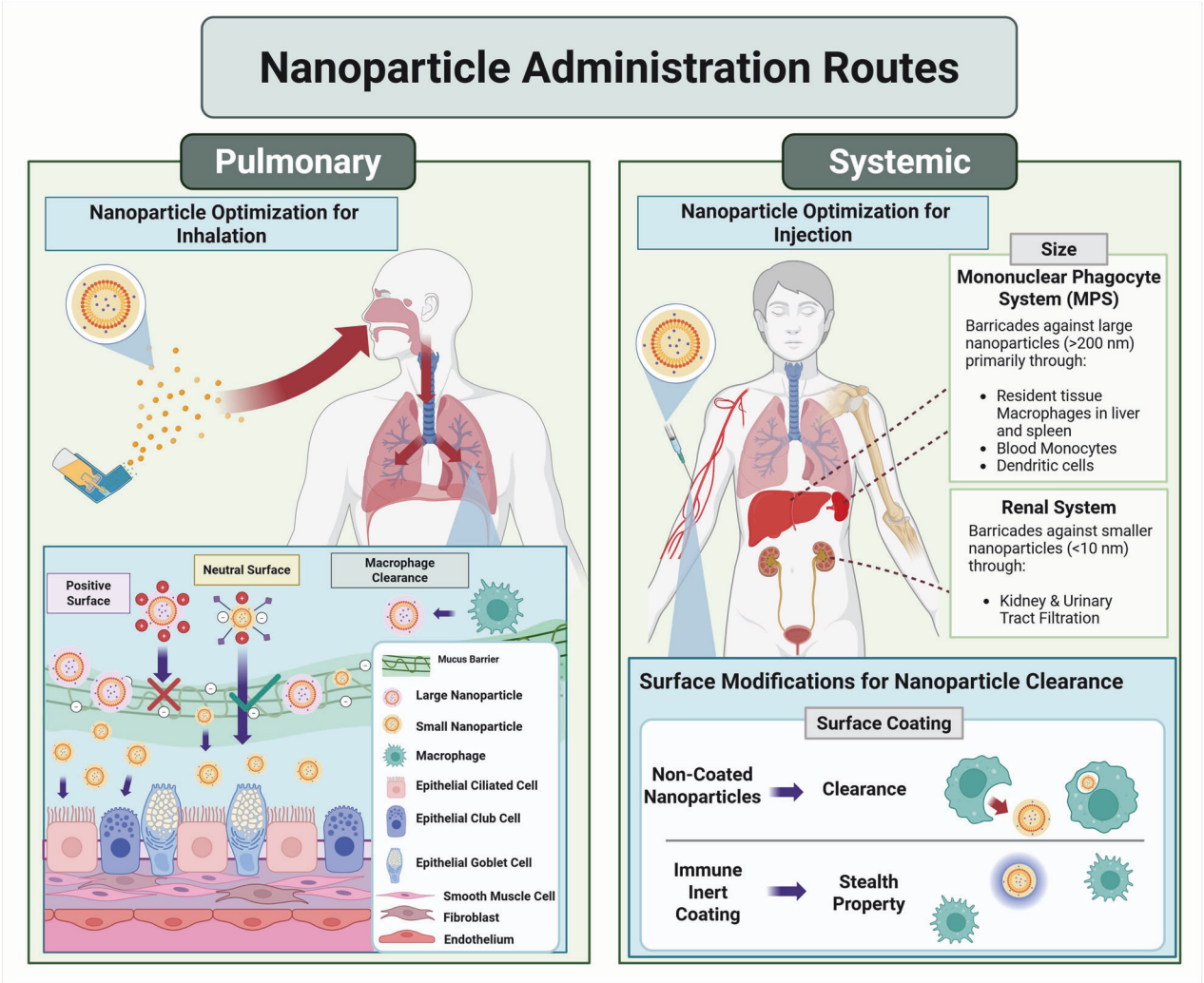

Fig. 3 Schematic overview comparing pulmonary and systemic nanoparticle administration routes. Pulmonary administration is based on inhalation, where nanoparticles encounter barriers such as airway mucus layers and alveolar macrophage clearance. Smaller nanoparticles with neutral surfaces are capable of overcoming these barriers. In systemic administration, intravenously injected nanoparticles are subject to clearance by the mononuclear phagocyte system (MPS) and renal filtration, depending on nanoparticle size and surface characteristics. Created with Biorender.com

conformation, which effectively shields the nanoparticle core from adhesive interactions with mucin, a primary component of mucus.[181,184] Despite the benefits of PEGylation, the clinical use of these formulations is challenged by the induction of anti-PEG antibodies. These antibodies can cause accelerated nanoparticle clearance from the peripheral blood, leading to decreased efficacy. Furthermore, PEGylated nanocarriers may trigger complement activation-related pseudoallergy, a non-IgE-mediated hypersensitivity reaction triggered by the release of inflammatory mediators. Addressing the "PEG dilemma" requires balancing the necessary benefits of stealth and stabilization against the risks of immunogenicity.[185] In addition to PEGylation, several alternative modifications have been reported to enhance mucus penetration. For instance, zwitterionic polymers, which contain both positively and negatively charged moieties, create balanced surface charges that resist protein and mucin adsorption, reducing mucociliary clearance and increasing mucus penetration.[186–188] Surfactant coatings can also alter nanoparticle penetration ability. One study demonstrated that dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylamine, and dipalmitoylphosphatidylglycerol-modified nanoparticles are capable of reducing mucus penetration, while dipalmitoylphosphatidylserine improves the mucus permeability of the nanoparticles.[189] Other biomolecules such as Chondroitin Sulfate A or cysteine-constrained heptapeptides have been reported to assist nanoparticle penetration of the mucus layer in pulmonary delivery due to their high hydrophilicity and net-neutral to slightly negative charge.[190] Mucolytic agents provide another strategy to enhance mucus penetration. These include reducing agents and proteolytic enzymes, which break disulfide bonds in mucins, thereby lowering viscosity and increasing pore size for easier diffusion of nanoparticles.[191] Moreover, recent studies showed that hydrogen-rich gas inhalation can disrupt non-covalent interactions between nanoparticles and mucins, effectively enhancing nanoparticle penetration and leading to improved therapeutic benefits in PF.[192]

Another major challenge in the pulmonary administration approach is the rapid clearance of nanoparticles by alveolar macrophages (AMs), which significantly limits nanoparticle delivery to lung tissues. Several physicochemical properties of nanoparticles determine macrophage clearance, including size, surface charge, and surface chemistry.[145] Nanoparticle size is one of the most critical factors influencing AMs clearance. AM uptake increased as particle size increased within the range of 100–1000 nm, with maximum uptake observed for particles with

a diameter of 1000 nm, whereas uptake of 100 nm particles was lower than 4%.[193] Surface charge also influences interactions between nanoparticles and AMs. Compared to anionic nanoparticles, cationic nanoparticles exhibit rapid clearance by mononuclear phagocytic systems since the positive charge promotes cell internalization.[194] Cationic nanoparticles can also be internalized by pulmonary dendritic cells (DCs), stimulating DC recruitment and maturation in the lung tissue. In contrast, anionic nanoparticles are typically immunologically inert.[195] Several polymeric materials have been used for nanoparticle modifications to help the nanoparticles escape from macrophage clearance. Among these polymers, PEGylation remains the gold standard for reducing AM clearance through its stealth properties. PEGylation surface modifications are the most established approaches to reduce AM clearance of nanoparticles in pulmonary delivery by creating a hydrophilic and neutral charged surface layer that minimizes interaction between nanoparticles and AMs.[196,197] Other hydrophilic polymers, such as polyvinyl alcohol (PVA)[198] and zwitterionic polymers,[199] can similarly reduce macrophage uptake. Lung surfactant proteins also play important roles in interactions of nanoparticles with AMs. Published studies have demonstrated that lung surfactant-associated proteins A and D (SPA and SPD) can enhance AM uptake of nanoparticles.[200] In specific pulmonary conditions such as non-tuberculous mycobacterial infections, ALIS (Arikayce) enhances drug uptake into the macrophages where these pathogens reside, thereby improving bactericidal efficacy compared to free drug delivery.[201] However, phosphatidylcholine modification on nanoparticles causes significant aggregation of SPA on their surface, and the SPA aggregation helps to delay and reduce AM clearance because macrophages typically do not internalize large aggregates of SPA.[202]

While both intranasal and intratracheal routes are categorized as pulmonary administration, they should be treated separately when designing and evaluating nanoparticle delivery due to several major differences. Intranasal administration is frequently used in preclinical studies because it is simple to use and minimally invasive. However, nanoparticles are initially deposited in the nasal cavity, where they may be lost in the upper respiratory tract. In contrast, intratracheal administration delivers nanoparticles directly to the trachea, bypassing the nasal compartment to provide more controlled pulmonary exposure. Because deposition profiles, clearance kinetics, and biodistribution may be different between the two routes, these factors must be considered when designing nanoparticles. Results from intranasal studies should not be directly applied to intratracheal administration without careful consideration of these route-specific differences.

The pulmonary administration of extracellular vesicles (EVs), particularly exosomes, provides an efficient route for direct delivery to the respiratory system.[203] These nanovesicles possess innate tissue-homing properties and low immunogenicity, enabling them to interact with the pulmonary microenvironment and evade immune detection more effectively compared to synthetic lipid-based carriers.[204] Cellular uptake of EVs occurs primarily through endocytosis, involving specialized pathways such as clathrin-mediated endocytosis, phagocytosis, and macropinocytosis. Alternatively, EVs may deliver their biological cargo by directly fusing with the target cell plasma membrane.[205] Upon internalization, these vesicles transfer functional mRNA, microRNA, and proteins that regulate recipient cell behavior and modulate intracellular signaling pathways. Inhaled EVs have shown potential in suppressing cytokine storms in ARDS, reducing collagen accumulation in PF, and providing targeted transport for lung cancer chemotherapy.[205]

The clinical efficacy of inhaled therapeutics depends upon complex interactions between the nanoparticle formulation containing therapeutic cargo, the delivery device, and the patient respiratory anatomy. A primary objective of this delivery system is the generation of an aerosol with a mass median aerodynamic diameter (MMAD) between 1 and 5 μm, which can efficiently reach the alveolar region. Different aerosolization mechanisms impose specific constraints on nanoparticle (NP) stability and delivered dose.[206] Air-jet nebulizers utilizing high-velocity gas streams through a small "venturi" nozzle, creating significant shear forces and air-liquid interface stresses that can induce NP aggregation or cargo degradation.[207] In contrast, vibrating mesh nebulizers (VMNs) utilize high frequency piezo-electric vibration to force liquid through tapered apertures to generate a fine, slow-moving mist. This method does not require a high-velocity gas stream, providing a gentler atomization process suitable for sensitive structures. VMNs do not heat the medication, making them suitable for thermolabile biologics. However, VMNs still face challenges such as aperture clogging and require frequent maintenance when delivering suspensions or viscous formulations.[207] Pressurized metered-dose inhalers (pMDIs) are also widely used due to the advantage of providing fixed doses and easy portability. These devices rely on rapid expansion of hydrofluoroalkane (HFA) propellants to generate an aerosol plume. The high aerosol exit velocity often results in significant oropharyngeal deposition.[207] Over the past two decades, significant progress has been made. Currently, pMDIs can achieve nearly 50% lung deposition, compared to just 10% during early development.[208] The success of a pMDI formulation depends on the drug stability and solubility in the propellants. Unstable suspensions can lead to erratic particle size distribution and poor dispersion.[209] Dry powder inhalers (DPIs), on the other hand, offer a propellant-free, environmentally friendly alternative with enhanced chemical stability in the solid state.[209] These devices can be classified as either passive (breath-actuated) or active (power-assisted) systems.[207] Passive DPIs mainly rely on the patient's inspiratory effort to drive the movement and de-agglomeration of particles into the respiratory tract. To assist patients with compromised respiratory function who may lack sufficient inspiratory flow, active DPIs have been developed. These devices are supplied with an internal energy source to aerosolize the powder, allowing for effective lung deposition at lower flow rates.[208] Despite these mechanisms, dry powder formulations remain sensitive to environmental heat and humidity, which can impair aerosol performance and reduce deposition efficiency.[207] Table 1 summarizes aerosol device types, specific formulation requirements, and representative clinical examples.

Systemic administration

Unlike pulmonary administration, systemic delivery introduces nanoparticles into the circulatory system, where they must navigate a distinct set of barriers before reaching lung tissues. This route inherently avoids the airway mucus barrier. However, it necessitates the design of nanoparticles that can effectively evade rapid systemic clearance and achieve excellent targeting efficiency towards lung-resident cell types. Intravenous systemic delivery is an alternative when aerosol-based pulmonary delivery methods are limited in reaching the distal lung parenchyma or interstitial compartments.[210]

One of the largest and most persistent challenges in systemic delivery of nanoparticles is rapid systemic clearance, driven by mechanisms such as immune surveillance, hepatic clearance and renal filtration, ultimately leading to low pulmonary concentrations. Nanoparticles with a size of ~60–80 nm are considered optimal for systemic delivery, including targeting pulmonary cells. This is because nanoparticles larger than 100 nm tend to be eliminated by the mononuclear phagocyte system (MPS), while those smaller than 10 nm are filtered out by the renal system.[211] These size-dependent clearance mechanisms represent major barriers limiting efficient nanoparticle accumulation in the lung following systemic administration. MPS, which is also known as the reticuloendothelial system (RES), constitutes the primary

Table 1. Type of aerosol devices used for drug delivery to lung tissues

Device type	Mechanism of action	Formulation constraints and considerations	Examples	Ref.
Air-jet nebulizer	Employs high-velocity gas streams through a venturi nozzle to generate an aerosol mist	High shear forces and air-liquid interface stresses can lead to nanoparticle aggregation or cargo degradation	PARI LC PLUS, PARI LC Sprint	482, 484
Vibrating mesh nebulizer (VMN)	Utilizes high-frequency piezo-electric vibration to extrude liquid through tapered apertures	Risks of aperture clogging when delivering viscous or concentrated formulations	Lamira™ Nebulizer System, eFlow® Technology	75, 211, 378, 483,
Pressurized metered-dose inhaler (pMDI)	Relies on the rapid expansion of hydrofluoroalkane (HFA) propellants to create an aerosol plume	Performance depends on drug solubility in propellants; unstable suspensions may cause erratic particle size distribution and increased oropharyngeal deposition	Ventolin®, Symbicort®, Asmanex® HFA, Dulera®, Xopenex®,	217
Dry powder inhaler (DPI)	Delivers solid-state particles through either breath-actuated (passive) or power-assisted (active) systems	Formulations require protection from environmental heat and humidity to maintain aerosol performance	RT234, GB0139, Technosphere®, Advair	498

barrier encountered by systemically administered nanoparticles.[212] MPS includes resident tissue macrophages and DCs primarily in the liver and spleen, along with circulating monocytes and myeloid progenitors in the bone marrow.[212] Less than 1% of the injected dose actually reaches the intended target tissue because of this rapid recognition and uptake by MPS cells.[212] Therefore, it is essential to consider strategies to evade clearance by the MPS when designing nanoparticles for systemic delivery. After i.v. injection, serum proteins rapidly adsorb onto the surface of nanoparticles, particularly Igs and complement system components, effectively "marking" the nanoparticles for clearance by macrophages in the MPS.[213] This critical process is known as opsonization.[214] To counteract this, specific nanoparticle surface coatings are used to achieve a "stealth" property, protecting the nanoparticles from blood protein absorption and macrophage recognition in circulation, thereby avoiding MPS clearance. To evade immune recognition, nanoparticles are coated with polymers that resist protein adsorption, such as PEG,[215] poly(-zwitterions),[216] and polyoxazolines.[217] These polymers create immune-inert surface coatings with steric and electrostatic barriers, effectively hindering protein adsorption and making these polymers ideal for nanoparticle modification to reduce MPS clearance.[218] In addition to polymers, cell-membrane coating on nanoparticles represents a revolutionary biomimetic strategy to prevent protein absorption and opsonization, thereby significantly reducing rapid clearance by macrophages and the MPS. Coating nanoparticles with natural cell membranes (e.g., from red blood cells, macrophages, platelets or stem cells) mimics their intrinsic biological properties. These cell membrane-coated nanoparticles inherit a "self" identity from the source cells, which helps minimize non-specific protein adsorption and opsonization, promoting the nanoparticle longevity in circulation.[219]

Because lung tissues possess extensive microvascular networks, many nanoparticle delivery systems utilizing systemic administration are specifically designed to target lung capillary endothelial cells. GALA peptide, a synthetic peptide, exemplifies this goal with its dual function: it targets sialic acid-terminated sugar chains on the surface of lung endothelial cells and simultaneously enhances endosomal escape, improving nanoparticle intracellular delivery to lung endothelial cells.[220] While it might intuitively be assumed that the presence of targeting ligands like GALA is the dominant factor for lung selectivity, published studies indicate that nanoparticle composition critically affects organ-specific targeting by masking non-target organ delivery (e.g., to the liver, spleen, kidney), thereby optimizing lung selectivity.[221,222] For instance, a recent study developed an amphiphilic Poly (β-Amino ester)-based nanoparticle system that can selectively deliver nanoparticles into lung tissue without targeting any other organs after intravenous injection.[223] This study also compared different polymer structures and nanoparticle/DNA encapsulation ratios, finding that only specific nanoparticle formulations can selectively target the lung.[223] Although the exact relationship between organ selectivity and nanoparticle formulation is not yet fully understood, the balance of size, charge, and hydrophobicity related to nanoparticle formulation is considered to play important roles in nanoparticle targeting to the lung tissue.

Selecting administration route

The choice between pulmonary and systemic administration routes significantly impacts therapeutic efficacy and requires a careful and thorough evaluation of the specific biological barriers and targeting mechanisms involved. When selecting an administration route for nanoparticle-targeted delivery to the lung, it is crucial to consider the specific cell types intended for targeting and the underlying disease pathophysiology.

Pulmonary administration is commonly employed as the first-line approach for targeting airway epithelial cells. This preference stems from the fact that airway epithelial cells, such as ciliated, goblet and club cells, are the first cell types that nanoparticles encounter after successfully overcoming the mucus barrier in the respiratory tract. This direct access makes pulmonary administration particularly suitable for treating diseases primarily characterized by epithelial pathology, such as CF, asthma, COPD, A1ATD, PCD, non-CF bronchiectasis, and bacterial, viral, and fungal infections. Many inhaled therapies are in development for lung cancers, pulmonary hypertension (PH), and IPF. However, for targeting pulmonary endothelial cells in PVDs, systemic administration is the preferred route since endothelial cells line the internal surfaces of blood vessels and are directly accessible via the circulation. In contrast, pulmonary administration becomes problematic for endothelial targeting as it would require nanoparticles to cross the epithelial barrier without premature cargo release. Interestingly, some specific modifications involving Transferrin have shown the potential to increase transcytosis and enhance nanoparticle transport through epithelial cells to other pulmonary cell types.[224] However, systemic delivery of nanoparticles remains the more common and effective route for endothelial cell targeting because these cells directly line the internal surfaces of blood vessels.[145]

For stromal (interstitial) cell populations such as fibroblasts and pericytes, the choice of delivery route is more complex. Nanoparticles administered via either pulmonary or systemic route may be nonspecifically sequestered by epithelial or endothelial barriers, preventing the targeting of stromal cells.[225] However, in certain disease states such as acute lung injury or PF, these barriers become disrupted, leading to impaired barrier integrity and increased permeability.[226] These compromised barriers may create gaps that allow nanoparticles to reach deeper

lung tissue. For example, in the case of acute lung injury, damage to endothelial cells leads to diffuse pulmonary interstitial and alveolar edema. Consequently, intravenous administration of nanoparticles after acute lung injury can achieve effective tissue penetration through a disrupted endothelial barrier, causing high accumulation of nanoparticles in the lung tissue.[210] On the other hand, disease conditions result in changes in cell biomarkers, offering new molecular targets for nanoparticle design. For example, the up-regulation of vascular cell adhesion molecule-1 (VCAM-1) on the surface of pulmonary endothelial cells in injured lungs can be explored because VCAM-1 exhibits high affinity to very late antigen-4 (VLA-4). Coating nanoparticles with engineered plasma membrane from cells genetically modified to constitutively express VLA-4 effectively targets pulmonary endothelial cells and delivers therapeutic payloads to injured lungs via systemic administration.[227] Therefore, it is important to tailor the nanoparticle design and administration strategy to both the biological environment and therapeutic objective.

For pulmonary administration, particles with a diameter smaller than 500 nm are deposited via Brownian diffusion and electrostatic attraction in the alveolar region, while nanoparticles smaller than 200 nm exhibit enhanced lung distribution. At the mucosal interface, nanoparticles smaller than 100 nm can efficiently penetrate the 100–200 nm mesh-like network of the airway mucus. Surface charge strongly influences mucus transport. Positively charged particles tend to interact with negatively charged mucin domains and become trapped within the mucus gel. Neutral or slightly negative nanoparticles generally exhibit improved mucus penetration. In contrast, for systemic administration, nanoparticle size primarily determines organ distribution and cellular interactions. Particles smaller than 10 nm are rapidly filtered by the kidneys, and those larger than 150 nm are quickly recognized by the MPS. Consequently, a hydrodynamic diameter of ~60–120 nm is often favorable for lung-targeted intravenous delivery because it balances prolonged circulation with reduced clearance by the liver and spleen. A specific challenge in systemic administration is the formation of the protein corona. Upon contact with blood plasma, the immediate adsorption of plasma proteins onto the nanoparticle surface occurs to reduce surface free energy at the nanoparticle-liquid interface.[228] The resulting corona can change the physicochemical properties of nanoparticles, including an increase in hydrodynamic radius and a shift toward a typically negative surface charge due to the anionic nature of most blood proteins. The corona facilitates recognition by the innate immune system through opsonization, which leads to rapid detection by circulating leukocytes and macrophages. Moreover, the physical presence of the corona can mask surface-conjugated ligands, limiting their access to target receptors and reducing targeting efficiency. Surface charge further influences cellular interactions. For example, cationic nanoparticles with ~ +20 mV surface charge are highly efficient at targeting endothelial cells through electrostatic interactions with the negatively charged glycocalyx, but excessive positive charge may increase nonspecific protein adsorption and immune cell uptake. Therefore, the optimal nanoparticle characteristics are context-dependent and can vary based on the nanomaterial platform, surface engineering strategy, target cell population, and disease state, highlighting the need for specific designs for different pulmonary diseases.

NANOPARTICLE TARGETING DELIVERY FOR PULMONARY DISEASES

Pulmonary fibrosis

Interstitial lung diseases, including idiopathic IPF, are progressive and irreversible lung disorders resulting from dysregulated lung repair after injury. There is substantial progress in diagnostic and clinical management of IPF, yet >100,000 patients suffer annually from this disease.[229] Early treatments for IPF, such as immunosuppressive therapies, showed limited therapeutic benefits with a high safety risk and adverse side effects.[230] Current antifibrotic drugs for IPF, such as pirfenidone and nintedanib, slow the progression of IPF but do not provide a curative outcome.[231] Therefore, IPF remains one of the most significant challenges in respiratory medicine, and there is an urgent need for novel therapeutics.

Aberrant fibroblast activation and excessive secretion of extracellular matrix (ECM) proteins are hallmarks of PF.[232] In cases of minor lung injury, wound-healing mechanisms can restore normal lung architecture. However, severe or repetitive lung injury results in persistent and excessive ECM accumulation, eventually leading to impaired lung function.[233] In addition to secretion of ECM proteins, fibroblasts are also involved in their maintenance and degradation. Through the secretion of proteolytic enzymes such as metalloproteinases, fibroblasts can degrade ECM proteins previously deposited during tissue repair.[234] Normally, an excess of fibroblasts is eliminated through apoptosis, but in PF, fibroblasts are resistant to cell death, leading to their aberrant accumulation.[231,235] Fibroblasts can also differentiate into myofibroblasts to produce more ECM proteins and α-smooth muscle actin. Myofibroblasts play a critical role in normal lung homeostasis, and lung tissue repair.[235] When myofibroblasts become dysregulated, as observed in lung fibrosis, excessive ECM production by these cells disrupts normal lung architecture. Among other subtypes of lung fibroblasts are lipofibroblasts, which are characterized by their lipid-storing capacity and are thought to be protective in fibrosis. Lipofibroblasts have been shown to transdifferentiate into myofibroblasts during fibrosis but revert to their original state after fibrosis resolution.[236]

In addition to fibroblasts, other pulmonary cell types also play important roles in the progression of lung fibrosis. Immune cells, including neutrophils, monocytes, and fibrocytes, can activate fibroblasts and promote their differentiation into myofibroblasts.[237] Type 2 cytokines and T-helper 2 (Th2) inflammatory responses promote fibrosis by activating the Transforming growth factor β (TGF-β) signaling pathway,[238] which is responsible for activation of fibroblasts to produce ECM and collagens, and for fibroblast-to-myofibroblast differentiation.[239] Recent evidence highlights the role of M2 macrophages that regulate myofibroblasts and inflammatory response. These M2 macrophages secrete insulin-like growth factor 1 (IGF-1), which prevents myofibroblast apoptosis.[240] Endothelial cells contribute to myofibroblast accumulation and lung fibrogenesis.[241] During fibrosis, endothelial cells can transdifferentiate into myofibroblasts in a process called endothelial-to-mesenchymal transition (EndMT).[234,241] EndMT involves the loss of endothelial markers and the acquisition of mesenchymal fibroblast-like characteristics.[242] Additionally, endothelial cells within fibrotic lesions (called fibrosis-associated endothelial cells) secrete profibrotic molecules and cytokines that contribute to lung inflammation and fibrosis.[241] Endothelial cells purified from fibrotic lungs showed upregulation of connective tissue growth factor (CTGF), a signaling mediator which contributes to the maintenance of the fibrotic phenotype during fibrogenesis.[243] Pericytes have recently garnered increasing attention in the research community. As perivascular cells, pericytes provide both biochemical and structural support to pulmonary circulation, working in conjunction with endothelial cells to maintain vascular integrity.[244] Their close interaction with the pulmonary endothelium is essential for preserving vascular homeostasis, and disruption of endothelial-pericyte coupling has been implicated in the progression of IPF.[245,246] Pro-inflammatory factors secreted by pericytes can trigger inflammatory responses and promote tissue remodeling, ultimately leading to excessive ECM deposition.[247] Moreover, studies have identified pericytes as a potential source of myofibroblasts. The pericyte-to-myofibroblast transition has been observed in bleomycin-

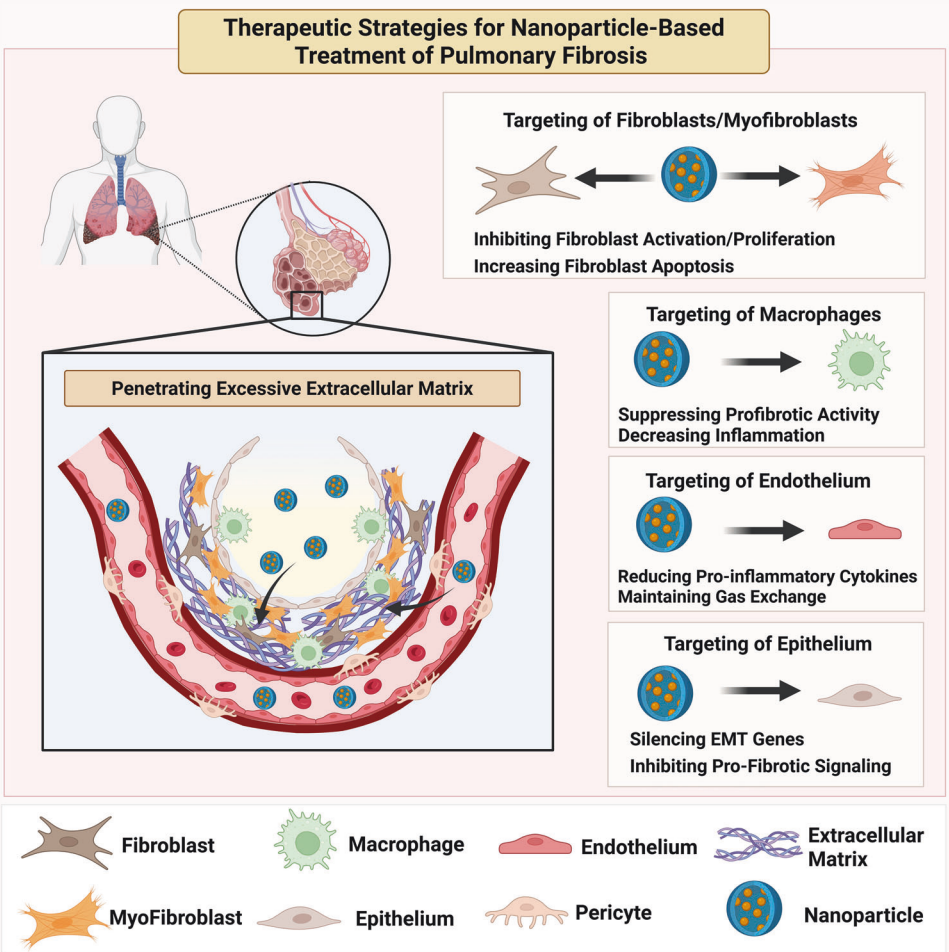

Fig. 4 Schematic shows therapeutic strategies for pulmonary fibrosis. Excessive extracellular matrix secretion creates a barrier, restricting nanoparticle accumulation in alveoli and fibrotic tissue. Fibroblasts and myofibroblasts are primary targets for nanoparticle delivery in fibrosis. Diverse cell types of the fibrotic microenvironment, including macrophages, endothelial cells, and epithelial cells, provide additional opportunities to target molecular mechanisms involved in pulmonary fibrosis. Created with Biorender.com

induced lung injury, a mouse model of lung fibrosis, contributing to ECM accumulation and the establishment of a pro-fibrotic environment.[248] Alveolar epithelial type II (ATII) cells play a critical role in the pathogenesis of PF. In IPF, ATII cells fail to regenerate properly after injury, leading to fibrosis instead of lung tissue repair.[249] Dysfunctional ATII cells exhibit aberrant communication with fibroblasts, contributing to fibrotic remodeling.[241,250] Epithelial cells undergo a process called epithelial-to-mesenchymal transition (EMT) in which epithelial cells differentiate into fibroblasts during lung fibrosis. There is a positive correlation between the degree of EMT and progression of PF.[251]

Given the multiple cell types and mechanisms involved in lung fibrosis, the design of nanoparticle-based delivery systems must account for the interactions between targeted cells (Fig. 4). Compared to other pulmonary diseases, IPF poses a unique challenge due to the dense ECM network, which forms an additional barrier alongside mucus, restricting nanoparticle accumulation in alveoli and fibrotic scars. To address this challenge, a recent study introduced a dual barrier-penetrating inhalable nanoparticle delivery system incorporating tris-(2-carboxyethyl)-phosphine (TCEP) and L-arginine.[252] TCEP disrupts disulfide bonds within mucus, while L-arginine promotes nitric oxide (NO) production, which activates endogenous matrix metalloproteinases (MMPs) to degrade excessive ECM.[252]

Fibroblasts and myofibroblasts are primary targets for therapeutic interventions in IPF.[253] One of the major therapeutic approaches focuses on delivering nucleic acids to regulate abnormal fibroblast activation. For instance, interleukin-11 (IL-11) has been identified as a profibrotic cytokine, and researchers have developed an inhalable lipid-polymer nanoparticle platform composed of polyethene glycol-conjugated poly(lactic-co-glycolic acid) (PEG-PLGA) and the lipid G0-C14 to deliver siRNA against IL11 (siIL11), thereby inhibiting fibroblast differentiation.[254] In another study, a human-derived cationic peptide complex with polyanionic polymeric antisense oligonucleotides that target TGF-β mRNA demonstrated inhibition of lung fibrosis in mice.[255] The synergistic therapy using multiple siRNAs co-loaded in the same nanoparticles is a promising strategy to maximize therapeutic effects. For example, siRNA targeting ADPH oxidase-4 (NOX4) suppresses (myo)fibroblast activation, while siRNA targeting protein tyrosine phosphatase-N13 (PTPN13) induces (myo)fibroblast apoptosis.[256] Co-delivery of these two siRNAs in micelle nanoparticles demonstrated significant antifibrotic effects in a bleomycin-induced PF mouse model.[256] In addition to fibroblasts and myofibroblasts, therapeutic targeting of other cell types within fibrotic lung tissue can inhibit fibrosis.[38] In IPF patients, the macrophage mannose receptor, CD206, is markedly upregulated.[257] Accordingly, mannose-coated nanoparticles delivering siRNA against TGF-β1 to profibrotic $CD206^{+}$ macrophages inhibited lung fibrosis.[257] In pulmonary endothelial cells, reduced expression of Forkhead Box F1 (FOXF1) transcription factor has been recently linked to aberrant fibroblast activation and the

endothelial release of proinflammatory cytokines such as interleukin-6 (IL-6) and C-C motif chemokine ligand 2 (CCL2).[38] Nanoparticle delivery of FOXF1 expression plasmid targeted to endothelial cells activated the transcription of RRAS, inhibited secretion of pro-inflammatory cytokines, and decreased PF in a mouse model.[38] Since alveolar epithelial type 2 cells (AT2) play an important role in IPF development, Wang et al. designed an inhalable γ-aminobutyric acid (GABA)-based lipid nanoparticle (LNP) for dual mRNAs (Membrane-bound cytochrome b5 reductase 3, CYB5R3, and Bone morphogenetic protein 4, BMP4) for co-delivery to AT2s. The nanoparticle treatment markedly promotes alveolar regeneration and prolongs the survival of mice after fibrotic lung injury.[258] Another fibrosis-associated gene, G2 and S phase-expressed 1 (GTSE1), is highly overexpressed in multiple cell types during disease progression. Because of the upregulation of mannose receptor (CD206) in fibrosis, delivery of siRNA targeting GTSE1 using mannose-modified lipid nanoparticles is an attractive strategy to reduce pro-EMT gene expression and improve lung function.[259]

In addition to gene delivery, nanoparticles can also encapsulate drugs for potential IPF treatment. For example, pirfenidone, an FDA-approved antifibrotic drug, can be encapsulated into chitosan-alginate nanoparticles and Au/mesoporous silica core/shell nanoparticles to enhance therapeutic potential in PF.[260] Activation of myofibroblasts via the Rho/myocardin-related transcription factor (MRTF)/serum response factor (SRF) signaling pathway can be inhibited by nanoparticle-mediated delivery of the MRTF/SRF pathway inhibitor CCG-1423 using PEG-PLGA nanoparticles. This nanoparticle treatment significantly suppressed myofibroblast activation.[261] Similarly, resolvins and their precursors have shown promising potential in the treatment of lung fibrosis. A recent study incorporated resolvins with phosphatidylcholine and PEG to form a "fish-oilsome (FOS)" nanoparticle, which showed improvement of pulmonary structure and function in fibrotic lungs.[262] Metal-organic frameworks (MOFs) are hybrid nanomaterials with a porous structure, allowing for high drug loading capacities and controlled release mechanisms. Using a MOF-based nanoparticle approach to deliver immune inhibitor sotuletinib (BLZ-945) to fibrosis-promoting macrophages, published studies demonstrated a significant therapeutic benefit.[263] Co-delivery strategies using nanoparticles to achieve synergistic effects are also gaining traction. Loading astaxanthin (AST) and trametinib (TRA) into nanoparticles and a subsequent treatment regimen significantly enhanced synergistic effects of both drugs by inhibiting myofibroblast activation and promoting proper lung repair.[264] Considering the profibrotic activity of M2 macrophages, a dual-targeting strategy has been developed using mannose-modified magnetic liposomal nanoparticles to deliver dexamethasone specifically to M2 macrophages under static magnetic field guidance, reducing M2 polarization and significantly alleviating lung fibrosis.[265] In another study, the researchers developed a nanoplatform loaded with nintedanib (NIN) and colchicine (COL) to modulate M1/M2 macrophage balance, ultimately suppressing fibroblast activation.[266] Notably, specifically designed nanoparticles can co-encapsulate nucleic acids and drugs for improved therapeutic effects. For example, antioxidant drug AST and siRNA targeting TGF-β1 (siTGF-β1) were co-loaded into liposomal nanoparticles and delivered to AT2 cells, resulting in inhibition of TGF-β signaling and activation of fibroblasts.[267]

In conclusion, nanomedicines for IPF target key molecular drivers to inhibit myofibroblast differentiation and apoptosis. By targeting profibrotic cytokines (TGF-β1, IL-11), transcription factors (FOXF1, GTSE1), and the M1/M2 macrophage balance, these therapies intervene at the molecular level to suppress myofibroblast activity and inhibit the transition of epithelial and endothelial cells into mesenchymal phenotypes. These targeted interventions are designed to decrease the excessive ECM deposition and restore impaired lung function.

Lung infections

Lung infections present a complex and multifactorial clinical challenge. The respiratory system is vulnerable to a wide array of pathogens because of the constant exposures to the environment. Despite shared clinical syndromes, pathogens utilize distinct strategies to initiate infection and cause lung disease. Bacterial pneumonia is a leading cause of morbidity and mortality worldwide, which is commonly caused by pathogens such as *Streptococcus pneumoniae, Haemophilus influenzae*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis*.[268,269] These bacteria produce virulence factors, including adhesins for epithelial entry and colonization,[270] polysaccharide capsules for immune evasion,[271] and toxins for inducing tissue damage.[272] Similarly, fungal lung infections, caused primarily by *Aspergillus* species, *Cryptococcus neoformans*, and *Pneumocystis jirovecii*, can also produce a variety of virulence factors.[273] Fungal adhesins such as Scf1 are structurally distinct from bacterial adhesins but functionally analogous.[274] Both bacterial and fungal pathogens can form biofilms composed of extracellular DNA, proteins, and polysaccharides. Biofilms act as physical and biochemical barriers that limit penetration of antimicrobial agents and enable immune system evasion.[275] Some pathogens have evolved strategies to invade and persist within host lung cells. For example, *Mycobacterium tuberculosis* infects macrophages, manipulating host signaling pathways to evade intracellular destruction and establish persistent infection.[276] Similarly, pathogenic fungi that reach the airways do not always remain extracellular. *Aspergillus fumigatus* conidia are internalized by alveolar epithelial cells and immune cells. By hijacking the host p11 protein, the fungus reroutes phagosomes into non-degradative pathways, thereby escaping intracellular destruction.[277] Unlike bacteria and fungi, viruses are always intracellular pathogens. Viruses must invade host cells and hijack the cellular machinery for replication. Viral replication disrupts normal cellular functions and induces apoptosis, inflammation, and immune responses.[278] In severe cases, viral infections can cause a cytokine storm, leading to ARDS associated with epithelial and endothelial barrier leakiness that predisposes the lung to secondary bacterial infections.[278]

Due to the significant differences between bacterial, fungal, and viral infections, the design of nanoparticle-based therapies must be infection-specific (Fig. 5). For bacterial and fungal infections, nanoparticle formulations administered by the pulmonary route are typically designed to penetrate deeply into the alveolar regions, where many pathogens reside. These nanoparticles must overcome biological barriers and protect encapsulated antimicrobial cargo (antibiotics, antivirals, or antifungals) from premature degradation.[279,280] Nanoparticles also provide the opportunity for co-delivery of multiple therapeutic agents, such as antibiotics combined with anti-inflammatory drugs or biofilm-disrupting mucolytics. This approach allows simultaneous suppression of microbial growth, virulence factor production, and pathological host responses, leading to synergistic efficacy, reduced dosage requirements, and mitigation of antimicrobial resistance.[281] In the case of viral infections, nanoparticle platforms are frequently employed for vaccine development. Vaccination remains the most cost-effective method to combat viral diseases, and nanoparticles can be engineered to produce stable, effective, and easily deliverable vaccines.[282]

A direct and widely explored nanoparticle-based approach for bacterial pneumonia involves the targeted delivery of antibiotics to the site of infection. For example, one study developed inhalable, pH-sensitive, natural polysaccharide-based nanoparticles to deliver the broad-spectrum antibiotic tobramycin.[283] The nanoparticles contained pH-sensitive imine bonds that allow tobramycin to be released preferentially in the acidic microenvironment of infection sites, effectively eradicating bacteria within biofilms.[283] Beyond antibiotic delivery, some nanoparticles can be designed to directly kill bacteria through physical and chemical

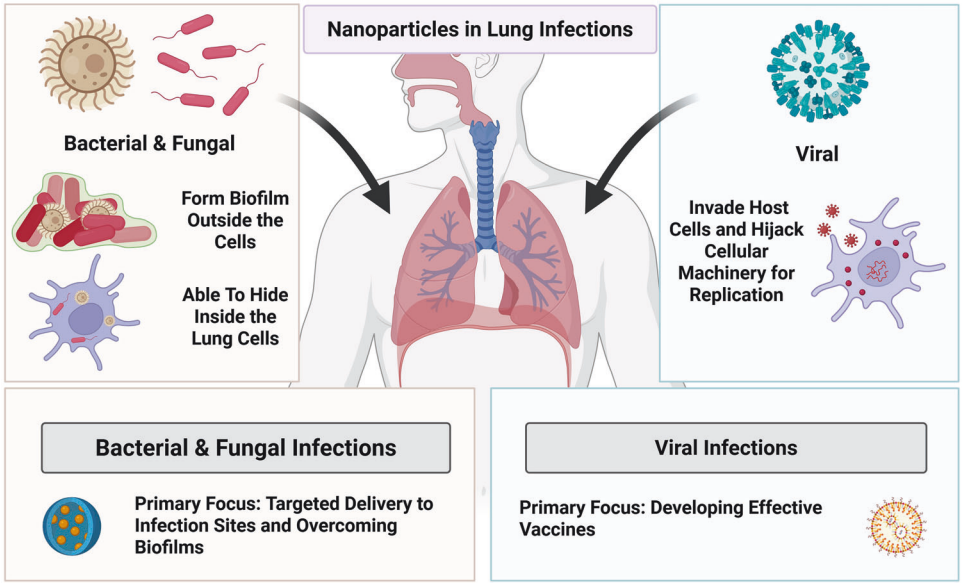

Fig. 5 Schematic shows the use of nanoparticles in pulmonary infectious diseases. Bacteria and fungi can form biofilms and persist both extracellularly and intracellularly. Nanoparticles are typically designed to target infection sites and penetrate or disrupt biofilms. Viruses invade and hijack the replication machinery in host cells, and nanoparticle-based approaches are primarily developed for vaccine delivery. Created with Biorender.com

mechanisms. Pan et al. created metal-organic framework (MOF)-derived carbon@TiO_2 nanoparticles that, when activated by ultrasound, generate reactive oxygen species (ROS).[284] The combination of ROS generation and mechanical disruption caused by ultrasound effectively eradicated even multidrug-resistant bacteria.[284] Recent studies have also explored the development of surface-biomimetic nanosystems. These systems exploit cell membrane coatings on nanoparticles to enable pathogen-specific targeting through natural receptor-ligand interactions without the need for synthetic ligands.[285] For example, Wang et al. constructed polymeric nanoparticles encapsulating an aggregation-induced emission (AIE) photothermal agent and coated the nanoparticles with membranes derived from *Mycobacterium*-stimulated macrophages. The macrophage membrane coating allowed selective targeting of granulomatous lesions and *M. tuberculosis*.[286] The photothermal agent, activated by NIR laser irradiation, produced localized hyperthermia, which enhanced bacterial eradication and reduced inflammation more effectively than conventional antibiotics.[286] Further innovations include dual-membrane coating strategies for bacterial infection therapy. A dual-membrane–coated nanosystem (MMHP NPs), created by fusion of bacterial outer membrane vesicles (OMVs) with macrophage membranes and containing an ROS-responsive hypericin prodrug, demonstrated enhanced immune evasion and prolonged circulation within inflamed lung tissue. ATP and ROS stimuli in infected areas enabled simultaneous immunomodulation and synergistic bacterial eradication in pneumonia models.[287] Additionally, Zhang et al. developed biofunctional lipid nanoparticles by coating norfloxacin-loaded lipid nanocarriers with hybrid vesicles composed of OMVs and neutrophil membrane vesicles (NMVs). This dual-targeting platform was capable of homing to inflamed endothelium and effective biofilm penetration, improving antibacterial efficacy.[288] Furthermore, the cell membrane coating can be combined with other microorganisms to enhance nanoparticle delivery efficiency. Gao et al. engineered hybrid microrobots by attaching neutrophil membrane-coated, antibiotic-loaded PLGA nanoparticles to motile microalgae (*Chlamydomonas reinhardtii*).[289] Powered by algal flagella, these microrobots navigated to deep lung regions and enabled targeted antibiotic release at infection sites. The neutrophil membrane conferred immune-evasive properties and attenuated the release of inflammatory cytokines, reducing lung inflammation. In a murine pneumonia model, a single dose of this microrobot treatment resulted in 100% survival, underscoring the therapeutic advantage of combining autonomous propulsion with anti-inflammatory surface camouflage.[289] The Hickey group developed Porous Nanoparticle-Aggregate Particles to effectively transport nanoparticles to the deep respiratory tract while avoiding the exhalation risks associated with individual nanoparticles.[290] Specifically, studies on rifampicin-loaded PLGA nanoparticle aggregates demonstrated that this formulation maintains sustained drug concentrations in lung tissue and AMs for over eight hours.[290,291] Additionally, the lab investigated spray-dried pyrazinoic acid salts, which utilize leucine or ammonium counterions to modulate local pH and improve bactericidal efficacy against *M. tuberculosis* within the intracellular environment of macrophages.[292]

Pulmonary aspergillosis, one of the most common fungal lung infections, affects immunocompromised individuals and poses major therapeutic challenges due to resilient fungal biofilms and the systemic toxicity of antifungals. To overcome these challenges, recent studies have developed nanoparticle-based delivery systems designed to improve antifungal drug targeting and efficacy. For instance, a PLGA-based polymeric nanoparticle platform encapsulating deferasirox (DFX) was shown to enhance drug accumulation in lung tissue after intravenous injection, significantly reducing fungal burden in comparison to free drug administration.[293] Another strategy employed lipid nanoparticles modified with lung-mimicking phospholipids, DPPC and dimyristoylphosphatidylglycerol (DMPG), to encapsulate the antifungal drug voriconazole (VRZ).[294] As DPPC and DMPG are components of pulmonary surfactant, their incorporation into the nanoparticle surface was capable of creating a biomimetic formulation that enhances alveolar retention and minimizes systemic drug absorption. In vivo studies demonstrated reduced fungal burden and preservation of lung architecture after the treatment, highlighting the potential of this approach for localized pulmonary antifungal therapy.[294] An alternative antifungal strategy used biosynthesized silver nanoparticles (AgNPs) derived from *Artemisia sieberi* leaf extract. These AgNPs were capable of adhering to

fungal membranes, disrupting fungal biofilms, and releasing Ag^+ ions that inhibited fungal enzymes and attenuated production of virulence factors such as gliotoxin.[295] If fungi adopt intracellular niches, targeted intracellular delivery becomes essential. For example, *Cryptococcus neoformans* can survive within macrophages, escape antifungal drugs and cause recurrent infection.[296] Amphotericin B (AMB)-functionalized polymeric nanoparticles with a unique patchy surface topology have been designed to enhance uptake by macrophages. These nanoparticles targeted intracellular *C. neoformans* via AMB binding to fungal ergosterol, enabling intracellular eradication of fungal microorganisms.[296] ALIS, marketed as Arikayce, is the only approved inhaled nanoparticle product and is indicated for refractory Mycobacterium avium complex lung disease.[58,201] This formulation effectively penetrates mycobacterial biofilms and increases amikacin concentrations in pulmonary macrophages by five to eight times compared to the free drug.[201] In addition, liposomal ciprofloxacin formulations like Pulmaquin have progressed through Phase 3 trials for non-CF bronchiectasis. These dual-release systems utilize a mixture of free and encapsulated antibiotics to provide an initial bolus of medication followed by a sustained-release component.[168]

The emergence of SARS-CoV-2 as a global health threat has highlighted the transformative potential of nanoparticle-based therapeutics in viral respiratory infections.[297] Lipid nanoparticles (LNPs) have become a central focus, driven by the success of mRNA-based COVID-19 vaccines. The BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) vaccines demonstrated outstanding clinical efficacy in phase III clinical trials, achieving 95% and 94.1% protection, respectively, against symptomatic COVID-19.[298] Key properties of LNPs contributed to this success: the nanoparticles protect and stabilize mRNA through a four-component structure consisting of ionizable lipids, phospholipids, cholesterol, and PEGylated lipids, forming a stable encapsulation matrix which maintains mRNA integrity during storage and delivery.[299] Currently, most vaccines are designed for intramuscular immunization, which is often inefficient in preventing virus infection in the upper respiratory tract due to the absence of mucosal immunity activation. Intranasal vaccines can induce strong mucosal and systemic immune responses, preventing initial infection and viral transmission at the entry site, while also offering practical benefits such as needle-free administration and ease of access for mass immunization.[300] For example, an intranasal nanoparticle vaccine comprised of cationic crosslinked carbon dots and a SARS-CoV-2 antigen elicited broad, potent, and durable mucosal and systemic immunity against Omicron and other SARS-CoV-2 variants. This vaccine activated DCs and nasal epithelial cells and demonstrated an excellent safety profile.[301] Beyond their role as delivery systems, LNPs can act as adjuvants by activating innate immune pathways through Toll-like receptors (TLR7/8), inducing type I interferons, stimulating inflammasome signaling, and creating an immunological environment favorable to adaptive immune responses.[302] Furthermore, the flexibility of the LNP-mRNA platform allows for rapid reprogramming against emerging pathogens by simply altering mRNA sequences without modifying the delivery vehicle. This is a key feature which has positioned nanoparticles as a promising tool in antiviral therapeutics. In addition to vaccines, nanoparticle-based antiviral strategies have expanded to include a range of innovative therapies. For instance, 71.[303] Antibody-conjugated LNPs, functionalized with F4/80 to target AMs, have been used to deliver siRNAs that suppress pro-inflammatory signaling pathways, thereby mitigating virus-induced pulmonary inflammation.[304] ACE2-decorated nanodecoys derived from human lung spheroid cells have been produced to act as competitive inhibitors of the virus, sequestering SARS-CoV-2 virions and blocking their entry into host cells.[305] Pulmonary delivery of AgNPs has been shown to "prime" AMs, triggering a biphasic immunomodulatory response by recruiting and activating lymphoid cells, and later suppressing excessive inflammation, thereby limiting viral replication while minimizing tissue damage.[306] These recent advancements collectively highlight the versatility and therapeutic promise of nanoparticle technologies in addressing current and future challenges in respiratory viral infections.

In conclusion, nanomedicines for lung infections regulate disease progression through various molecular strategies. Antibacterial and fungal therapies target biofilm integrity and virulence factors, while antiviral platforms utilize mRNA to activate innate immune pathways, such as TLR7/8, or siRNA to suppress pro-inflammatory signaling. These therapies reduce microbial burden and prevent tissue damage associated with respiratory infections.

Chronic obstructive pulmonary disease

COPD is one of the leading causes of morbidity and mortality from respiratory disease worldwide, with data from 2021 demonstrating its burden on public health.[307] The primary etiologic factor is prolonged exposure to inhaled toxicants, most notably cigarette smoke, which causes a persistent and dysregulated inflammatory response within the lungs.[308] Additional risk factors also influence the susceptibility to and progression of COPD, including A1ATD.[309] Chronic exposure to noxious stimuli initiates a cascade of pathogenic events, including oxidative stress, epithelial barrier dysfunction, and the aberrant activation of immune system. Oxidative stress, driven by both exogenous sources (e.g., cigarette smoke) and endogenous production by activated immune cells, not only exacerbates tissue injury but also impairs antiprotease defenses and promotes glucocorticoid resistance via histone deacetylase 2 (HDAC2) inactivation.[310] This sustained oxidative and inflammatory milieu contributes to protease-antiprotease imbalance, leading to progressive alveolar wall destruction (emphysema), and induces EMT and airway remodeling. The resulting clinical characteristics include a combination of fixed airflow obstruction and impaired alveolar gas exchange capacity specific to COPD. Importantly, neutrophil-dominated inflammation further amplifies this tissue destruction cycle through the release of proteolytic enzymes such as neutrophil elastase and MMPs, along with reactive oxygen species (ROS), contributing to alveolar and airway remodeling.[311]

Because airflow limitation in COPD is largely irreversible,[312] current pharmacologic treatments are directed at symptom management without reversing the disease course. Inhaled corticosteroids (ICS) can modestly reduce the frequency of exacerbations and improve symptoms in a subset of COPD patients. However, their overall efficacy is limited, particularly in the context of neutrophilic inflammation, which is less responsive to corticosteroid therapy. Moreover, long-term ICS use has been associated with an increased risk of pneumonia, raising safety concerns.[313,314] Phosphodiesterase-4 inhibitors, such as roflumilast, have shown modest improvements in lung function and quality of life, with improvements in FEV1 (forced expiratory volume) falling below clinically meaningful thresholds. These agents often exhibit delayed onset of action and are associated with undesirable side effects such as gastrointestinal discomfort, weight loss, and neuropsychiatric symptoms.[315] Bronchodilators, including LABAs (Long-Acting Beta-2 Agonists) and LAMAs (Long-Acting Muscarinic Antagonists), remain the cornerstone of symptom control by promoting airway smooth muscle relaxation. However, these drugs do not alter the underlying inflammatory processes or slow the disease progression.[316] Combination treatment regimens (e.g., LABA/ICS or LABA/LAMA) improve lung function and reduce COPD exacerbations but fail to meaningfully modify long-term disease trajectory.[317] Thus, while current therapies provide symptomatic relief and reduce exacerbation

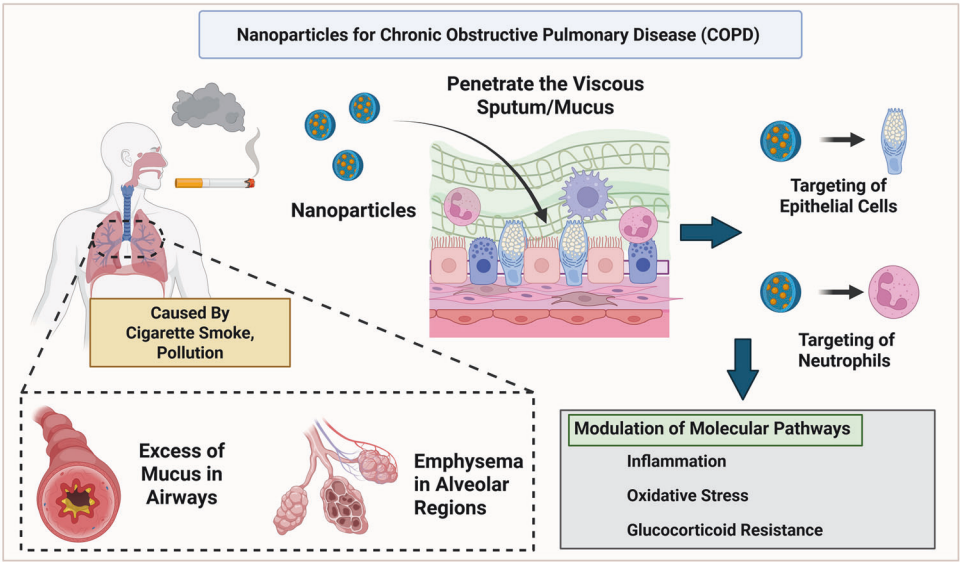

Fig. 6 Schematic shows the use of nanoparticles in chronic obstructive pulmonary disease (COPD). COPD is primarily caused by cigarette smoke and environmental pollution. In the diseased lung, excessive accumulation of viscous mucus and chronic airway inflammation create barriers for delivery of therapeutic agents. Nanoparticles are designed to penetrate the mucus layers, achieve targeted delivery to epithelial and/or inflammatory cells, and modulate key pathological processes such as inflammation, oxidative stress, and glucocorticoid resistance. Created with Biorender.com

risk, they do not prevent the progression of COPD, highlighting the urgent need for novel therapeutic strategies that specifically target the underlying pathobiological mechanisms of the disease.

Recent advances in nanoparticle-based drug delivery provide promising new avenues for future COPD treatments by enabling targeted, efficient, and sustained delivery of therapeutics to key pathological sites. The pathobiological changes in COPD, characterized by reduced airflow and increased velocities and collisions due to disturbances at airway bifurcations, intricately complicate nanoparticle deposition because of the interplay of diffusion and inertia forces.[318] Nanoparticle design for COPD therapy primarily focuses on two objectives: (1) targeted delivery to specific pulmonary cells involved in COPD pathogenesis, and (2) modulation of fundamental pathological processes such as inflammation, oxidative stress, and glucocorticoid resistance (Fig. 6).

For cell-specific targeting, nanoparticles have been engineered to direct drugs to airway epithelial cells and neutrophils, both of which are central to COPD pathogenesis. The airway epithelium acts as both a structural barrier and an immune sentinel, regulating mucociliary clearance and innate immunity.[319] Neutrophils, although critical for host defense, exacerbate COPD pathology through release of proteolytic enzymes and oxidative mediators.[320] Several studies have capitalized on these mechanisms to direct nanoparticle therapy. For instance, liposomal formulations encapsulating corticosteroids have been developed to directly target the airway epithelium. Leo et al. engineered unilamellar liposomes (40–65 nm) capable of penetrating the viscous sputum of COPD patients and delivering anti-inflammatory agents directly to dysfunctional airway epithelial cells.[321] Similarly, neutrophil-targeting nanoparticles, such as PLGA-based nanoparticles functionalized with polyethene glycol and conjugated with neutrophil-specific antibodies (PINPs), have been employed to selectively deliver drugs to activated neutrophils, thereby mitigating lung tissue injury.[322]

A major therapeutic approach involves nanoparticle-mediated modulation of key molecular pathways underlying COPD pathogenesis. Inflammation, oxidative stress, and glucocorticoid resistance remain central, unresolved issues in disease progression. To counter inflammation, nanocomposite microparticles (NCMPs) loaded with miR-146a microRNA were formulated using spray-drying techniques. MiR-146a inhibits inflammatory signaling by targeting IRAK1 and TRAF6, suppressing cytokine expression in preclinical models.[323] To address oxidative stress, dimethyl fumarate, a potent Nrf2 activator,[324] has been incorporated into inhalable microparticulate/nanoparticulate systems to restore antioxidant defense mechanisms by activating the Nrf2/Keap-1 pathway. These particles were engineered for improved aerosol dispersion with deep deposition into the lung tissue.[325] Natural compounds with anti-inflammatory and antioxidant properties, such as agarwood oil and berberine, are limited by poor bioavailability when delivered conventionally. To overcome this limitation, nanoemulsions based on poloxamers have been created for agarwood oil.[326] Berberine has been incorporated into liquid crystalline nanoparticles, improving pulmonary absorption and therapeutic efficiency.[327] Another innovative strategy is based on targeting glucocorticoid resistance by delivering plasmid DNA encoding HDAC2 using lipid-polymer hybrid nanoparticles. By restoring HDAC2 expression, these nanoparticles re-sensitize immune cells to corticosteroids and increase anti-inflammatory efficacy.[328]

Acute exacerbations of COPD are frequently triggered by bacterial infections, further accelerating lung injury. Thickened mucus and biofilm formation facilitate bacterial colonization and persistence. To address this issue, bacteria-specific delivery platforms have been developed. Zhu et al. developed hollow mesoporous silica nanoparticles loaded with ceftazidime and coated with a pH-responsive polypeptide. These nanoparticles remain stable during aerosolization but undergo conformational changes in the acidic biofilm environment. After interaction with biofilm, the nanoparticles become cationic, disrupt the biofilm, and release the antibiotic payload.[329] Similarly, black phosphorus quantum dots have been used in mucus/biofilm-penetrating systems. Once the quantum dots enter the biofilm microenvironment, these nanoparticles release phosphate and protons, locally acidifying the environment. This pH change destabilizes the PEG shell on nanoparticles and accelerates drug release (e.g., amikacin) at the site of infection.[330] Another approach utilizes inhalable nanoparticles coated with neutrophil membranes that retain most native membrane proteins. These biomimetic nanoparticles

exhibited enhanced mucus penetration, prolonged retention in the lung tissue, decreased macrophage phagocytosis, and efficient delivery of levofloxacin.[331] Furthermore, the nanoparticles neutralized pro-inflammatory cytokines, decreased inflammation, and reduced lung remodeling in murine COPD models.[331]

In conclusion, nanomedicines for COPD focus on targeting specific molecular pathways to decrease disease progression. By delivering agents such as miR-146a to inhibit IRAK1 and TRAF6, Nrf2 activators to restore antioxidant defenses, and HDAC2 plasmid to restore HDAC2 expression, these nanoparticles address the primary drivers of neutrophilic inflammation, oxidative stress, and glucocorticoid resistance. These molecular-level interventions target the protease-antiprotease imbalance and EMT, ultimately aiming to reduce airway remodeling and improve pulmonary function.

Pulmonary vascular diseases

PVDs represent a diverse group of life-threatening conditions that involve structural and functional alterations in pulmonary arteries, veins, lymphatic vessels, or capillaries and are characterized by abnormal vascular remodeling and dysfunction of the pulmonary vessels. These disorders, particularly PH, affect millions worldwide and continue to pose substantial therapeutic challenges despite significant advances in the understanding of their molecular mechanism. PH affects ~1% of the global population and is a frequent and clinically important consequence of many chronic respiratory and cardiovascular diseases.[332] Hemodynamically, PH is defined by an elevated mean pulmonary arterial pressure (mPAP) greater than 20 mmHg, measured by right heart catheterization.[333] Among PVDs, PH represents the most extensively investigated and clinically significant form. Based on pathophysiological mechanisms, clinical presentation, hemodynamic characteristics, and therapeutic strategies, PH is classified into five major groups: Group 1, PAH; Group 2, PH associated with left heart disease; Group 3, PH associated with chronic lung diseases and/or hypoxia; Group 4, PH due to pulmonary artery obstructions; and Group 5, PH with unclear and/or multifactorial mechanisms.[334] PH occurs due to pulmonary embolism (PE) and pulmonary veno-occlusive disease (PVOD). PE causes an acute rise in pulmonary artery pressure, and in ~2.7% of survivors, this may progress to chronic thromboembolic pulmonary hypertension (CTEPH).[335] PVOD is a rare form of PAH characterized by remodeling of small pulmonary veins and capillaries, which inherently presents with pulmonary hypertension and carries a poor prognosis.[336]

Endothelial cells lining the pulmonary vasculature are the first to respond to hemodynamic stress, environmental injury, and inflammatory insults, placing them at the center of PVD pathogenesis, particularly in PH.[337] Under normal physiological conditions, endothelial cells maintain vascular homeostasis by producing vasodilatory mediators such as nitric oxide and prostacyclin, regulating vascular permeability, and exerting antithrombotic effects. In PVD, one of the earliest pathological events is the development of endothelial dysfunction. During this process, endothelial cells lose their normal functional properties, including vasodilation, barrier integrity, antithrombotic properties, and undergo functional reprogramming.[338,339] This maladaptive reprogramming triggers a cascade of molecular changes that drive pathological vascular remodeling which can result in occlusion of pulmonary blood vessels. Multiple signaling pathways and transcriptional regulators act in synchrony to accelerate endothelial dysfunction, cellular phenotype alterations, and progress vascular damage in PH. Understanding these molecular mechanisms has revealed potential therapeutic targets. One well-characterized mechanism in PH is the endothelial-to-mesenchymal transition (EndMT), in which endothelial cells lose their canonical markers and gain fibroblast-like, migratory properties.[340] Transforming growth factor-beta (TGF-β) signaling plays a central role in PH pathogenesis, particularly in PAH.[341] Mutations in BMPRII, a receptor from the TGF-β superfamily, have been found in over 70% of familial PAH cases and approximately 20% of sporadic cases, highlighting the importance of the BMPRII signaling pathway.[341] SOX17, a transcription factor from the SRY-box family, protects against PAH by suppressing EndMT. Decreased expression of SOX17 seen in PAH patients and PH animal models leads to the activation of Rho-associated protein kinase 1 (ROCK1), which promotes EndMT and contributes to PAH progression.[342] Similarly, FOXF1, a transcriptional regulator expressed in pulmonary endothelial cells, is markedly diminished in PH associated with neonatal PVDs.[343] Loss of FOXF1 contributes directly to persistent DNA damage,[344] defective angiogenesis,[345,346] and impaired cell migration.[347] Restoration of FOXF1 levels in endothelial cells from PAH patients repairs DNA damage, reinstates angiogenic capacity, and improves cell motility.[344] These restorative effects position FOXF1 as a promising target for reversing PH-related endothelial injury.[344] Beyond endothelial cells, other vascular cell types also play a key role in PH pathogenesis. Pulmonary artery smooth muscle cells undergo phenotypic transformation from a quiescent, contractile phenotype to a proliferative, apoptosis-resistant state. This process drives pulmonary vascular remodeling and muscularization of distal pulmonary arterioles. The phenotypic shift arises primarily from an imbalance between downregulated antiproliferative bone morphogenetic protein signaling and upregulated proliferative TGF-β signaling.[348]

Due to the central role of endothelial cells in the development and progression of PVD, particularly PH, most nanoparticle-based delivery systems are designed to specifically target the pulmonary endothelium and perivascular stromal cells. PH is frequently associated with underlying genetic mutations or dysregulated gene expression, including the loss of endothelial-specific markers, disrupting normal vascular homeostasis. As a result, nanoparticles have emerged as promising vehicles for gene therapy and gene editing applications in PVD (Fig. 7). By delivering therapeutic nucleic acids, nanoparticles can restore the expression of protective genes or repair disease-causing mutations. These approaches aim to correct molecular defects at their source, potentially offering long-term or curative solutions.

A key pathological process in PH involves the dysregulation of the TGF-β signaling pathway. Oxidative stress, characterized by increased reactive oxygen species (ROS), plays a critical role in PH pathogenesis by inducing cell proliferation, DNA damage, and activating the TGF-β1/BMP pathway. Non-metallic nanozymes, such as melanin-polyvinylpyrrolidone-polyethylene glycol nanoparticles, are designed to combat oxidative stress because of their strong antioxidant capacity, effectively scavenging ROS in pulmonary artery smooth muscle cells. The beneficial effects of these nanoparticles, including reducing PASMC proliferation and migration, attenuating pulmonary vascular remodeling, and improving right ventricular function, are attributed to inhibiting the ROS-driven activation of the TGF-β1 pathway.[349] In another study, hydrogen-generated MOF nanoparticles with high hydrogen storage and capacity for controlled and continuous release of hydrogen gas were used.[350] The hydrogen gas eliminates excessive ROS, prevents DNA damage and reverses excessive proliferation and migration of dysfunctional endothelial cells.[350] Gene therapy strategies targeting the TGF-β/BPM superfamily signaling pathway have also demonstrated promise. For instance, lipid nanoparticles engineered for targeted delivery of BMPR2 mRNA to pulmonary endothelial cells increase BMPR2 downstream signaling through phosphorylation of SMAD1/5/9 and induction of ID1 expression that are key regulators of vascular homeostasis.[351] In addition to the TGF-β/BMP pathway, EndMT is recognized as a major contributor to PH progression. Downregulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) has been observed in pulmonary

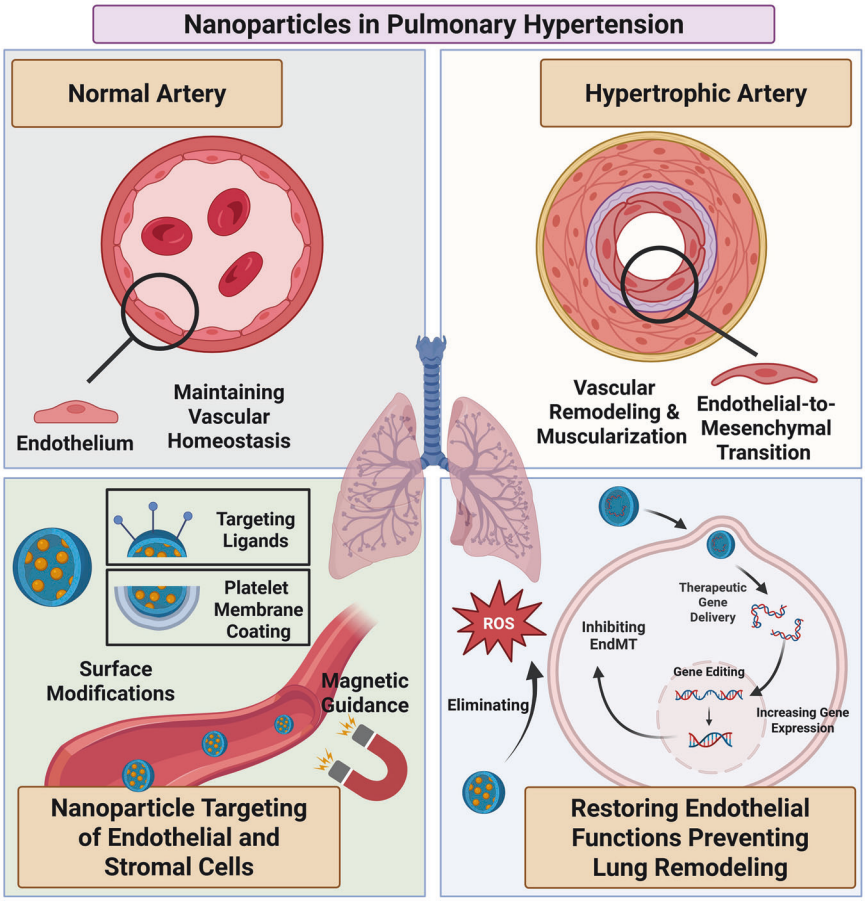

Fig. 7 Schematic shows the use of nanoparticles in Pulmonary Hypertension. In healthy pulmonary arteries, endothelial cells maintain vascular homeostasis. In pulmonary hypertension, endothelial dysfunction promotes vascular remodeling, muscularization, and endothelial-to-mesenchymal transition (EndMT). Nanoparticles can be engineered to selectively deliver therapeutics to pulmonary endothelial and perivascular stromal cells. These nanoparticles can restore normal cellular functions by reducing oxidative stress (ROS), inhibiting EndMT, and preventing vascular remodeling. Created with Biorender.com

arterial endothelial cells from PH patients and in preclinical animal models.[352] Recently, nanoparticles have been constructed to deliver PGC-1α plasmids under control of endothelial-specific promoters, stimulating PGC-1α expression and inhibiting EndMT. This strategy aimed to preserve endothelial identity, restore endothelial nitric oxide synthase expression, and increase nitric oxide production that is critical to reduce pulmonary vascular stiffness and remodeling.[352]

To improve therapeutic applicability, several nanoparticle systems were designed to incorporate specific targeting mechanisms. E-selectin, which is upregulated on pulmonary endothelial cells under hypoxic conditions, has been explored for selective targeting in hypoxia-induced PAH. In this context, sialic acid-modified, hypoxia-responsive nanoparticles were developed to anchor to E-selectin, facilitating intracellular transport to pulmonary artery smooth muscle cells and releasing the endothelin receptor antagonist, ambrisentan, in response to hypoxia-induced nitroreductase activity. This dual-targeted, stimuli-responsive nanoparticle system effectively reduces smooth muscle proliferation, media thickening, and pulmonary vasoconstriction, while minimizing systemic toxicity.[353] Similarly, hypoxia leads to increased expression of von Willebrand factor (vWF) and exposure of subendothelial collagen in injured endothelial cells. Platelet membrane-coated nanoparticles have been employed to exploit this pathological change. The membrane proteins GPIbα and GPVI on platelets bind specifically to vWF and exposed collagen, respectively, thereby enabling precise binding to injured endothelium in hypoxic PH.[354] Magnetic targeting offers another promising strategy for enhancing delivery precision for pulmonary vascular disorders. In pulmonary vein stenosis (PVS), rapamycin-loaded SPIONs were utilized with an external magnetic field to achieve targeted delivery. This approach aims to deliver the mTOR inhibitor, rapamycin, precisely to the site of neointimal proliferation (e.g., at the bifurcation point) to inhibit vascular cell overgrowth, reducing restenosis and preventing systemic side effects.[355] Notably, recent findings showed that nanoparticles with carefully tuned physicochemical properties can achieve pulmonary endothelial cell targeting even without biological ligands or external guidance.[223] These findings underscore the potential of physicochemical design principles as a viable strategy for targeted delivery in PVD therapy.

Treprostinil palmitil (TP) is a hydrophobic prodrug initially developed as a nebulized lipid nanoparticle suspension (TPIS).[356] To enhance patient convenience and reduce administration time, the formulation was transitioned to a dry powder inhaler format (TPIP), which maintains a sustained release of the active vasodilator treprostinil in the lung over a 24-hour period.[356,357] Data from Phase 1 clinical trials demonstrated that TPIP is well-tolerated with a pharmacokinetic profile supporting once-daily dosing, leading to current Phase 2 clinical trials in patients with PAH and pulmonary hypertension associated with interstitial lung disease (PH-ILD).[357] These advancements represent a significant effort to overcome critical biological barriers in PVD.

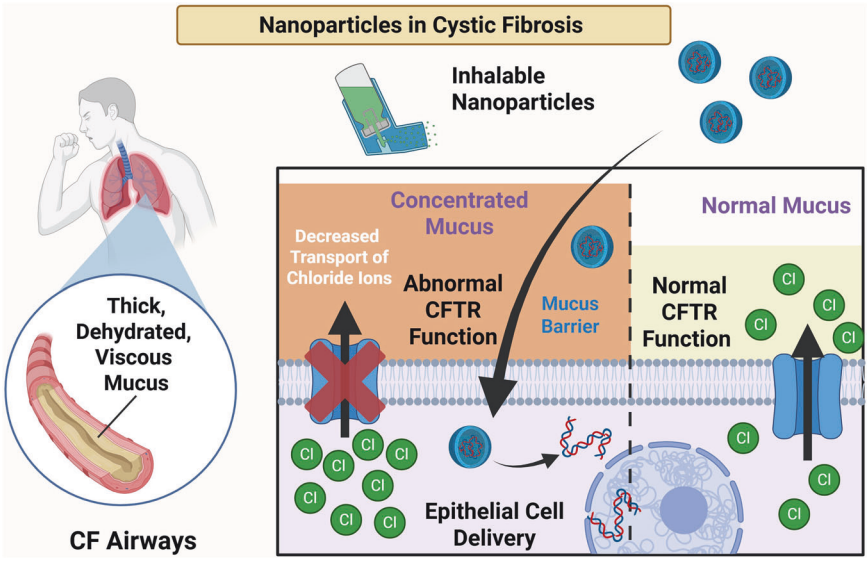

Fig. 8 Schematic shows the use of nanoparticles in cystic fibrosis (CF). Nanoparticles are engineered as inhalable carriers that stabilize therapeutic nucleic acids, penetrate the thickened mucus barrier, and deliver genetic material to airway epithelial cells. Successful expression of functional CFTR restores chloride ion transport across the epithelial membrane, thereby improving airway hydration and mucus clearance. Created with Biorender.com

In conclusion, nanomedicines for PVD therapy prioritize the regulation of key signaling pathways and transcriptional factors critical for pulmonary vascular development and homeostasis. Specifically, nanoparticles designed to restore FOXF1 levels target the repair of DNA damage and angiogenic capacity, whereas those delivering PGC-1α plasmids aim to suppress EndMT and restore nitric oxide levels in pulmonary endothelial cells. Systems that deliver BMPR2 mRNA or scavenge ROS via non-metallic nanozymes target the TGF-β/BMP signaling axis, which is central to smooth muscle cell proliferation and vessel wall thickening. By intervening at these specific molecular levels, these nanomedicines provide a means to attenuate smooth muscle proliferation and mitigate the progressive vascular remodeling that characterizes PH.

Cystic fibrosis

CF is an autosomal recessive genetic disease caused by loss-of-function mutations in the CFTR. This disease mostly affects epithelial compartments in the digestive, reproductive and respiratory systems. The CFTR protein functions as a chloride anion channel that is activated via phosphorylation by cyclic adenosine monophosphate (cAMP)-dependent protein kinases. Upon activation, this channel facilitates the transport of chloride ions out of the cell. Mutations in the CFTR gene result in a dysfunctional protein that either fails to properly transport chloride ions or does not localize to the cell membrane. Consequently, aberrant chloride ion accumulation occurs within epithelial cells, creating an osmotic gradient that dehydrates mucus.[358] The thick mucus in the airways of CF patients leads to repeated episodes of infection and inflammation, progressing to respiratory failure. Recently, there was a breakthrough in the development of modulator therapy. Trikafta modulators, which combine Tezacaftor (VX-661, type I corrector), Ivacaftor (VX-770, channel potentiator), and Elexacaftor (VX-445, dual-function modulator), are beneficial for CF caused by the predominant CF mutation, a deletion of phenylalanine 508 in the CFTR gene.[359] Approximately 85.5% of patients with CF in the United States have the phenylalanine 508 gene variant, also known as F508del.[19,360] Although, the novel modulator therapy provided unprecedented clinical benefits for over 90% of patients with CF, ~10% of CF patients (including those with large CFTR deletions that include the promoter and intronic regions of the gene) are ineligible for CFTR modulator therapy.[361] To address this clinical problem, enormous efforts are being put into gene replacement and gene editing strategies to restore CFTR expression and function.

Nanoparticle-based gene delivery represents a promising approach to correct the genetic defect in CF by restoring the expression of functional CFTR.[362] The primary focus of nanoparticle design for CF is the development of inhalable formulations capable of stabilizing CFTR-encoding nucleic acids, penetrating the thickened airway mucus layers, and delivering the nanoparticle's payload to airway epithelial cells (Fig. 8).

Lipid nanoparticles (LNPs) are among the most widely investigated nanocarriers for CF gene therapy.[363] Intranasal delivery of LNPs encapsulating CFTR mRNAs to CFTR knockout mice restored up to 55% of net chloride efflux, demonstrating the therapeutic potential of LNP-based nucleic acid delivery.[364] However, effective translation of this strategy into clinical therapy requires a careful design of LNPs to overcome the unique biophysical properties of CF mucus, which is substantially more adhesive, hyperviscoelastic, and structurally heterogeneous than healthy airway mucus. CF mucus is more concentrated and viscous, which reduces pore sizes and restricts the mobility and diffusion of nanoparticles in human airways.[365] Compared to normal airways, LNPs show little to no movement in CF airways free of mucus, making the smaller size of nanoparticles pivotal for delivery.[365] Furthermore, the density of PEG on the LNP surface significantly influences mucus penetration. Higher PEG density increases shear resistance and enhances the ability of LNPs to diffuse through thick mucus. For example, LNPs incorporating β-sitosterol and enriched with PEG lipids demonstrated superior diffusion through the mucus.[366] Nevertheless, some studies suggest that in highly heterogeneous and polymicrobial CF mucus, PEGylation may act as a lubricant without adding a substantial advantage for mucus penetration.[367] Particle size and intrinsic surface chemistry may play a more dominant role in overcoming mucus obstruction.[367] In addition to nanoparticle engineering, mucolytic agents, such as dornase alfa (Pulmozyme®), an FDA-approved nebulized DNase I enzyme, have been shown to degrade extracellular DNA in CF mucus and decrease sputum viscoelasticity.[368] Therefore, mucolytic agents can be

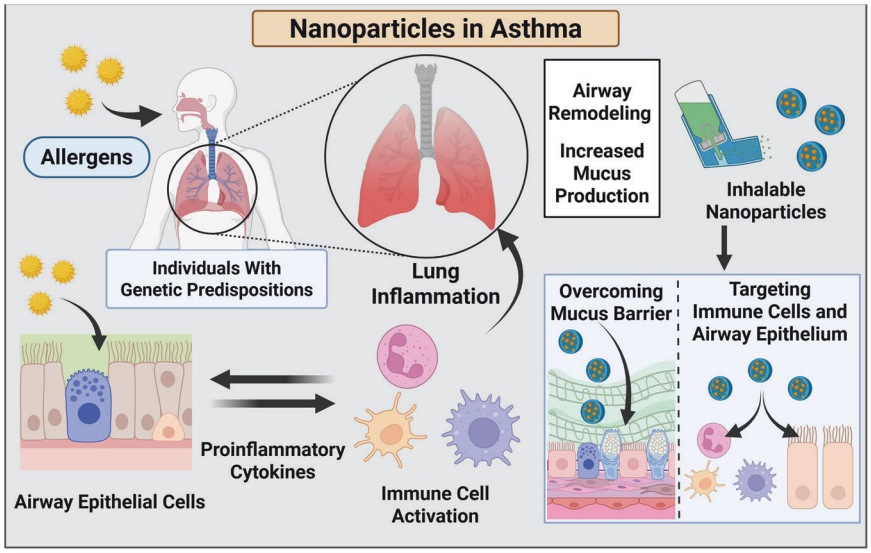

Fig. 9 Schematic shows the use of nanoparticles in asthma. Allergens trigger the release of proinflammatory cytokines by immune epithelial cells, particularly in individuals with a genetic predisposition, leading to immune cell responses and lung inflammation. This aberrant inflammatory signaling results in airway remodeling and increased mucus production. Inhalable nanoparticles are capable of overcoming the mucus barrier and selectively target immune cells and airway epithelium, improving therapeutic outcomes in asthma. Created with Biorender.com

effective in improving the diffusion and targeting efficiency of LNP-based therapies.

Beyond LNPs, polymeric nanoparticles are also being explored as gene delivery systems for CF therapy. Guan et al. developed a nanocarrier composed of poloxamine-based block copolymers combined with targeting peptides to deliver CFTR plasmids or CFTR mRNA. This system achieved a long-term increase of CFTR function in bronchial epithelial cells from CF patients carrying the F508del mutation (CFBE-delF cells), as well as in murine models of CF.[369] While pulmonary administration is the preferred route for nanoparticle delivery in CF due to localized targeting, some researchers are exploring systemic delivery to bypass the thick mucus barrier entirely. For example, PLGA nanoparticles encapsulating peptide nucleic acids (PNAs, a hybrid molecule with a peptide backbone and nucleobases that can undergo base pairing with DNA and RNA) were designed to specifically target CF with the F508del mutation.[370] Intravenous administration of these nanoparticles successfully corrected CFTR function and restored transepithelial chloride secretion in preclinical models, demonstrating that systemic delivery can also be a viable approach in CF.[370] This study demonstrated that systemic delivery of nanoparticles is feasible to develop future therapies for CF patients.

In conclusion, nanomedicines for CF therapy focus on restoring functional CFTR expression by delivering CFTR mRNA or DNA to airway epithelial cells. These therapies restore transmembrane chloride transport and improve mucus hydration, thereby mitigating the production of thick mucus and subsequent cycles of infection and inflammation in CF.

Asthma

Asthma is a chronic respiratory disease characterized by airway inflammation and remodeling driven by a complex interplay of immune and inflammatory mechanisms.[371] The process typically begins when airway epithelial cells encounter allergens or irritants and release innate cytokines such as IL-33, IL-25, and TSLP, which activate T helper 2 (TH2) cells that serve as central orchestrators of the allergic response. These TH2 cells produce key inflammatory mediators including IL-4, IL-5, and IL-13, which drive eosinophilic inflammation, promote IgE synthesis, and induce goblet cell metaplasia leading to increased mucus production.[372,373] Macrophages play a dual role in asthma pathogenesis. Initially recruited to clear allergens, these macrophages later adopt an activated M2 phenotype that produces pro-inflammatory mediators, recruits additional immune cells and contributes to airway remodeling through secretion of TGF-β and MMPs.[374] Mast cells migrate from the submucosa to the airway epithelium where they degranulate in response to allergen exposure, releasing histamine and proinflammatory mediators that cause bronchoconstriction and increased vascular permeability.[375] Neutrophils, particularly prominent in severe and steroid-resistant asthma, contribute to pathogenesis through release of neutrophil elastase and formation of extracellular DNA traps (NETs) that damage airway epithelium and promote mucus hypersecretion.[376] Current asthma treatment follows a stepwise approach with inhaled corticosteroids serving as the most effective long-term control medications to reduce airway inflammation, combined with bronchodilators such as albuterol for quick symptom relief.

Nanoparticle-based therapeutic strategies have emerged as promising alternatives to current asthma treatment with the overall goal of overcoming existing treatment limitations, including suboptimal drug delivery, insufficient control of inflammation, and the persistence of steroid-resistant disease.[377] Approximately 5–10% of patients exhibit severe, corticosteroid-resistant asthma, which fails to respond to conventional therapies.[378] In addition, the thickened mucus barrier characteristic of asthmatic airways traps conventional drug particles, leading to rapid mucociliary clearance and limited drug deposition at the intended sites of action.[177] Specialized nanoparticle formulations offer multiple advantages, including the ability to penetrate mucus barriers, target specific cell types, and provide sustained and localized drug release. These features improve therapeutic efficacy and reduce systemic adverse effects (Fig. 9).

Because immune cells are central to asthma pathogenesis, nanoparticle-based therapies for asthma have been developed to selectively modulate these cell populations. One example is the use of hybrid nanoparticles loaded with dexamethasone and coated with monophosphoryl lipid A to specifically target DCs and macrophages. These nanoparticles induce the differentiation of tolerogenic DCs and M2 macrophages, promote regulatory T-cell generation, and significantly alleviate airway inflammation in murine models of asthma by suppressing immune responses.[379] Another innovative strategy involves targeting M2 macrophages directly. Researchers have engineered exosome membrane-

coated polylactic-co-glycolic acid (PLGA) nanoparticles (EM-PLGA) that mimic natural surface properties of M2 macrophages. These biomimetic carriers specifically home to M2 macrophages. When loaded with a Dnmt3aos smart silencer, these EM-PLGA@Dnmt3aos nanoparticles significantly reduced lung inflammation and decreased the abundance of M2 macrophages in a murine model of allergic asthma.[380] Additionally, airway epithelial cell-specific delivery has been achieved through the development of lipid nanoparticles (LNPs) targeting intercellular adhesion molecule-1 (ICAM-1) receptors on the apical surface of airway epithelial cells.[381] The targeting strategy mimics the natural entry of rhinoviruses, a major trigger of asthma exacerbations, that enter airway epithelial cells via ICAM-1 receptor-mediated endocytosis, making this adhesion molecule an ideal target for therapeutic intervention. When loaded with siRNA against thymic stromal lymphopoietin (TSLP), these LNPs can reduce the secretion of proinflammatory cytokines including IL-4 and IL-13, decreasing inflammatory cell infiltration and mucus production in ovalbumin-challenged mice.[381]

In addition to targeting specific cell types, nanoparticles have been employed to overcome physical barriers in the inflamed lung. Gold nanoparticle-loaded macrophages have emerged as cellular carriers capable of crossing lung barriers and delivering therapeutic agents to inflamed sites.[382] The procedure involves loading macrophages with gold nanoparticles ex vivo, followed by the macrophage transfer to ovalbumin-challenged mice, where the nanoparticle-loaded macrophages can navigate through lung tissues and accumulate at sites of inflammation. This cellular delivery system capitalizes on the inherent ability of macrophages to cross biological barriers and their natural tropism for inflammatory sites, making them ideal vehicles for targeted drug delivery in respiratory diseases.[382] Furthermore, hydrophilic PEG nanoparticles have been employed to enhance mucus penetration. The PEG shield prevents interaction with mucin fibers, preventing particle adhesion to mucus.[383] Fc receptor-binding peptide (FcBP)-functionalized PEG nanoparticles have been developed to penetrate both mucus and epithelial barriers through receptor-mediated transcytosis.[384] By combining mucus-penetrating properties with epithelial transcytosis capabilities, these dual-functional nanoparticles first diffuse through the mucus layer and then engage Fc receptors on airway epithelial cells to undergo receptor-mediated transcytosis, thereby significantly improving transepithelial transport and pulmonary retention of therapeutic agents.[384].

In conclusion, nanomedicines for asthma focus on modulating immune responses by targeting specific cell populations, such as DCs, M2 macrophages, and airway epithelial cells. By delivering payloads like dexamethasone, Dnmt3aos smart silencers, and TSLP-specific siRNA, these therapies target the molecular drivers of pulmonary inflammation. These interventions directly address asthma pathophysiology by suppressing pro-inflammatory cytokine secretion and decreasing mucus production.

Lung cancer

Lung cancer remains the leading cause of cancer-related deaths worldwide, responsible for 18% of all cancer-related mortalities and representing 11% of global cancer cases.[385] Lung cancer can be broadly categorized into two major histological subtypes: NSCLC and small cell lung cancer (SCLC).[386] NSCLC accounts for ~85% of all cases and consists of adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. In contrast, SCLC, which comprises ~15% of lung cancer cases, is characterized by rapid growth, early metastasis, and a strong association with smoking.[386] The etiologies for lung cancers include cigarette smoking and exposure to other carcinogens and environmental pollutants.[387,388] On the other hand, mutations and genetic predispositions are important etiologic factors in subjects who develop lung cancer but are not exposed to pollutants or smoke.[387,389]

At the molecular level, lung cancers arise from a complex interplay of genetic and epigenetic alterations. Tumorigenesis is often driven by the loss of tumor suppressor genes, such as TP53,[390] PTEN,[391] and NF1,[392] and the activation of oncoproteins such as KRAS[393] and EGFR,[394] resulting in the dysregulation of cell proliferation and survival pathways. In lung adenocarcinomas, the simultaneous inactivation of NF1, RASA1,[395] and PTEN contributes to synergistic oncogenic signaling and tumor development. In SCLC, frequent mutations in TP53 and RB1 facilitate cell cycle dysregulation and resistance to apoptosis.[396] Moreover, intratumor heterogeneity, especially pronounced in squamous cell carcinoma, poses significant challenges for effective targeted therapy due to diverse mutational landscapes.[397]

Treatment options for lung cancers vary and are based on tumor subtype and stage. For early-stage NSCLC, surgical resection remains the primary treatment.[398] However, it is often combined with adjuvant therapies such as chemotherapy or immunotherapy to improve the likelihood of cure and reduce the risk of recurrence.[399] For advanced NSCLC, treatments often include platinum-based chemotherapy, radiation therapy, immunotherapy (e.g., PD-1/PD-L1 inhibitors), and targeted therapies such as tyrosine kinase inhibitors (TKIs) for tumors harboring mutations in EGFR and ALK.[389,400] SCLC is typically managed with a combination of platinum-based chemotherapy and radiotherapy.[401] Although immune checkpoint inhibitors such as pembrolizumab and nivolumab, have improved outcomes in subsets of patients, the overall prognosis for lung cancer remains poor.[402] This is largely due to late-stage diagnosis, limited drug efficacy, and side effects of available therapies. In SCLC, despite initial responsiveness to treatment, relapses are common and long-term survival remains rare.[403]

Current research efforts are directed toward addressing the major limitations of conventional lung cancer therapies, including high systemic toxicity, poor tumor specificity, and the emergence of drug resistance.[404] Consequently, the optimization of nanoparticles has become a central focus. Key strategies involve the design of novel, multifunctional nanoparticles capable of selectively targeting tumor cells, navigating biological barriers through tailored administration routes, enabling controlled and site-specific release of therapeutic agents, and supporting early-stage detection of lung cancer to enhance synergistic treatment outcomes (Fig. 10).

Since lung cancer cells often express specific biomarkers distinguishable from normal cells, nanoparticles with tumor-targeting ligands have been designed to enhance targeting efficiency. For example, programmed death ligand 1 (PD-L1) is commonly upregulated in NSCLC, which enables immune evasion through checkpoint suppression. Using nanoparticles conjugated with PD-L1 antibody, PLK1 inhibitors can be delivered to target PD-L1-expressing lung cancer cells. The enhanced delivery significantly reduced tumor growth, prolonged survival, and decreased drug resistance in immune checkpoint inhibitor-refractory lung cancer models.[405] Similarly, CD47 is overexpressed in NSCLC, assisting tumor cells in evasion of macrophage phagocytosis. To explore these mechanisms, an antibody-conjugated drug-loaded nanotherapeutic platform has been recently developed using antibodies against CD47 and PD-L1.[406] These nanocarriers conjugated with both anti-CD47 and anti-PDL1 for lung cancer cell recognition were encapsulated with PI-103, a dual PI3K/AKT/mTOR pathway inhibitor, to suppress tumor growth.[406] In addition to antibodies, other molecules can be functionalized onto nanoparticles for lung cancer targeting. For example, epithelial cell adhesion molecule (EpCAM) is moderately to highly expressed in over 70% of NSCLC tumors. The modification of three-way junction RNA nanoparticles with EpCAM aptamers can enable targeted siRNA delivery to EpCAM-positive epithelial tumors residing in the lung.[407] FA is widely used for nanoparticle modifications due to the overexpression of FR α on

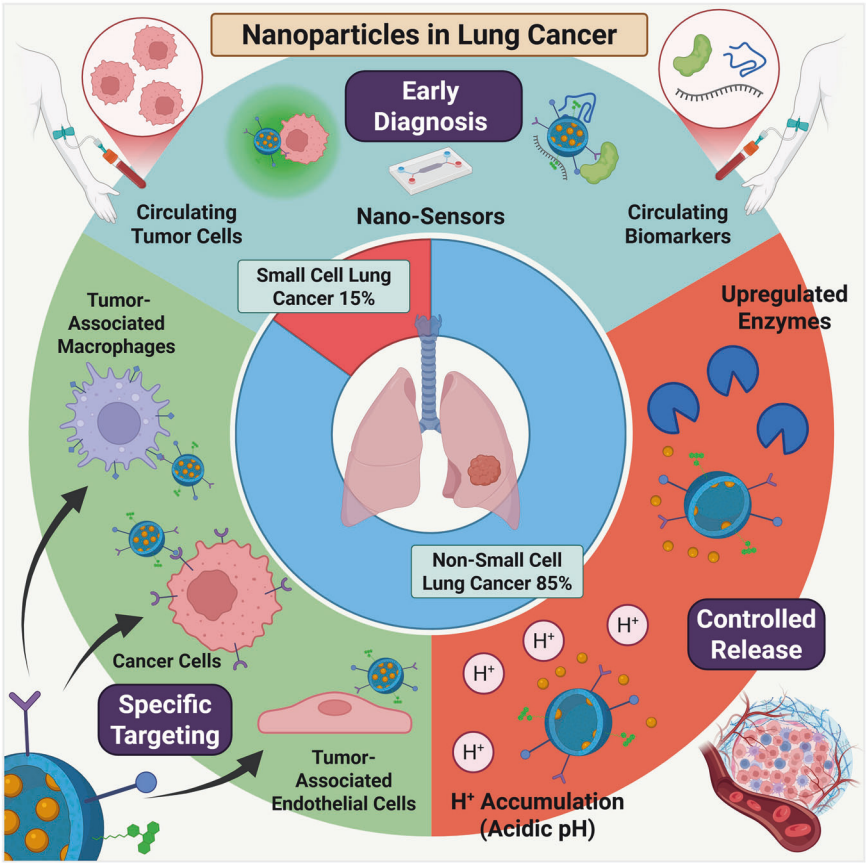

Fig. 10 Schematic shows the use of nanoparticles in lung cancer. Nanoparticle delivery systems enable precise targeting of tumor cells and the tumor microenvironment, including tumor-associated macrophages and endothelial cells, for precision therapy. Nanoparticles can also control cargo release at the tumor site, responding to lower pH and specific enzymes. Specially designed nanoparticles can be used for early detection of lung cancers. Created with Biorender.com

lung cancer cells.[408] FA-functionalized nanoparticles were used to co- deliver gefitinib and capsaicin, which led to enhanced efficiency of drug targeting, and increased apoptosis of cancer cells.[409] Ferroptosis is a regulated cell death pathway that can be utilized to enhance tumor death. To induce this pathway, folate-modified liposomes were used to deliver the lncRNA pseudogene metallothionein 1D to enhance Erastin activity, a known inducer of ferroptosis.[410] Similarly, hyaluronic acid is known to specifically bind to CD44, which is overexpressed in many types of malignant cancers, including lung cancers. Using hyaluronic acid modification, CD44-mediated active targeting resulted in an ~3.8-fold higher accumulation of docetaxel in tumor tissues, improving tumor suppression in vivo.[411] Using this CD44-hyaluronic acid-mediated endocytosis pathway, enhanced targeting of iron-platinum nanoparticles was capable of overcoming tyrosine kinase inhibitor resistance by inducing ferroptosis in metastatic lung cancer cells.[412] Chondroitin sulfate is a natural glycosaminoglycan which also binds to CD44 receptors. Self-assembling chondroitin sulfate-based nanoparticles can be used for drug delivery in lung cancers.[413] Additionally, these CD44-chondroitin sulfate nanoparticles were capable of targeting the Golgi complex and inhibiting production of multiple metastasis-associated proteins, which significantly inhibited tumor metastasis.[414]

Pulmonary administration provides high local nanoparticle delivery to lung tumors while minimizing systemic exposure to anti-cancer drugs. A recent study discussed five essential elements for nanoparticle targeting of lung cancers. The pulmonary administration route enables the local accumulation of therapeutic agents in the lung tissue, significantly minimizing systemic toxicity and protecting healthy tissues.[415] Many studies have focused on developing nanoparticles suitable for pulmonary delivery in lung cancer therapy. For example, a recent study reported the production of liposomal nanoparticles co-delivering osimertinib (a third-generation EGFR tyrosine kinase inhibitor) and a gene therapy payload.[416] This formulation was optimized for inhalation-based delivery to treat primary lung tumors and lung metastases. The nanoparticle size was between 100 and 200 nm, which is considered optimal for deep lung deposition where tumors commonly reside. This inhalable nanoparticle system significantly inhibited tumor growth in both primary and metastatic lung cancer models.[416] The targeting ligands are also used with inhalable nanoparticles to further improve targeting efficiency. For example, one research group has developed biodegradable PLGA-PEG nanoparticles that can maintain the structural integrity, spherical morphology, and sizes between 170 and 200 nm during nebulization.[417] To enhance nanoparticle efficiency during pulmonary administration, a recent study developed thiolated mussel adhesive protein (MAP)-based nanoparticles. These nanoparticles possess a superior adhesive ability to mucosal tissues, enabling prolonged retention in lung tissue and resisting mucociliary clearance. Furthermore, their small size (<200 nm) and increased hydrophilicity (enhanced by moisture absorption from the mucosal layer) facilitate their diffusion into the mucus network, thereby improving therapeutic performance.[418] Conjugation of luteinizing hormone-releasing hormone (LHRH) to the end of the PEG molecule as a tumor-targeting moiety demonstrated excellent lung tumor deposition and tumor cell uptake after delivery of the nanoparticles through the pulmonary administration route.[417] Another study compared phenylboronic acid-modified nanoparticles with different

targeting modifications for pulmonary administration. Compared to mannose modification, which causes nanoparticle interception by lung macrophages, the FA modification significantly enhanced lung cancer cell targeting and the accumulation in tumor tissues.[419]

As another anti-cancer strategy, immune cells associated with tumors can also be targeted. Tumor-associated macrophages (TAMs) play important roles in carcinogenesis due to their significant involvement in promoting tumor initiation, growth, invasion, and metastasis. The accumulation of these macrophages is associated with poor prognosis and thus, represents a promising target for nanomedicine.[420] One study reported a biodegradable polymeric multilayer capsule. Fabricated using layer-by-layer assembly of poly-L-arginine (cationic) and dextran sulfate (anionic) on $CaCO_3$ templates, this enabled encapsulation of chemotherapeutic agents such as gemcitabine and clodronate to suppress tumor growth.[421] FR β, another type of receptor in the FR family, is primarily expressed in TAMs. Thus, the use of folate-modified liposomal complexes to deliver pro-apoptotic genes can effectively inhibit tumor growth by inducing apoptosis in both cancer cells and TAMs.[422] Similarly, Park et al. successfully developed folate-conjugated, pH-sensitive multi-drug liposomes capable of delivering a synergistic combination of doxycycline and docetaxel to FR β-expressing cancer cells and TAMs, significantly inhibiting tumor growth and macrophage infiltration.[423] In addition to macrophages, other immune cells can be targeted during lung cancer therapy. For example, a research group developed an inhalable nebulized nanoemulsion system designed to specifically target the Lymphocyte-activation gene 3 (LAG-3 or CD223) on immune cells within lung tumors and tumor-draining lymph nodes. This innovative approach effectively promoted DC maturation and T-cell activation, led to robust systemic antitumor immune responses, prolonged survival, and the establishment of immunologic memory in lung cancer models.[424] A recent study reported on the development of hyaluronic acid-lipid hybrid multilamellar nanoparticles (HLHC) that demonstrated superior mucus penetration via pulmonary delivery. Further optimization of these nanoparticles through arginine modification resulted in Arg-HLHC that significantly improved cytosolic drug delivery and targeted antigen-presenting cells (APCs), including macrophages and DCs. Delivery of Arg-HLHC enabled complete eradication of lung metastases in preclinical cancer models by activating local immune cells without inducing systemic inflammation.[425] In addition to immune cells, endothelial cells have been recently shown to be a promising target for nanoparticle-based therapies. A recent study found that low levels of the FOXF1 transcription factor promote lung cancer progression by impairing FZD4/Wnt/β-catenin signaling in tumor-associated endothelial cells, leading to abnormal, leaky tumor vessels. The study demonstrated that precise nanoparticle delivery of Fzd4 cDNA to endothelial cells effectively restores FZD4 expression, normalizes tumor vasculature, and significantly inhibits lung tumor progression.[426]

The lung tumor microenvironment differs markedly from normal tissue environments, exhibiting unique biochemical and physiological features such as altered pH, redox status, and enzymatic activity.[427,428] These distinct characteristics provide a valuable basis for designing nanoparticles that can specifically respond to tumor-specific stimuli to release therapeutic payloads in a controlled manner. Given the acidic microenvironment of lung tumors, pH-responsive systems are particularly promising. A recent study reported on the development of a TME-responsive-nanoimmunomodulator containing pH-sensitive amphiphilic amino acid fluorenylmethoxycarbonyl-L-histidine that can disassemble and release encapsulated drugs in the acidic tumor microenvironment to promote chimeric antigen receptor T (CAR-T) cell infiltration, reducing immunosuppression in lung cancer therapy.[429] To improve the therapeutic outcome, nanoparticles can be triggered by external stimuli for precise controlled release. In a recent study, researchers designed supramolecular therapeutic nanoparticles that contained photosensitizers generating reactive oxygen species (ROS). ROS cleavage of thioketal bonds led to disintegration of the nanoparticles and delivery of therapeutic payload.[430] Additionally, enzymes upregulated by tumors can be used for payload delivery. For example, Cathepsin B, which is upregulated in tumors, cleaves the GFLG peptide linker in prodrugs delivered by the nanoparticles.[430]

The early diagnosis of lung cancer is challenging, and a significant percentage of patients are diagnosed at advanced stages.[431] This late detection is a primary reason for high mortality rates associated with lung cancers.[432] Early detection of lung cancer is critical for therapies because it significantly improves patient survival by enabling early treatment interventions. Nanoparticle-based detection methods have emerged as a transformative approach for early lung cancer detection, offering superior sensitivity, signal amplification, and multimodal detection capabilities for low-abundance lung cancer biomarkers. Recent studies have utilized nanoparticles conjugated with specific recognition elements, such as antibodies, aptamers, or peptides, to enable precise imaging of cancer cells and detection of key molecular biomarkers. These platforms harness fluorescent nanomaterials, including quantum dots, for high-resolution imaging.[433] The use of magnetic nanoparticles facilitates the enrichment of rare cell populations, such as circulating tumor cells (CTCs),[434] or enables the capture of low-concentration biomarkers like NSCLC-associated miRNA-21.[435] The combination of molecular targeting and signal enhancement allows for the detection of cancer-specific signals even at trace levels, enabling early-stage diagnosis. For example, a group of studies focused on detecting tumor-associated proteases and other cancer-related biomarkers by monitoring electrical or electrochemical changes.[436] These platforms leverage highly conductive nanomaterials, such as multiwalled carbon nanotubes,[437] graphene-based composites, MOF hybrids,[438] and biotinylated DNA barcode-conjugated nanosensors,[436] to convert molecular recognition events into quantifiable electrical outputs. Such sensors can detect cancer biomarkers in various biological matrices, including exhaled air, urine, blood and serum, and offer non-invasive and rapid diagnostic solutions. The integration of ML algorithms into these systems enhances diagnostic accuracy by enabling sophisticated signal interpretation and classification of disease-specific patterns.[439,440] Many of these nanoparticle-based detection systems have been successfully validated using patient-derived samples, demonstrating high sensitivity, low limits of detection, and promising potential for real-world applications for lung cancer diagnostics.[434]

In conclusion, nanomedicines for lung cancers use a wide range of molecules, including PD-L1, CD47, EpCAM, and FRs, to target tumor cells. By delivering therapeutic agents directly to tumor cells, these nanomedicines inhibit oncogenic signaling and tumor growth, induce tumor cell apoptosis, promote anti-cancer immune responses, and normalize tumor vasculature.

Neonatal pulmonary diseases

Neonatal pulmonary diseases represent a distinct category of respiratory conditions that are fundamentally different from adult lung pathology due to the ongoing developmental processes and inherent vulnerabilities of the immature respiratory system. The human lung follows a complex developmental process which extends well beyond birth, creating multiple critical windows of vulnerability. One of the most vital stages is alveolar development, which is crucial for establishing the mature gas-exchange apparatus. This phase of lung development involves rapid alveolarization, which is highly associated with pulmonary vascular development, creating the intricate alveolar capillary networks essential for efficient gas exchange. Disruption of normal pulmonary vascular development leads to severe lung diseases.

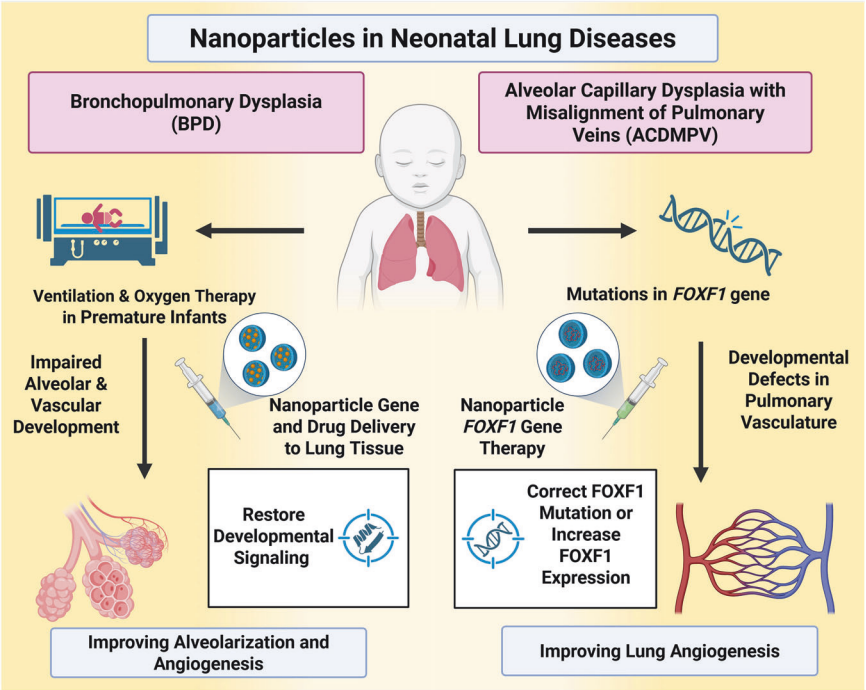

Fig. 11 Schematic shows the use of nanoparticles in neonatal lung diseases. Bronchopulmonary dysplasia (BPD) and alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) are severe neonatal lung diseases with different developmental origins. Nanoparticle delivery systems can enable targeted delivery of therapeutic agents to treat these conditions by restoring developmental signaling in the developing lung. FOXF1 is forkhead box F1. Created with Biorender.com

Due to the lack of proper protective mechanisms, immature pulmonary vasculature is particularly susceptible to injury from hyperoxia, mechanical ventilation, and inflammatory mediators.[441] These insults can lead to endothelial dysfunction, altered vasoreactivity, and pathological vascular remodeling which contributes to long-term pulmonary complications.[442] The vulnerability of the developing pulmonary vasculature represents a key factor distinguishing neonatal from adult lung disease patterns. The neonatal lung exists in a state of active development. This creates specific pathological conditions that rarely or never occur in adults, with BPD, Congenital Diaphragmatic Hernia (CDH), and ACDMPV serving as examples of neonatal pulmonary diseases (Fig. 11).

BPD is one of the most common complications of preterm birth with lifelong consequences associated with adverse respiratory, cardiovascular and neurodevelopmental outcomes.[42,443] With improved survival of neonates born at less than 28 weeks' gestation, the number of survivors with BPD is increasing. BPD results from the premature lung's vulnerability to stress during the neonatal developmental period. Multiple factors such as ventilation-induced lung injury, oxygen toxicity, inflammation secondary to infections, or any other stress result in additional injury to the developing lung.[444] Many transcription factors and molecular pathways are involved in BPD pathogenesis.[445] For example, in infants with BPD, and particularly in its most severe form accompanied by pulmonary hypertension (BPD + PH), the transcription factor FOXF1 is significantly decreased within the capillary endothelium.[346] The decrease of FOXF1 is associated with deficits in VEGF signaling, NOTCH-dependent cell interactions, and semaphorin guidance.[446–450] Disruption of these pathways is associated with abnormal alveologenesis and vasculogenesis following preterm birth.[445,451–453]

ACDMPV represents one of the most severe and lethal developmental lung disorders affecting neonates.[345,442,454] This congenital lethal lung disease is caused by mutations affecting 16q24.1 locus.[455–458] Most ACDMPV cases are caused by de novo single-nucleotide variants or copy-number variant deletions involving the FOXF1 gene, altering the expression or activity of the FOXF1 protein.[455] Most patients develop symptoms within the first 24 h after birth, and the lungs of ACDMPV patients exhibit loss of alveolar capillaries, lung hypoplasia, and respiratory failure.[459] A published study demonstrated that the S52F FOXF1 mutation causes ACDMPV by disrupting FOXF1-STAT3 protein-protein interactions and inhibiting *Stat3* gene transcription. This disruption reduces STAT3 signaling, a critical transcriptional regulator of angiogenesis, and its downstream effects on gene expression and endothelial cell proliferation, ultimately leading to severe defects in pulmonary vascular development.[454]

Nanoparticle delivery systems are emerging as a promising avenue for treating neonatal pulmonary diseases. Published studies have used a murine model of ACDMPV to demonstrate that intravascular delivery of positively charged polymeric nanoparticles carrying the STAT3 expression plasmid effectively prevents pulmonary hypertension and improves survival by enhancing alveolar capillary density and arterial oxygenation in ACDMPV mice.[343] Similarly, in a model of BPD, the polymeric nanoparticles have been successfully employed for the delivery of plasmids encoding either FOXM1 or FOXF1, both of which are key regulators of lung development and repair after lung injury.[38,458,460–463] This approach stimulated lung angiogenesis and endothelial cell proliferation, thereby inhibiting alveolar simplification and preserving lung function.[146,152] In addition to targeting the transcription factors, nanoparticle-mediated gene delivery has been applied to modulate other critical molecular pathways implicated in BPD-associated PH. For example, a reduction of endothelial carnitine palmitoyltransferase 1a (Cpt1a) expression is a major contributor to the development of pulmonary hypertension in BPD. Targeted delivery of Cpt1a plasmid to lung endothelial cells via nanoparticles significantly restored Cpt1a expression, attenuated pulmonary vascular remodeling, decreased right ventricular hypertrophy, and corrected dysregulated metabolic pathways.[464] Moreover, lipid-based

Table 2. Comparative performance and safety profiles of nanoparticle types for pulmonary diseases

NP types	Lipid (LNPs, liposomes)	Polymeric (polyethylenimine, chitosan)	Inorganic (gadolinium, gold, hafnium oxide, iron oxide)	Cell-derived (exosomes)
Stability	Thermodynamically unstable in certain conditions; high shear forces in nebulizers may cause liposome degradation	Stability is highly dependent on the type of the polymer; polymer degradation can be engineered from hours to months	Stability is highly dependent on surface chemistry of the inorganic nanoparticles	Specific techniques such as cryopreservation and freeze-drying are needed to maintain the integrity of modified exosomes
Payload types	Nucleic acids and small molecule drugs	Nucleic acids and small molecule drugs	Peptides and hydrophobic drugs	Nucleic acids and proteins
Mucus penetration	Usually request particle size smaller than 200 nm.[179]	Surface modification like Zwitterionic polymer conjugation and PEGylation can improve mucus penetration[183,199]	In acidic mucus, PEGylated silica nanoparticles exhibit a 42.7-fold higher mean squared displacement compared to carboxyl-modified silica nanoparticles[181]	Enzyme-modified exosomes can reach an apparent permeability coefficient over 5×10^{-7} cm/s[203]
Macrophage clearance propensity	Liposome uptake by AM cells after 2 h was 14% (2000 nm) and <4% (100 nm).[193]	Reduced via PEGylation or zwitterionic polymer coatings[183,199]	For nanodiamonds, a high-density polyglycerol coating reduces macrophage uptake from ~12% to ~10%[214]	Specific exosomes can reduce the immune cell clearance rate to 5–10%[204]
Typical lung accumulation	Specific liposomal systems achieve lung-specific accumulation without affecting other organs via intravenous injection[222]	Specific liposomal systems achieve lung-specific accumulation without affecting other organs via intravenous injection[223]	Magnetic guidance of iron oxide increases deposition into the lung tissue[163]	Inhalation typically shows higher accumulation compared to intravenous delivery[205]
Major safety concerns	Generally biocompatible, repeated use of PEGylated lipids may trigger anti-PEG antibodies	Monomers from hydrolysis must be biocompatible; cationic density must be controlled to prevent membrane rupture	Concerns include biopersistence, toxic ion release, and reactive oxygen species generation	Generally biocompatible. Risks include transferring pro-inflammatory signals and immunogenicity from repeated administrations
Clinical stage	FDA-approved (Arikayce), multiple Phase 1/2/3 trials, preclinical studies	Phase 1/2 trials. Preclinical studies	Phase 1/2 trials. Preclinical studies	Phase 1/2 trials. Preclinical studies

nanoparticles incorporating ursolic acid have been recently developed. In a BPD rat model, the lipid nanoparticles encapsulating NR1D1 mRNA have demonstrated notable therapeutic efficacy. NR1D1 is an intracellular circadian clock protein which is crucial for regulating inflammation and oxidative stress during lung injury. Nanoparticle delivery of NR1D1 mRNA reduced inflammatory cytokines and oxidative stress in a BPD model, decreasing alveolar simplification and promoting neonatal lung angiogenesis.[465]

Prenatal nanoparticle therapy is also being investigated for its potential to prevent neonatal lung diseases. One compelling example is the treatment of Congenital Diaphragmatic Hernia (CDH), a life-threatening congenital defect characterized by pulmonary hypoplasia and compromised vascularization. In a recent study, prenatal administration of Vascular Endothelial Growth Factor (VEGF) immobilization nanodiamonds significantly improved lung growth and vascular development in animal CDH models, offering a novel therapeutic opportunity to enhance or complement current interventions such as fetal tracheal occlusion. This early intervention strategy highlights the potential of nanomedicine for prenatal therapeutic applications in neonatal pulmonary diseases, opening new frontiers for precision-based pediatric care.[466]

In summary, nanomedicines for neonatal pulmonary diseases target key developmental pathways and transcription factors, such as FOXF1, FOXM1, STAT3, Cpt1a, and NR1D1. Through the nanoparticle-mediated delivery of plasmids or mRNA, these therapies aim to restore normal lung development and repair after neonatal lung injury. These interventions regulate the pathophysiology of BPD and ACDMPV by promoting angiogenesis, enhancing endothelial cell proliferation, and regulating inflammatory and oxidative stress responses.

A technical comparison of the performance characteristics, biological barrier interactions, and safety profiles for major nanoparticle classes is provided in Table 2.

SAFETY CONSIDERATIONS FOR TRANSLATIONAL NANOMEDICINE

Nanoparticle safety assessment is a fundamental requirement for clinical translation because the unique physicochemical properties of these systems, such as their small size and high surface energy, allow them to navigate biological barriers.[467] These characteristics usually lead to the formation of a protein corona upon contact with physiological fluids, which can alter the nanoparticle biological identity and trigger unpredictable immune recognition or rapid systemic clearance.[468] Safety profiles may vary significantly across nanoparticle platforms. Inorganic materials like Cadmium or silver are prone to biopersistence and the release of toxic ions,[468] whereas MOF nanoparticles can generate ROS, resulting in oxidative stress and cellular damage.[469] Additionally, nanostructures with rigid surfaces or sharp edges, such as carbon nanotubes or graphene oxide sheets, can physically disrupt biological membranes.[468] Therefore, carefully addressing potential safety concerns during the initial design phase is essential. Rational nanoparticle design should avoid the use of inherently toxic components, such as heavy metals or hazardous chemicals. Lipid and polymer-based systems are generally considered more biocompatible or biodegradable; employing these materials can prevent the leakage of toxic metal ions and minimize the long-term nanoparticle accumulation in human tissues. Furthermore, the size and structure of the carrier must be optimized to prevent excessive interaction with biological entities or membranes that might cause damage to tissues or cells. Cationic nanoparticles are

specifically noted for their ability to rupture lysosomal membranes via the "proton sponge effect", which facilitates endosomal escape and the release of cargo. However, an excessive "proton sponge effect" may trigger the release of degradative enzymes into the cytoplasm.[468] Therefore, researchers must balance cationic density and working concentrations to maintain safety. Finally, surface modifications with stealth molecules, such as PEG or zwitterionic polymers, create an inert hydrophilic layer that masks nanoparticles from the immune system, thereby reducing the potential for inflammatory responses.[218]

Evaluating nanoparticle safety for clinical use requires a multitiered assessment strategy that integrates physicochemical characterization, in vitro screening, and in vivo validation in preclinical animal models. Essential physicochemical parameters, including size (hydrodynamic diameter), shape, surface charge (zeta potential), purity, and stability, must be carefully characterized.[467] These properties act as critical determinants of biological effects and must be monitored to ensure batch-to-batch consistency.[470] In vitro assessments utilizing human cell lines serve as an initial biosafety screening phase to evaluate cytotoxicity through assays measuring cell viability, metabolic activity, membrane integrity, hemolytic activity, and the generation of ROS. In vivo animal studies further assess the toxicokinetic profiles of nanoparticles across various species, typically transitioning from rodents to non-human primates. Evaluation of toxicity typically includes monitoring animal weight, blood chemistry, hematology, and serum cytokines to detect systemic inflammation or organ injury.[471] Furthermore, in vivo imaging systems may be used to track real-time biodistribution and organ accumulation. Comprehensive in vivo preclinical studies can determine the maximum tolerated dose and identify dose-limiting toxicities, serving as vital guidelines for potential clinical translation.[471] To improve predictive accuracy and minimize animal use, innovative strategies such as iPSC-derived human organoids and organ-on-a-chip devices that mimic in vivo environments, control fluid mechanical forces, and allow real-time monitoring are increasingly employed in nanomedicine safety assessments.[467] Integrating all available methods to ensure nanomedicine safety is necessary prior to potential clinical translation.

While general nanotoxicology provides a baseline for safety, the unique physiological environment of the lungs requires route-specific pulmonary safety assessments. In preclinical validation, it is essential to move beyond systemic toxicity and focus on local pulmonary interactions. A critical component of this evaluation is the analysis of bronchoalveolar lavage fluid (BALF). Monitoring BALF cell counts and cytokine profiles provides a direct window into the immediate inflammatory response and the recruitment of immune cells to the lung. Furthermore, assessing airway hyperresponsiveness and bronchospasm is vital for understanding how nanoparticles may impact pulmonary mechanics and function of airway smooth muscle cells. Beyond functional metrics, local histopathology remains the gold standard for identifying structural changes in lung remodeling. It is necessary to evaluate signs of airway epithelial injury, mucociliary clearance impairment, and the severity of PF. By integrating these route-specific parameters, researchers can more accurately predict the safety profile of lung-directed nanoparticles in humans.

CLINICAL APPLICATIONS AND CURRENT CHALLENGES

The clinical development of nanoparticle-based therapeutics for pulmonary diseases represents a transformative approach over the past several years, with multiple formulations reaching various stages of human testing. The field encompasses diverse nanoparticle types, administration routes, and therapeutic applications, ranging from infectious diseases to cancer and genetic disorders. Among the most notable clinical successes is ARIKAYCE® (ALIS), a liposomal formulation of the antibiotic amikacin. Delivered directly to the lungs via aerosolized nebulization, ARIKAYCE® received approval in 2018 as part of a combination regimen for treating *Mycobacterium avium* complex lung disease.[58,201,368,472] Another notable example is Linhaliq™, a liposomal ciprofloxacin formulation developed by Aradigm for nebulized delivery using a jet nebulizer system (PARI LC®). Linhaliq™ underwent extensive clinical testing, including two Phase 3 ORBIT clinical trials, for the treatment of non-CF bronchiectasis associated with chronic *Pseudomonas aeruginosa* infections.[473,474] Current regulatory assessments of nanotherapeutics and inhaled gene editing platforms are conducted within established pharmaceutical and biological frameworks.[475] The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) use case-by-case evaluations to assess the safety and efficacy of these therapeutic agents. The "case-by-case" methodology is primarily driven by the structural complexity of these products, where multiple components simultaneously influence the pharmacological behavior of the active ingredient. Consequently, standardized tests cannot be applied universally to all nanoparticle formulations. Regulators instead evaluate each nanomedicine-specific characteristics relative to its intended use through several actions: (1) classifying the product by its primary mode of action; (2) adapting established pharmaceutical quality assessments; (3) requesting data based on the unique architecture and surface interactions of nanoparticles; (4) conducting stepwise comparisons with reference products; and (5) applying real-time safety monitoring for risks during clinical trials.[475]

The COVID-19 pandemic posed an unprecedented global health crisis, resulting in millions of deaths worldwide and necessitating the rapid development of effective countermeasures. Among the most impactful biomedical achievements during the pandemic era was the deployment of mRNA-based COVID-19 vaccines, which represent a landmark application of lipid nanoparticle (LNP) technology in human medicine. Both the Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) vaccines utilize LNPs to encapsulate and deliver mRNA encoding the SARS-CoV-2 spike protein.[476,477] In a multinational, placebo-controlled, observer-blinded, pivotal efficacy trial involving 43,548 randomized participants, the BNT162b2 vaccine demonstrated 95% efficacy in preventing COVID-19 with onset at least 7 days after the second dose.[476] Similarly, the mRNA-1273 COVID-19 vaccine demonstrated over 94% efficacy against COVID-19 in the phase 3 clinical trial.[478] These remarkable achievements not only underscore the rapid translational potential of nanoparticle platforms but also validate their utility for large-scale prophylactic and therapeutic applications.

Currently, numerous nanoparticle-based therapeutic agents undergo clinical trials for potential treatment of pulmonary diseases (Table 3). Despite the clinical potential, numerous challenges continue to hinder the broader development and clinical translation of nanoparticle-based therapies. Although a wide variety of nanoparticle systems have been reported in preclinical literature, only a small subset has progressed to human clinical trials. Currently, most nanoparticles used in clinical studies are lipid-based. These nanoparticles lack specific targeting capabilities or controlled-release functionalities. The lack of these capabilities reflects the broader translational gap and highlights persistent obstacles in bringing more advanced nanocarrier systems into the clinic.

One significant barrier is the difficulty of large-scale production, particularly for nanoparticles with complex designs or intricate chemistries. While these structural complexities may enhance the functionality of the nanoparticles, such as improved targeting, cargo release kinetics, or immune modulation, the complex structure often results in low production yields and poor reproducibility, making the complex nanoparticles unsuitable for industrial-scale manufacturing and global deployment. For

Table 3. Nanoparticles in clinical trials (active or completed in past 3 years)

NCT numbers	Investigational product name(s)	Phase	Delivery route	Primary endpoint	Published result	Most recent update	Last verification date
N	Irinotecan liposome, anlotinib	Not Applicable	Intravenous injection	Objective response rate (ORR)	No results posted	08/20/2025	07/05/2026
N	Irinotecan liposome	Phase 2	Intravenous injection	Objective response rate (ORR)	No results posted	07/10/2024	07/05/2026
N	Irinotecan hydrochloride liposome	Phase 4	Intravenous infusion	Intracranial objective response rate	No results posted	06/21/2024	07/05/2026
N	Irinotecan Liposome, Ivonescimab	Phase 2	Intravenous injection	6-month progression-free survival rate	No results posted	06/26/2026	07/05/2026
N	Irinotecan Liposome, Adebrelimab, Famitinib	Phase 1 & 2	Intravenous Infusion	6-month progression-free survival	No results posted	03/27/2024	07/05/2026
N	Irinotecan Liposome, Platinum, Tislelizumab, Anlotinib	Phase 4	Intravenous injection	Progression-free survival (PFS)	No results posted	04/30/2025	07/05/2026
N	Irinotecan Hydroochloride Liposome, Topotecan	Phase 3	Intravenous injection	Overall Survival	No results posted	03/15/2024	07/05/2026
N	Irinotecan Liposome, Adebellizumab	Phase 2	Intravenous infusion	Progression-free survival (PFS)	No results posted	06/08/2025	07/05/2026
N	Irinotecan Hydrochloride Liposome, Carboplatin, Serplulimab	Not Applicable	Intravenous injection	Progression-free survival (PFS)	No results posted	06/28/2024	07/05/2026
N	Irinotecan Liposome, Apatinib	Phase 2	Intravenous infusion	6-month progression-free survival	No results posted	12/27/2024	07/05/2026
N	Irinotecan Liposome, Adebrelimab, Carboplatin, Etoposide	Phase 2	Intravenous infusion	1 year overall survival rate	No results posted	12/18/2024	07/05/2026
N	Irinotecan Liposome, Ivonescimab	Phase 2	Intravenous injection	Progression-free survival (PFS)	No results posted	02/11/2025	07/05/2026
N	Paclitaxel Liposome, Serplulimab, Carboplatin, Pemetrexed, Nab-paclitaxel	Phase 2	Intravenous infusion	Event-free survival (EFS)	No results posted	09/19/2024	07/05/2026
N	Gemcitabine Liposome, Pembrolizumab	Phase 2	Intravenous injection	Determine the incidence of Treatment Emergent Adverse Events, Duration of stable disease in monotherapy, duration of stable disease in combination therapy	No results posted	09/04/2025	07/05/2026
N	Transcrocetin Liposome	Phase 3	Intravenous injection	Days free from invasive mechanical ventilation, 30 Day All-Cause Mortality	No results posted	10/15/2024	07/05/2026
N	Amphotericin B Liposome, Posaconazole	Phase 2	Intravenous infusion	Proportion of participants achieving a successful outcome at completion of six weeks.	No results posted	05/22/2026	07/05/2026
N	Annamycin Liposome	Phase 1 & 2	Intravenous infusion	Dose Limiting Toxicity	No results posted	10/24/2024	07/05/2026
N	Amikacin Liposome Inhalation Suspension	Phase 2	Inhalation delivery	Variation of sputum conversion rate	No results posted	11/19/2025	07/05/2026
N		Phase 3	Inhalation delivery		No results posted		07/05/2026

Table 3. continued

NCT numbers	Investigational product name(s)	Phase	Delivery route	Primary endpoint	Published result	Most recent update	Last verification date
	Amikacin Liposome Inhalation Suspension, Azithromycin, Ethambutol			Change from Baseline in Respiratory Symptom Score at Month 13		02/03/2026	
N	Iloprost Liposome Inhalation Solution	Phase 1	Inhalation delivery	The incidence of dose-limiting toxicity (DLT), the incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs), Frequency and severity of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs)	No results posted	10/16/2024	07/05/2026
N	Treprostinil Liposome Inhalation Suspension	Phase 3	Inhalation delivery	Safety/Tolerability assessed by incidence of treatment-emergent AEs/SAEs	No results posted	04/01/2026	07/05/2026
N	Quaratusugene Ozeplasmid (DNA plasmid lipid), osimertinib, platinum-based chemotherapy	Phase 1 & 2	Intravenous injection	Recommended Phase 2 Dose (RP2D)—Phase 1, Overall Response Rate (ORR)—Phase 2a, Progression-free Survival (PFS)—Phase 2b	No results posted	01/21/2026	07/05/2026
N	BNT162b2 (mRNA Lipid)	Phase 4	Intramuscular injection	Geometric Mean Titer of SARS-CoV-2 serum neutralizing antibodies	No results posted	05/15/2026	07/05/2026
N	Moderna COVID-19 Vaccine	Phase 3	Intramuscular injection	Efficacy of Moderna COVID-19 Vaccine Against SARS-CoV-2 Infection, Effect of Moderna COVID-19 Vaccine on Mean Peak Nasal Viral Load, Effect of Moderna COVID-19 Vaccine on Median Peak Nasal Viral Load	No results posted	08/02/2023	07/05/2026
N	Moderna mRNA-1273, Moderna mRNA-1273.222	Phase 2 & 3	Intramuscular injection	Evaluate primary vaccine efficacy by tracking symptomatic COVID-19 cases up to Month 6 (Part A), and booster efficacy by comparing cases following a Monovalent vs. Bivalent booster at Month 6 (Part B).	Results Posted PMID: 39902315	04/21/2026	07/05/2026
N	mRNA-1273, mRNA-1273.351	Phase 1	Intramuscular injection	Evaluate the safety and reactogenicity of the mRNA-1273 and mRNA-1273.351 vaccines in both COVID-19 native and previously vaccinated adults	Results Posted PMID: 35547849	03/18/2024	07/05/2026
N	mRNA-1273	Phase 1	Intramuscular injection	Evaluate the safety and reactogenicity of a 2-dose vaccination schedule of mRNA-1273, administered 28 days apart, across 5 different dosages (10 mcg, 25 mcg, 50 mcg, 100 mcg, and 250 mcg) in healthy adults.	Results Posted PMID: 40632654 PMID: 35547849 PMID: 35126362 PMID: 35072628 PMID: 34642699 PMID: 34090865 PMID: 32991794 PMID: 32663912	03/18/2024	07/05/2026
N	ARCT-032 (CFTR mRNA Lipid Nanoparticle)	Phase 2	Inhalation delivery	Incidence, severity and dose relationship of adverse events	No results posted	05/08/2026	07/05/2026

Table 3. continued

NCT numbers	Investigational product name(s)	Phase	Delivery route	Primary endpoint	Published result	Most recent update	Last verification date
N	ARCT-032 (CFTR mRNA Lipid Nanoparticle)	Phase 1	Inhalation delivery	Incidence, severity and dose-relationship of AEs	No results posted	11/13/2024	07/05/2026
N	Paclitaxel Albumin-Stabilized Nanoparticle Formulation	Phase 2	Intravenous injection	Overall Response Rate (Complete and Partial Response) Defined by RECIST 1.1 Criteria	Results Posted In this trial of 26 participants, no complete responses were observed. 34.6% had a partial response, 23.1% had stable disease, 23.1% had progressive disease, and 30.8% experienced grade ≥3 toxicity. No publication posted	07/16/2021	07/05/2026
N	Paclitaxel, Nab-Paclitaxel, Carboplatin, Cisplatin, Pemetrexed	Phase 3	Intravenous infusion	Cycle 1 Area Under the Curve (AUC) From 0–3 Weeks of Pembrolizumab, Cycle 6 Model-Based Minimal Concentration (Ctrough) of Pembrolizumab	Results Posted In this study, serious adverse events were reported in 37.1% (132/358) of participants in Arm A (Pembrolizumab Subcutaneous + Platinum Doublet Chemotherapy) and 36.1% (62/173) in Arm B (Pembrolizumab IV + Platinum Doublet Chemotherapy). No publication posted	12/03/2024	07/05/2026
N	Cabozantinib S-malate, Docetaxel, Gemcitabine Hydrochloride, Nab-paclitaxel, Paclitaxel	Phase 2	Intravenous injection	Progression-free survival (PFS) for patient population with non-squamous non-small cell lung cancer (NSCLC)	No results posted	07/02/2026	07/05/2026
N	MPDL3280A, Carboplatin, Nab-paclitaxel	Phase 2	Intravenous infusion	Number of Subjects With Major Pathologic Response (MPR)	Results Posted PMID: 22481232 PMID: 21296855 PMID: 25559415 PMID: 21079145 PMID: 17762336 PMID: 18506026 PMID: 20338627 PMID: 24576776	12/12/2023	07/05/2026
N	Carboplatin, Nab-paclitaxel,Pemetrexed	Phase 1 & 2	Intravenous injection	Incidence of adverse events, Best overall response rate	No results posted	10/17/2024	07/05/2026
N	Carboplatin, Nab-paclitaxel, Paclitaxel, Pemetrexed	Phase 3	Intravenous infusion	Overall Survival (OS)	No results posted	07/02/2026	07/05/2026
N	GBP510, ChAdOx1-S	Phase 3	Intramuscular injection	Geometric Mean Titer(GMT) of neutralizing antibody to the SARS-CoV-2 measured by wild-type virus neutralization assays, Percentage of participants with ≥ fourfold rise in wild-type virus neutralizing antibody titer from baseline, Geometric Mean Titer(GMT) of neutralizing antibody to SARS-CoV-2 measured by wild-type virus neutralization assays	No results posted	04/08/2024	07/05/2026

Table 3. continued

NCT numbers	Investigational product name(s)	Phase	Delivery route	Primary endpoint	Published result	Most recent update	Last verification date
N	GBP510	Phase 1 & 2	Intramuscular injection	Evaluate the vaccine safety and tolerability in Stage 1, followed by measuring its ability to trigger a strong immune response in Stage 2.	No results posted	04/18/2023	07/05/2026
N	qNIV Nanoparticle Vaccine2 in-clinic mixed with Matrix-M1 Adjuvant, SARS-CoV-2 rS Nanoparticle Vaccine in-clinic mixed with Matrix-M1 Adjuvant	Phase 1 & 2	Intramuscular injection	Number of Participants with Solicited Local and Systemic Adverse Events (AE's), Number of Participants Reporting All AE's, Number of Participants With MAAEs, AESIs (Including PIMMCs), SAEs	Results Posted In this study of 637 participants, local adverse events were reported in 84.3% of participants, while systemic adverse events were reported in 66.4%. No publication posted	04/10/2025	07/05/2026
N	qNIV vaccine with matrix-M Adjuvant, Influenza Vaccine, CIC vaccine with Matrix-M adjuvant, SARS-CoV-2 rS vaccine with matrix-M adjuvant	Phase 2	Intramuscular injection	Number of participants with solicited local and systemic Adverse Events (AEs), Percentage of participants with all AEs, Percentage of participants with Medically Attended Adverse Events (MAAEs), Adverse Events of Special Interest (AESIs) and Serious Adverse Events (SAEs)	No results posted	07/16/2024	07/05/2026
N	NVX-CoV2373	Phase 2	Intramuscular injection	Evaluate the safety (by tracking adverse events) and the immunogenicity (by measuring antibody and immune responses) of the NVX-CoV2373 vaccine in both HIV-positive and HIV-negative participants.	No results posted	03/16/2023	07/05/2026
N	SARS-CoV-2 rS/Matrix M1-adjuvant, licensed seasonal influenza vaccine	Phase 3	Intramuscular injection	Participants with Symptomatic Mild, Moderate, or Severe Coronavirus Disease 2019 (COVID-19)	No results posted	05/08/2025	07/05/2026
N	SARS-CoV-2 rS/Matrix-M1 adjuvant	Phase 3	Intramuscular injection	Prevention of PCR-confirmed, symptomatic COVID-19 in participants with onset at least 7 days after receiving their second dose of the vaccine.	Results Under Review	12/21/2023	07/05/2026
N	SARS-CoV-2 rS/Matrix-M1 adjuvant	Phase 2	Intramuscular injection	Evaluate the efficacy of the vaccine in preventing symptomatic disease in both HIV-negative and HIV-positive adults. Additionally, the study measures the vaccine safety and its immunogenicity.	Results under review	02/17/2022	07/05/2026
N	CIC vaccine co-formulated tNIV2, SARSCoV-2 rS, Matrix-M Adjuvant, Novavax COVID-19 Vaccine, tNIV Vaccine, Fluzone	Phase 3	Intramuscular injection	Evaluate the safety of the combination vaccine and its immunogenicity.	No results posted	06/05/2026	07/05/2026
N	Sodium chloride, SpFN_1B-06-PL + ALFQ (QS21 Adjuvant)	Phase 1	Intramuscular injection	Number of participants with local and systemic reactions, Incidents of treatment-adverse events as assessed by FDA Toxicity grading scale, Number of participants with humoral immune response at Study Day 43 (±2)	No results posted	06/17/2025	07/05/2026

Table 3. continued

NCT numbers	Investigational product name(s)	Phase	Delivery route	Primary endpoint	Published result	Most recent update	Last verification date
N	Hafnium oxide-containing nanoparticles NBTXR3	Phase 1	Intranodal/ Intratumorally Injection	Incidence of dose-limiting toxicity (DLT), determination of the recommended phase II dose (RP2D), determination of the maximum tolerated dose (MTD)	No results posted	03/11/ 2026	07/05/2026
N	AGuIX	Phase 1 & 2	Intravenous injection	Maximum tolerated dose (MTD) Phase 1, Compare local control at 12 months of maximum tolerated dose MTD—Phase 2	No results posted	05/14/ 2026	07/05/2026
N	LD Vehicle-GNP, LD PepGNP-Covid19, HD Vehicle-GNP, HD PepGNP-Covid19	Phase 1	Intradermal Injection	Solicited local & systemic AEs, unsolicited AEs, SAEs, adverse events of special interest (AESI)	No results posted	02/12/ 2025	07/05/2026
N	EP0057, Olaparib	Phase 1 & 2	Intravenous injection	Maximum tolerated dose (MTD)/ recommended phase 2 dose	Results posted PMID: 32897402	06/30/ 2026	07/05/2026
N	hMSC-Exos	Phase 1 & 2	Inhalation delivery	Incidence of adverse reaction, TTCI, 28-day mortality	No results posted	07/22/ 2024	07/05/2026
N	EXO 1, EXO 2	Phase 1 & 2	Inhalation delivery	Number of participants with non-serious and serious adverse events during trial, number of participants with non-serious and serious adverse events during inhalation procedure	Results Posted In this study of 30 participants, no adverse events were reported, and mean clinical recovery times were 13.8, 14.8, and 14.1 days for the EXO-1, EXO-2, and placebo groups, respectively. No publication posted	11/04/ 2020	07/05/2026
N	HDT-301	Phase 1	Intramuscular injection	Solicited AE, Unsolicited AE, Lab abnormalities, medically-attended AE, AESI and SAE	No results posted	10/23/ 2024	07/05/2026

Table 3 methodology: for the identification of relevant clinical trials, we used ClinicalTrials.gov as the primary database. We conducted a targeted keyword search using terms “nanoparticle”, “liposome”, “lipid nanoparticle (LNP)”, and “exosome” to identify nanoparticle-based interventions. These were combined with keywords related to pulmonary conditions, including “pulmonary disease”, “pulmonary fibrosis”, “lung infections”, “Chronic Obstructive Pulmonary Disease (COPD)”, “Pulmonary Vascular Disease (PVD)”, “cystic fibrosis”, “asthma”, “lung cancer”, “Non-Small Cell Lung Cancer (NSCLC)”, and “small cell lung cancer (SCLC)”. Both search fields were applied simultaneously. We included trials from the previous three years (January 2022 to August 2025) to capture recent developments. A search was also conducted in July 2026 to confirm that the status of the included clinical trials had not changed since the initial manuscript submission. Clinical trials that included nanoparticles as a secondary element rather than a primary element were excluded

example, microfluidics is highly effective in the lab because rapid mixing at the microscale ensures that nanoparticles are nearly uniform in size. However, industrial scale-up demands higher throughput, which often involves increased flow rates. These changes can alter the Reynolds number and other hydrodynamic parameters, shifting the balance between inertial and viscous forces. Even if the flow remains nominally laminar, differences in shear stress, mixing efficiency, solvent exchange rates, and residence time distributions can significantly impact nanoparticle formation. Such variations can lead to nonuniform self-assembly, aggregation, inconsistent drug loading, and changes in size, PDI, surface coating, or ligand presentation. These issues are particularly severe for complex platforms like multilayer and cell membrane-coated nanoparticles, as their assembly is highly sensitive to local fluid conditions. Furthermore, nanoparticles incorporating expensive or scarce biological components may also face economic hurdles in commercialization. In addition, biodegradable nanoparticles, which are preferred for clinical use due to their safety profile, often suffer from instability during long-term storage. The breakdown of these nanoparticles leads to alterations in physicochemical properties, degradation of therapeutic cargo, loss of biological function, or even potential safety risks.

The translation of pulmonary nanomedicines from laboratory discovery to clinical application currently follows a product-focused, science-based regulatory approach outlined in the FDA's finalized 2022 guidance entitled "*Drug Products, Including Biological Products, that Contain Nanomaterials*".[479] This framework applies to materials engineered within the 1–100 nm range or those up to 1000 nm that exhibit size-dependent functional properties. This classification requires the strict monitoring of critical quality attributes (CQAs), including size, surface charge, and morphology, to ensure consistent aerosol performance and stability. Translating these therapies for pulmonary diseases often involves multi-decade timelines due to significant hurdles like the manufacturing process, GMP compliance, and the understanding of complex bio-nano interactions. To mitigate clinical risks and bridge the translational gap, researchers increasingly utilize strategic models like the DELIVER framework.[480] This methodology includes seven core principles: defining the target product profile (D), essential characterization (E), lead candidate optimization (L), intellectual property patenting (I), validated efficacy and safety (V), economical and scalable production (E), and the regulatory and clinical pathway (R). By establishing a quality target product profile early in the design phase, nanomedicines can be rationally engineered to bypass specific biological barriers while balancing therapeutic efficacy with requirements for manufacturing and biocompatibility.[480] The integrated stages of this translational roadmap are visually summarized in Fig. 12.

Another critical limitation in the field of nanoparticle-based therapeutics arises from the preclinical models currently employed. ARDS, a severe complication of acute lung injury (ALI), remains a significant cause of morbidity and mortality in both adults and children, with especially high vulnerability in the neonatal population.[481,482] Epidemiological studies reveal key differences in ARDS incidence and outcomes between pediatric and adult populations, particularly in neonates who often

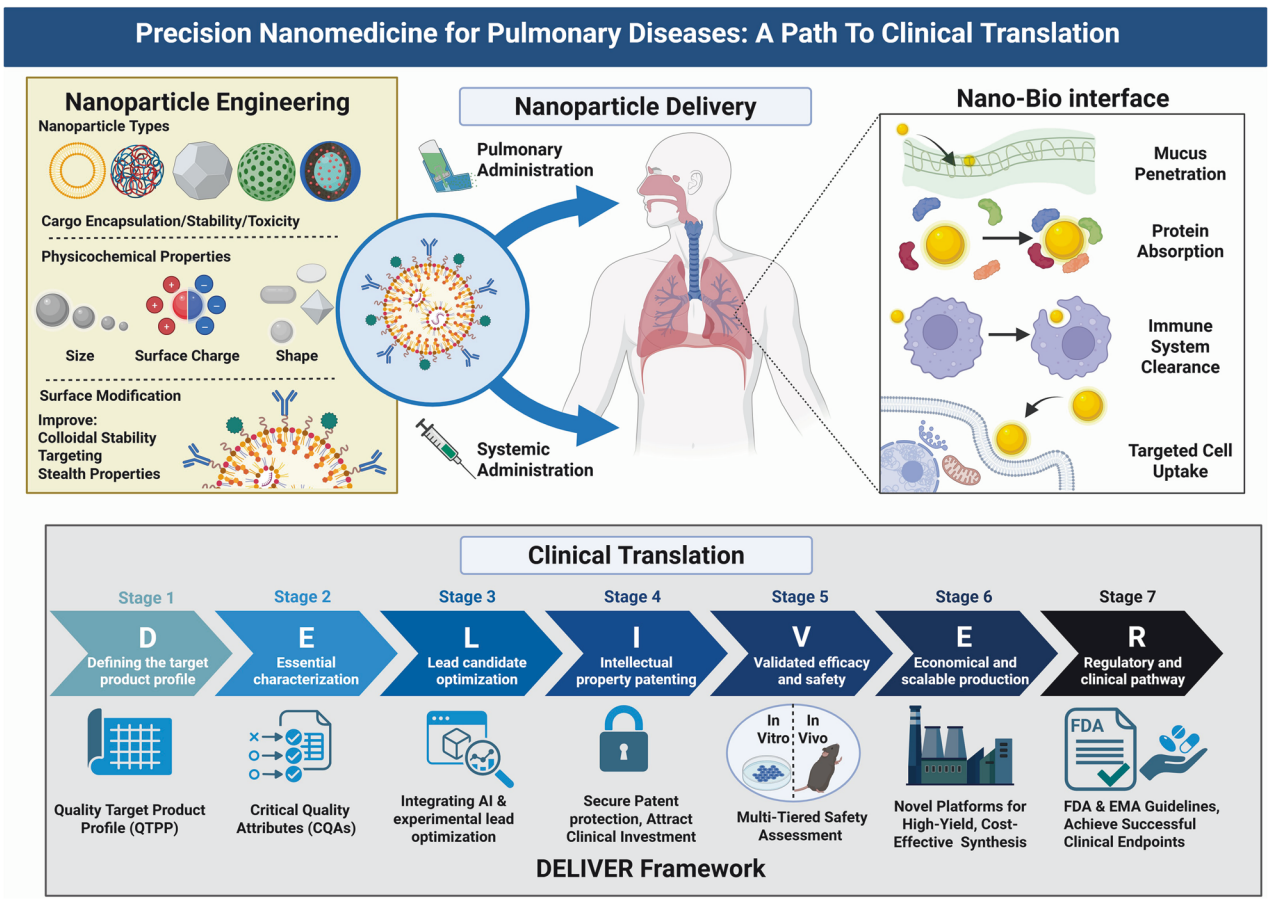

Fig. 12 Schematic shows a comprehensive overview of the progression from nanoparticle engineering to clinical translation. The design of nanoparticle delivery systems for pulmonary diseases requires the optimization of particle types, physicochemical properties, and surface modifications to facilitate specific administration routes and overcome biological barriers. The DELIVER framework serves as a seven-stage strategic roadmap for clinical translation, extending from initial product profiling and characterization to safety assessment, scalable manufacturing, and regulatory approval. Created with Biorender.com

experience distinct pathophysiological responses to lung injury compared to adults.[483,484] Despite these differences, most therapeutic development and preclinical studies rely on adult models, leading to a significant underrepresentation of pediatric-specific research and therapeutic development.[481,485] Moreover, existing preclinical models frequently rely on in vitro studies or short-term rodent models. These models often fail to capture the long-term biodistribution, degradation behavior, and potential toxicity of nanoparticles after multiple applications. This must be considered because many nanoparticles exhibit longer retention times in vivo compared to conventional small-molecule drugs. Consequently, long-term risks such as bioaccumulation, off-target effects, and immune-related toxicities remain insufficiently understood, representing potential safety liabilities in human translation.

CONCLUSION AND FUTURE PERSPECTIVES

To overcome these complex, multi-level challenges, interdisciplinary collaboration among researchers in medicine, chemistry, materials science, and biomedical engineering is essential. A deep understanding of disease-specific pathophysiology is critical for guiding rational nanoparticle design tailored to specific cellular targets and microenvironments. For instance, emerging technologies such as click chemistry offer powerful tools for nanoparticle functionalization and lung-targeting ligand conjugation. While several prior studies have used antibody-conjugated nanoparticles to target lung cells, most have relied on traditional coupling chemistries. There is an unmet need to explore next-generation chemistries, including click reactions, that enable more efficient, stable, and site-specific conjugation of ligands to nanoparticle surfaces. Furthermore, scalable nanoparticle production will require optimization of synthesis parameters to balance nanoparticle functionality, reduce cost, increase yield, and establish robust quality control metrics to ensure batch-to-batch reproducibility. Additional efforts are needed to perform long-term toxicity and pharmacokinetic studies, with the primary focus on elucidating degradation pathways, clearance mechanisms, and immunogenicity profiles under clinically relevant conditions. The implementation of Quality by Design (QbD) principles during early process development facilitates the identification of high-risk parameters to establish a stable manufacturing process and reproducible manufacturing outcomes. In the field of nanomedicine, the QbD framework uses CQAs as measurable benchmarks that link complex nanoparticle properties to their ultimate clinical performance and patient safety. These CQAs typically include particle size distribution, morphology, surface charge, and polydispersity to ensure batch uniformity and scientific reproducibility.[475] To produce specialized carriers for lung delivery, various particle engineering technologies are employed to achieve the precise aerodynamic properties required for deep airway deposition.[486] Supercritical fluid (SCF) technology uses supercritical carbon dioxide as a sustainable solvent to crystallize active ingredients, effectively reducing organic solvent residues while achieving the narrow particle size distribution required for inhalable powders. Complementary approaches include spray drying (SD), which generates porous particles with consistent sizes, and spray freeze drying (SFD), a method suited for thermolabile and lipid-based materials that yields low-density particles with high surface areas. Furthermore, emerging technologies such as thin-film freezing (TFF), hot-melt extrusion (HME), and proprietary platforms like TechnoSphere and PulmoSphere provide solvent-free or specialized processing routes to improve the solubility and stability of inhaled formulations.[486,487] High-resolution methods like particle replication in non-wetting templates (PRINT) further allow for the design of particles with regulated NMADs to ensure uniform distribution throughout the respiratory system.[486,487] To overcome the structural constraints of centralized, batch-based manufacturing, next-generation systems such as BioNTainer and Ntensify offer decentralized, continuous-flow solutions that increase throughput and reduce process variability.[488] For example, the BioNTainer system utilizes a modular, container-based architecture to enable the rapid local production of high-quality mRNA vaccines. Similarly, the Ntensify platform achieves mRNA synthesis yields of 5.6 mg/mL and reduces overall production costs by 60% compared to traditional manufacturing.[488]

Beyond conventional drug and mRNA delivery, the next frontier in pulmonary nanomedicine is the permanent correction of genetic defects through advanced gene-editing tools. Recent preclinical studies have evaluated the feasibility of pulmonary CRISPR/Cas9 delivery using non-viral platforms.[489] The CRISPR/Cas9 system is a programmable gene-editing technology which utilizes guide RNAs to direct the Cas9 endonuclease toward specific genomic sequences for precise DNA modification. Local pulmonary delivery of these components requires a carrier to protect CRISPR/Cas9 components from degradation and overcome biological barriers. Published studies developed PEGylated chitosan as a delivery platform and demonstrated that these nanocomplexes were stable during nebulization-induced shear stress and effectively permeated mucus layers.[490] Other studies employing high-throughput screening identified biodegradable ionizable lipid nanoparticles (LNPs) that enabled efficient gene editing in mouse lung epithelium and were suitable for repeated intratracheal administration.[491] This approach combining AAV-mediated guide RNA delivery with LNP-mediated Cas9 mRNA expression showed editing efficiencies near 17%, which is comparable to dual-AAV systems but offers a safer, transient expression profile.[491] In addition to DNA-targeted genome editing, polymeric formulations have been used to deliver Cas13a mRNA to lung epithelial cells to target SARS-CoV-2 RNA, demonstrating therapeutic efficacy in infected mice.[489] Despite these new advances, significant constraints remain, including physiological barriers such as mucociliary clearance, AM phagocytosis, and the thick mucus characteristic of chronic respiratory diseases. Technical challenges also include the potential for cargo degradation during aerosolization and overall limited progress in standardized inhalation formulations for clinical applications.

In addition to designing nanoparticles for specific pulmonary cell targeting, the clinical application of precision nanomedicine can be improved through the identification of molecular endotypes and patient-specific biomarkers. Identifying reproducible disease endotypes provides a foundation for clinical stratification. For example, in ARDS, multi-omics integration has defined two primary endotypes: a hyperinflammatory C1 phenotype and a hypoinflammatory C2 phenotype.[492] Because these ARDS endotypes exhibit differential responses to standard care, it is important to determine whether a nanoparticle system should deliver anti-inflammatory payloads or focus on tissue-specific repair. Similarly, severe asthma can be subdivided into T2-high and T2-low endotypes using sputum cytometry and "breathomics" signatures.[493] These biomarkers will allow clinicians to distinguish various inflammatory processes and select specific nanoparticle-mediated agents, such as anti-IL-5 for T2-high disease or macrolide antibiotics for neutrophilic bronchitis. Furthermore, recent advances in single-cell transcriptomics and next-generation sequencing enable the discovery of additional biomarkers that guide the development of personalized nanomedicine. In ARDS, ML applied to transcriptomic datasets has identified four-gene signatures for diagnosis and an eight-protein panel for early prognosis,[492] both of which can be used to determine the timing and dosage of nanoparticle-based interventions. In lung cancer, development of precision nanovaccines utilizes exome sequencing to identify patient-unique neoantigens.[494] This genotype-guided approach facilitates the creation of individualized mRNA-based or peptide vaccines targeting specific KRAS mutations, ensuring that the nanoparticle cargo is tailored to the patient's

unique genetic profile. By incorporating these patient-specific treatments, pulmonary nanomedicine can move beyond generalized delivery to a truly individualized therapeutic framework.

To speed up the design of complex new therapies, the field is increasingly using data-driven methodologies to optimize nanoparticles. Recent advances in AI and ML offer promising avenues for accelerating nanoparticle design, optimization, and prediction of biological effects. A recent study introduced a novel AI-guided deep learning strategy to design and optimize ionizable lipids for LNPs. The strategy successfully identified high-potency candidates that significantly enhanced mRNA delivery efficiency for pulmonary gene therapy and vaccine applications.[495] As nanoparticle datasets become more comprehensive and standardized, AI/ML platforms are expected to enable predictive modeling of key properties such as cellular uptake, biodistribution, endosomal escape, transfection efficiency, toxicity, and even age-specific differences in therapeutic responses. These AI/ML tools may facilitate rational design of safer and more effective nanoparticle systems, ultimately streamlining the translation of nanomedicine from bench to bedside for both adult and pediatric pulmonary conditions.

In conclusion, this review provides a systematic overview of precision nanomedicine for pulmonary diseases, focusing on how nanoparticles can overcome the limitations of conventional therapies. It highlights the critical role of physicochemical properties in targeted delivery, explores advanced design strategies, and compares pulmonary and systemic administration routes. The manuscript also covers therapeutic applications for various conditions, including PF, respiratory infections, COPD, PVD, CF, asthma, lung cancer, and neonatal disorders. Finally, this review discusses translational challenges and provides future perspectives to guide the development of future respiratory therapies.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (NHLBI) under grant numbers HL141174, HL152973, and HL158659.

AUTHOR CONTRIBUTIONS

Zicheng Deng: conceptualization, methodology, writing—original draft, writing—review & editing, visualization; Wen Gao: methodology, writing—original draft; Jonathan Do: writing—review & editing; Shravya Aragam: methodology, writing—review & editing; Orshina Dawd: methodology, writing—review & editing; Meghana Belthur: methodology, writing—review & editing; Donglu Shi: supervision; Tanya V. Kalin: supervision; Vladimir V. Kalinichenko: writing—review & editing, supervision. All authors have read and approved the article.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Sikkema, L. et al. An integrated cell atlas of the lung in health and disease. *Nat. Med.* **29**, 1563–1577 (2023).
2. Wang, C. C. et al. Airborne transmission of respiratory viruses. *Science* **373**, eabd9149 (2021).
3. Mitchell, M. J. et al. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **20**, 101–124 (2021).
4. Bulcha, J. T. et al. Viral vector platforms within the gene therapy landscape. *Signal Transduct. Target Ther.* **6**, 53 (2021).
5. Lundstrom, K. Viral vectors in gene therapy: where do we stand in 2023? *Viruses* **15**, 698 (2023).
6. Fan, D. et al. Nanomedicine in cancer therapy. *Signal Transduct. Target Ther.* **8**, 293 (2023).
7. Sepp, A. et al. The physiological limits of bispecific monoclonal antibody tissue targeting specificity. *MAbs* **17**, 2492236 (2025).
8. Hill, D. B., Button, B., Rubinstein, M. & Boucher, R. C. Physiology and pathophysiology of human airway mucus. *Physiol. Rev.* **102**, 1757–1836 (2022).
9. Sun, L. et al. Clodronate-loaded liposomal and fibroblast-derived exosomal hybrid system for enhanced drug delivery to pulmonary fibrosis. *Biomaterials* **271**, 120761 (2021).
10. Pramanik, S. et al. Nanoparticle-based drug delivery system: the magic bullet for the treatment of chronic pulmonary diseases. *Mol. Pharm.* **18**, 3671–3718 (2021).
11. World Health Organization. *Global Tuberculosis Report 2024* https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024 (2024).
12. Cadena, A. M., Fortune, S. M. & Flynn, J. L. Heterogeneity in tuberculosis. *Nat. Rev. Immunol.* **17**, 691–702 (2017).
13. D'Agnillo, F. et al. Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19. *Sci. Transl. Med.* **13**, eabj7790 (2021).
14. Villalba, J. A. et al. Vasculopathy and increased vascular congestion in fatal COVID-19 and acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* **206**, 857–873 (2022).
15. Christenson, S. A., Smith, B. M., Bafadhel, M. & Putcha, N. Chronic obstructive pulmonary disease. *Lancet* **399**, 2227–2242 (2022).
16. Al Wachami, N. et al. Estimating the global prevalence of chronic obstructive pulmonary disease (COPD): a systematic review and meta-analysis. *BMC Public Health* **24**, 297 (2024).
17. Holtjer, J. C. S. et al. Identifying risk factors for COPD and adult-onset asthma: an umbrella review. *Eur. Respir. Rev.* **32**, 230009 (2023).
18. Elborn, J. S. Cystic fibrosis. *Lancet* **388**, 2519–2531 (2016).
19. Ong, T. & Ramsey, B. W. Cystic fibrosis: a review. *JAMA* **329**, 1859–1871 (2023).
20. de la Bastida-Casero, L., Garcia-Leon, B., Tura-Ceide, O. & Oliver, E. The Relevance of the endothelium in cardiopulmonary disorders. *Int. J. Mol. Sci.* **25**, 9260 (2024).
21. GBD 2021 Pulmonary Arterial Hypertension Collaborators Global, regional, and national burden of pulmonary arterial hypertension, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Respir. Med.* **13**, 69–79 (2025).
22. Cuthbertson, I., Morrell, N. W. & Caruso, P. BMPR2 mutation and metabolic reprogramming in pulmonary arterial hypertension. *Circ. Res.* **132**, 109–126 (2023).
23. Zhu, M. M. et al. GCN2 kinase activation mediates pulmonary vascular remodeling and pulmonary arterial hypertension. *JCI Insight* **9**, e177926 (2024).
24. Galambos, C. et al. Phenotype characterisation of TBX4 mutation and deletion carriers with neonatal and paediatric pulmonary hypertension. *Eur. Respir. J.* **54**, 1801965 (2019).
25. Wu, D. & Birukov, K. Endothelial cell mechano-metabolomic coupling to disease states in the lung microvasculature. *Front. Bioeng. Biotechnol.* **7**, 172 (2019).
26. Fregonese, L. & Stolk, J. Hereditary alpha-1-antitrypsin deficiency and its clinical consequences. *Orphanet J. Rare Dis.* **3**, 16 (2008).
27. Seixas, S. & Marques, P. I. Known mutations at the cause of alpha-1 antitrypsin deficiency an updated overview of SERPINA1 variation spectrum. *Appl Clin. Genet.* **14**, 173–194 (2021).
28. Despotes, K. A., Zariwala, M. A., Davis, S. D. & Ferkol, T. W. Primary ciliary dyskinesia: a clinical review. *Cells* **13**, 974 (2024).
29. Wee, W. B. et al. Primary ciliary dyskinesia. *Pediatrics* **153**, e2023063064 (2024).
30. Melen, E. et al. Asthma inception: epidemiologic risk factors and natural history across the life course. *Am. J. Respir. Crit. Care Med.* **210**, 737–754 (2024).
31. Aghapour, M. et al. Role of air pollutants in airway epithelial barrier dysfunction in asthma and COPD. *Eur. Respir. Rev.* **31**, 210112 (2022).
32. Komlosi, Z. I. et al. Cellular and molecular mechanisms of allergic asthma. *Mol. Asp. Med.* **85**, 100995 (2022).
33. Thai, A. A. et al. Lung cancer. *Lancet* **398**, 535–554 (2021).
34. Benusiglio, P. R. et al. Lung cancer is also a hereditary disease. *Eur. Respir. Rev.* **30**, 210045 (2021).
35. Malhotra, J. et al. Risk factors for lung cancer worldwide. *Eur. Respir. J.* **48**, 889–902 (2016).
36. Pardo, A. & Selman, M. The interplay of the genetic architecture, aging, and environmental factors in the pathogenesis of idiopathic pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **64**, 163–172 (2021).
37. Shin, S. Y. et al. From epithelium to therapy: transitional cells in lung fibrosis. *Am. J. Respir. Cell Mol. Biol.* **72**, 472–483 (2025).
38. Bian, F. et al. Lung endothelial cells regulate pulmonary fibrosis through FOXF1/R-Ras signaling. *Nat. Commun.* **14**, 2560 (2023).

39. Moss, B. J., Ryter, S. W. & Rosas, I. O. Pathogenic mechanisms underlying idiopathic pulmonary fibrosis. *Annu. Rev. Pathol.* **17**, 515–546 (2022).
40. Etzel, R. A. The special vulnerability of children. *Int. J. Hyg. Environ. Health* **227**, 113516 (2020).
41. Bishop, N. B., Stankiewicz, P. & Steinhorn, R. H. Alveolar capillary dysplasia. *Am. J. Respir. Crit. Care Med.* **184**, 172–179 (2011).
42. Gilfillan, M., Bhandari, A. & Bhandari, V. Diagnosis and management of bronchopulmonary dysplasia. *BMJ* **375**, n1974 (2021).
43. Gie, A. & Toelen, J. Chronic neonatal lung disease: time to look beyond the alveolus. *Thorax* **77**, 1161–1162 (2022).
44. Donovan, J. et al. Improving anti-tumor efficacy of low-dose Vincristine in rhabdomyosarcoma via the combination therapy with FOXM1 inhibitor RCM1. *Front. Oncol.* **13**, 1112859 (2023).
45. Willis, J. R. et al. Vaccination with mRNA-encoded nanoparticles drives early maturation of HIV bnAb precursors in humans. *Science* **389**, eadr8382 (2025).
46. Zhang, H. et al. A hepatocyte-targeting nanoparticle for enhanced hepatobiliary magnetic resonance imaging. *Nat. Biomed. Eng.* **7**, 221–235 (2023).
47. Zhao, Y., Zhang, Z., Pan, Z. & Liu, Y. Advanced bioactive nanomaterials for biomedical applications. *Exploration* **1**, 20210089 (2021).
48. Li, Z. et al. Hollow nanomaterials in advanced drug delivery systems: from single- to multiple shells. *Adv. Mater.* **35**, e2203890 (2023).
49. Bussin, B. et al. Discovering nanoparticle corona ligands for liver macrophage capture. *Nat. Nanotechnol.* **20**, 914–925 (2025).
50. Zheng, B. et al. Rare-earth doping in nanostructured inorganic materials. *Chem. Rev.* **122**, 5519–5603 (2022).
51. Fu, D. et al. Self-assembling nanoparticle engineered from the ferritinophagy complex as a rabies virus vaccine candidate. *Nat. Commun.* **15**, 8601 (2024).
52. Liu, X. et al. Comprehensive understanding of magnetic hyperthermia for improving antitumor therapeutic efficacy. *Theranostics* **10**, 3793–3815 (2020).
53. Joseph, T. M. et al. Nanoparticles: taking a unique position in medicine. *Nanomaterials* **13**, 574 (2023).
54. Jeon, M., Halbert, M. V., Stephen, Z. R. & Zhang, M. Iron oxide nanoparticles as T(1) contrast agents for magnetic resonance imaging: fundamentals, challenges, applications, and prospectives. *Adv. Mater.* **33**, e1906539 (2021).
55. Zhi, D. et al. Targeting strategies for superparamagnetic iron oxide nanoparticles in cancer therapy. *Acta Biomater.* **102**, 13–34 (2020).
56. Deng, Z. et al. Dual targeting with cell surface electrical charge and folic acid via superparamagnetic Fe(3)O(4)@Cu(2-x)S for photothermal cancer cell killing. *Cancers* **13**, 5275–5275 (2021).
57. Wareing, T. C., Gentile, P. & Phan, A. N. Biomass-based carbon dots: current development and future perspectives. *ACS Nano.* **15**, 15471–15501 (2021).
58. Li, Z., Perkins, W. & Cipolla, D. Robustness of aerosol delivery of amikacin liposome inhalation suspension using the eFlow(R) Technology. *Eur. J. Pharm. Biopharm.* **166**, 10–18 (2021).
59. Li, X. et al. Multifunctional nanoparticle-mediated combining therapy for human diseases. *Signal Transduct. Target Ther.* **9**, 1 (2024).
60. Kalepu, S. & Nekkanti, V. Insoluble drug delivery strategies: review of recent advances and business prospects. *Acta Pharm. Sin. B.* **5**, 442–453 (2015).
61. Liu, Y. et al. Development of high-drug-loading nanoparticles. *Chempluschem* **85**, 2143–2157 (2020).
62. Beach, M. A. et al. Polymeric nanoparticles for drug delivery. *Chem. Rev.* **124**, 5505–5616 (2024).
63. Sun, S. et al. Targeting and regulating of an oncogene via nanovector delivery of MicroRNA using patient-derived xenografts. *Theranostics* **7**, 677–693 (2017).
64. Trzeciak, K., Chotera-Ouda, A., Bak, S. II & Potrzebowski, M. J. Mesoporous silica particles as drug delivery systems-the state of the art in loading methods and the recent progress in analytical techniques for monitoring these processes. *Pharmaceutics* **13**, 950 (2021).
65. Kumar, R. et al. Polymeric delivery of therapeutic nucleic acids. *Chem. Rev.* **121**, 11527–11652 (2021).
66. Mendes, B. B. et al. Nanodelivery of nucleic acids. *Nat. Rev. Methods Primers* **2**, 24 (2022).
67. Zhang, H. et al. Nucleic acid degradation as barrier to gene delivery: a guide to understand and overcome nuclease activity. *Chem. Soc. Rev.* **53**, 317–360 (2024).
68. Micura, R. & Hobartner, C. Fundamental studies of functional nucleic acids: aptamers, riboswitches, ribozymes and DNAzymes. *Chem. Soc. Rev.* **49**, 7331–7353 (2020).
69. Ho, W. et al. Next-generation vaccines: nanoparticle-mediated DNA and mRNA delivery. *Adv. Health. Mater.* **10**, e2001812 (2021).
70. Eygeris, Y., Gupta, M., Kim, J. & Sahay, G. Chemistry of lipid nanoparticles for RNA delivery. *Acc. Chem. Res.* **55**, 2–12 (2022).
71. Puri, S. et al. Evolution of nanomedicine formulations for targeted delivery and controlled release. *Adv. Drug Deliv. Rev.* **200**, 114962 (2023).
72. Zhu, K. et al. Berberin sustained-release nanoparticles were enriched in infarcted rat myocardium and resolved inflammation. *J. Nanobiotechnol.* **21**, 33 (2023).
73. Mahar, R. et al. Application of PLGA as a biodegradable and biocompatible polymer for pulmonary delivery of drugs. *AAPS PharmSciTech.* **24**, 39 (2023).
74. Liu, J. Y. & Sayes, C. M. Lung surfactant as a biophysical assay for inhalation toxicology. *Curr. Res. Toxicol.* **4**, 100101 (2023).
75. Zhou, Y. et al. Molecularly stimuli-responsive self-assembled peptide nanoparticles for targeted imaging and therapy. *ACS Nano.* **17**, 8004–8025 (2023).
76. Gowda, B. H. J. et al. Stimuli-responsive microneedles as a transdermal drug delivery system: a demand-supply strategy. *Biomacromolecules* **23**, 1519–1544 (2022).
77. Rosenkrans, Z. T., Ferreira, C. A., Ni, D. & Cai, W. Internally responsive nanomaterials for activatable multimodal imaging of cancer. *Adv. Health. Mater.* **10**, e2000690 (2021).
78. Khan, M. I. et al. Recent progress in nanostructured smart drug delivery systems for cancer therapy: a review. *ACS Appl. Bio Mater.* **5**, 971–1012 (2022).
79. Zhang, J. et al. Stimuli-responsive nanoparticles for controlled drug delivery in synergistic cancer immunotherapy. *Adv. Sci.* **9**, e2103444 (2022).
80. Liu, X. et al. GSH-responsive nanoprodrug to inhibit glycolysis and alleviate immunosuppression for cancer therapy. *Nano Lett.* **21**, 7862–7869 (2021).
81. Sun, L. et al. Smart nanoparticles for cancer therapy. *Signal Transduct. Target Ther.* **8**, 418 (2023).
82. Zhang, Q. et al. Stimuli-responsive gene delivery nanocarriers for cancer therapy. *Nanomicro Lett.* **15**, 44 (2023).
83. Wang, S. et al. A novel stimuli-responsive injectable antibacterial hydrogel to achieve synergetic photothermal/gene-targeted therapy towards uveal melanoma. *Adv. Sci.* **8**, e2004721 (2021).
84. Kasinski, A., Zielinska-Pisklak, M., Oledzka, E. & Sobczak, M. Smart hydrogels—synthetic stimuli-responsive antitumor drug release systems. *Int. J. Nanomed.* **15**, 4541–4572 (2020).
85. Tu, L. et al. Ultrasound-controlled drug release and drug activation for cancer therapy. *Exploration.* **1**, 20210023 (2021).
86. Wu, D. et al. The blood-brain barrier: structure, regulation, and drug delivery. *Signal Transduct. Target Ther.* **8**, 217 (2023).
87. Mosquera, J., Garcia, I. & Liz-Marzan, L. M. Cellular uptake of nanoparticles versus small molecules: a matter of size. *Acc. Chem. Res.* **51**, 2305–2313 (2018).
88. Patton, J. S., Fishburn, C. S. & Weers, J. G. The lungs as a portal of entry for systemic drug delivery. *Proc. Am. Thorac. Soc.* **1**, 338–344 (2004).
89. Patton, J. S. et al. The particle has landed-characterizing the fate of inhaled pharmaceuticals. *J. Aerosol. Med. Pulm. Drug Deliv.* **23**, S71–87 (2010).
90. Gandek, T. B., van der Koog, L. & Nagelkerke, A. A comparison of cellular uptake mechanisms, delivery efficacy, and intracellular fate between liposomes and extracellular vesicles. *Adv. Health. Mater.* **12**, e2300319 (2023).
91. Pei, D. How do biomolecules cross the cell membrane? *Acc. Chem. Res.* **55**, 309–318 (2022).
92. Roberts, T. C., Langer, R. & Wood, M. J. A. Advances in oligonucleotide drug delivery. *Nat. Rev. Drug Discov.* **19**, 673–694 (2020).
93. Wu, S., Lin, L., Shi, L. & Liu, S. An overview of lipid constituents in lipid nanoparticle mRNA delivery systems. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **16**, e1978 (2024).
94. Linnane, E. et al. The uptake of metal-organic frameworks: a journey into the cell. *Chem. Soc. Rev.* **51**, 6065–6086 (2022).
95. Li, Y. et al. A computational study of the influence of nanoparticle shape on clathrin-mediated endocytosis. *J. Mater. Chem. B.* **11**, 6319–6334 (2023).
96. E, S. et al. Tunable organelle imaging by rational design of carbon dots and utilization of uptake pathways. *ACS Nano.* **15**, 14465–14474 (2021).
97. Sousa de Almeida, M. et al. Understanding nanoparticle endocytosis to improve targeting strategies in nanomedicine. *Chem. Soc. Rev.* **50**, 5397–5434 (2021).
98. Rennick, J. J., Johnston, A. P. R. & Parton, R. G. Key principles and methods for studying the endocytosis of biological and nanoparticle therapeutics. *Nat. Nanotechnol.* **16**, 266–276 (2021).
99. Herd, H. et al. Nanoparticle geometry and surface orientation influence mode of cellular uptake. *ACS Nano.* **7**, 1961–1973 (2013).
100. Zhang, S., Gao, H. & Bao, G. Physical principles of nanoparticle cellular endocytosis. *ACS Nano.* **9**, 8655–8671 (2015).
101. Hinde, E. et al. Pair correlation microscopy reveals the role of nanoparticle shape in intracellular transport and site of drug release. *Nat. Nanotechnol.* **12**, 81–89 (2017).
102. He, W. et al. Nanocarrier-mediated cytosolic delivery of biopharmaceuticals. *Adv. Funct. Mater.* **30**, 1910566 (2020).
103. Thamizhchelvan, A. M. et al. Shape-dependent cellular uptake of iron oxide nanorods: mechanisms of endocytosis and implications on cell labeling and cellular delivery. *Nanoscale* **16**, 21398–21415 (2024).
104. Zheng, L., Bandara, S. R., Tan, Z. & Leal, C. Lipid nanoparticle topology regulates endosomal escape and delivery of RNA to the cytoplasm. *Proc. Natl. Acad. Sci. USA* **120**, e2301067120 (2023).

105. Wonder, E. et al. Competition of charge-mediated and specific binding by peptide-tagged cationic liposome-DNA nanoparticles in vitro and in vivo. *Biomaterials* **166**, 52–63 (2018).
106. Zhou, M. et al. The role of cell-penetrating peptides in potential anti-cancer therapy. *Clin. Transl. Med.* **12**, e822 (2022).
107. Gori, A. et al. Cell penetrating peptides: classification, mechanisms, methods of study, and applications. *ChemMedChem* **18**, e202300236 (2023).
108. Pirhaghi, M. et al. Cell-penetrating peptides: promising therapeutics and drug-delivery systems for neurodegenerative diseases. *Mol. Pharm.* **21**, 2097–2117 (2024).
109. Pei, D. & Buyanova, M. Overcoming endosomal entrapment in drug delivery. *Bioconjug Chem.* **30**, 273–283 (2019).
110. Qiu, C. et al. Advanced strategies for overcoming endosomal/lysosomal barrier in nanodrug delivery. *Research* **6**, 0148 (2023).
111. Lee, J. et al. DNA-inspired nanomaterials for enhanced endosomal escape. *Proc. Natl. Acad. Sci. USA* **118**, e2104511118 (2021).
112. Wang, Y., Ukwattage, V., Xiong, Y. & Such, G. K. Advancing endosomal escape of polymeric nanoparticles: towards improved intracellular delivery. *Mater. Horiz.* **12**, 3622–3632 (2025).
113. Bajracharya, R. et al. Functional ligands for improving anticancer drug therapy: current status and applications to drug delivery systems. *Drug Deliv.* **29**, 1959–1970 (2022).
114. Bi, D. et al. Deconvolving passive and active targeting of liposomes bearing LDL receptor binding peptides using the zebrafish embryo model. *Small* **20**, e2310781 (2024).
115. Matsumoto, M. L. Molecular mechanisms of multimeric assembly of IgM and IgA. *Annu Rev. Immunol.* **40**, 221–247 (2022).
116. Marques, A. C., Costa, P. J., Velho, S. & Amaral, M. H. Functionalizing nanoparticles with cancer-targeting antibodies: a comparison of strategies. *J. Control Release* **320**, 180–200 (2020).
117. Juan, A. et al. An overview of antibody conjugated polymeric nanoparticles for breast cancer therapy. *Pharmaceutics* **12**, 802 (2020).
118. Kanthe, A. et al. No ordinary proteins: sdsorption and molecular orientation of monoclonal antibodies. *Sci. Adv.* **7**, eabg2873 (2021).
119. Jazayeri, M. H. et al. Various methods of gold nanoparticles (GNPs) conjugation to antibodies. *Sens. Bio-Sens. Res.* **9**, 17–22 (2016).
120. Lee, N. K. et al. Impact of the conjugation of antibodies to the surfaces of polymer nanoparticles on the immune cell targeting abilities. *Nano Converg.* **8**, 24 (2021).
121. Bruckner, M., Simon, J., Landfester, K. & Mailander, V. The conjugation strategy affects antibody orientation and targeting properties of nanocarriers. *Nanoscale* **13**, 9816–9824 (2021).
122. Juan, A. et al. Antibody conjugation of nanoparticles as therapeutics for breast cancer treatment. *Int. J. Mol. Sci.* **21**, 6018 (2020).
123. Agrahari, A. K. et al. Cu(I)-catalyzed click chemistry in glycoscience and their diverse applications. *Chem. Rev.* **121**, 7638–7956 (2021).
124. Kaur, J., Saxena, M. & Rishi, N. An overview of recent advances in biomedical applications of click chemistry. *Bioconjug. Chem.* **32**, 1455–1471 (2021).
125. Yoon, H. Y. et al. Copper-free click chemistry: applications in drug delivery, cell tracking, and tissue engineering. *Adv. Mater.* **34**, e2107192 (2022).
126. Kumar, G. S. & Lin, Q. Light-triggered click chemistry. *Chem. Rev.* **121**, 6991–7031 (2021).
127. Smedley, C. J. et al. Accelerated SuFEx click chemistry for modular synthesis. *Angew. Chem. Int. Ed. Engl.* **61**, e202112375 (2022).
128. Zhang, Q. et al. Site-specific antibody assembly on nanoparticles via a versatile coating method for improved cell targeting. *Adv. Sci.* **10**, e2206546 (2023).
129. Rosenblum, D. et al. CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. *Sci. Adv.* **6**, eabc9450 (2020).
130. Yadav, B. et al. RGD-decorated PLGA nanoparticles improved effectiveness and safety of cisplatin for lung cancer therapy. *Int. J. Pharm.* **633**, 122587 (2023).
131. Sheikh, A., Md, S. & Kesharwani, P. RGD engineered dendrimer nanotherapeutic as an emerging targeted approach in cancer therapy. *J. Control Release* **340**, 221–242 (2021).
132. Abstiens, K., Gregoritza, M. & Goepferich, A. M. Ligand DenSity And Linker Length Are Critical Factors For Multivalent Nanoparticle-receptor Interactions. *ACS Appl. Mater. Interfaces* **11**, 1311–1320 (2019).
133. Biscaglia, F. et al. Gold nanoparticle aggregates functionalized with cyclic RGD peptides for targeting and imaging of colorectal cancer cells. *Acs Appl. Nano Mater.* **2**, 6436–6444 (2019).
134. Siani, P., Frigerio, G., Donadoni, E. & Di Valentin, C. Molecular dynamics simulations of cRGD-conjugated PEGylated TiO(2) nanoparticles for targeted photodynamic therapy. *J. Colloid Interface Sci.* **627**, 126–141 (2022).
135. Zhao, Z., Ukidve, A., Kim, J. & Mitragotri, S. Targeting strategies for tissue-specific drug delivery. *Cell* **181**, 151–167 (2020).
136. Srinivasarao, M. & Low, P. S. Ligand-targeted drug delivery. *Chem. Rev.* **117**, 12133–12164 (2017).
137. Merjaneh, N. et al. FOXM1 inhibitor, RCM-1, enhances venetoclax mediated apoptosis through downregulation of ATP2B4 in rhabdomyosarcoma. *Int. J. Oncol.* **68**, 52 (2026).
138. Goksen, S., Varan, G., Bilensoy, E. & Esendagli, G. Folate receptor beta (FRbeta) expression on myeloid cells and the impact of reticuloendothelial system on folate-functionalized nanoparticles' biodistribution in cancer. *Mol. Pharm.* **21**, 4688–4699 (2024).
139. Mignani, S., Shi, X., Cena, V. & Majoral, J. P. Dendrimer- and polymeric nanoparticle-aptamer bioconjugates as nonviral delivery systems: a new approach in medicine. *Drug Discov. Today* **25**, 1065–1073 (2020).
140. Fu, Z. & Xiang, J. Aptamer-functionalized nanoparticles in targeted delivery and cancer therapy. *Int. J. Mol. Sci.* **21**, 9123 (2020).
141. Ren, J. et al. Chemical and biophysical signatures of the protein corona in nanomedicine. *J. Am. Chem. Soc.* **144**, 9184–9205 (2022).
142. Carney, C. P. et al. Impact of targeting moiety type and protein corona formation on the uptake of Fn14-targeted nanoparticles by cancer cells. *ACS Nano.* **17**, 19667–19684 (2023).
143. Blanco, E., Shen, H. & Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **33**, 941–951 (2015).
144. Xu, M. et al. Size-dependent in vivo transport of nanoparticles: implications for delivery, targeting, and clearance. *ACS Nano* **17**, 20825–20849 (2023).
145. Deng, Z., Kalin, G. T., Shi, D. & Kalinichenko, V. V. Nanoparticle delivery systems with cell-specific targeting for pulmonary diseases. *Am. J. Respir. Cell Mol. Biol.* **64**, 292–307 (2021).
146. Dunn, A. W., Kalinichenko, V. V. & Shi, D. Highly efficient in vivo targeting of the pulmonary endothelium using novel modifications of polyethylenimine: an importance of charge. *Adv. Health. Mater.* **7**, e1800876 (2018).
147. Peng, Y. et al. Size-controlled DNA tile self-assembly nanostructures through caveolae-mediated endocytosis for signal-amplified imaging of MicroRNAs in living cells. *Adv. Sci.* **10**, e2300614 (2023).
148. Dolai, J., Mandal, K. & Jana, N. R. Nanoparticle size effects in biomedical applications. *ACS Appl. Nano Mater.* **4**, 6471–6496 (2021).
149. Fan, J., Zheng, S., Wang, M. & Yuan, X. The critical roles of caveolin-1 in lung diseases. *Front. Pharmacol.* **15**, 1417834 (2024).
150. Yang, C. et al. The role of caveolin-1 in the biofate and efficacy of anti-tumor drugs and their nano-drug delivery systems. *Acta Pharm. Sin. B.* **11**, 961–977 (2021).
151. Gillich, A. et al. Capillary cell-type specialization in the alveolus. *Nature* **586**, 785–789 (2020).
152. Bolte, C. et al. Nanoparticle delivery of proangiogenic transcription factors into the neonatal circulation inhibits alveolar simplification caused by hyperoxia. *Am. J. Respir. Crit. Care Med.* **202**, 100–111 (2020).
153. Kohram, F. et al. Demonstration of safety in wild type mice of npFOXF1, a novel nanoparticle-based gene therapy for alveolar capillary dysplasia with misaligned pulmonary veins. *Biologics* **17**, 43–55 (2023).
154. Fu, L. et al. The role of the cell surface glycocalyx in drug delivery to and through the endothelium. *Adv. Drug Deliv. Rev.* **184**, 114195 (2022).
155. Wang, J. P. et al. Targeting endothelial cell junctions with negatively charged gold nanoparticles. *Chem. Mater.* **30**, 3759–3767 (2018).
156. Chen, B. et al. Targeting negative surface charges of cancer cells by multifunctional nanoprobes. *Theranostics* **6**, 1887–1898 (2016).
157. Warburg, O. On the origin of cancer cells. *Science* **123**, 309–314 (1956).
158. Liberti, M. V. & Locasale, J. W. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci.* **41**, 211–218 (2016).
159. Han, X. et al. Biomarkerless targeting and photothermal cancer cell killing by surface-electrically-charged superparamagnetic Fe(3)O(4) composite nanoparticles. *Nanoscale* **9**, 1457–1465 (2017).
160. Zhao, J. et al. Electrical-charge-mediated cancer cell targeting via protein corona-decorated superparamagnetic nanoparticles in a simulated physiological environment. *ACS Appl Mater. Interfaces* **10**, 41986–41998 (2018).
161. Ostrovski, Y., Hofemeier, P. & Sznitman, J. Augmenting regional and targeted delivery in the pulmonary acinus using magnetic particles. *Int. J. Nanomed.* **11**, 3385–3395 (2016).
162. Reczynska, K. et al. Stimuli-sensitive fatty acid-based microparticles for the treatment of lung cancer. *Mater. Sci. Eng. C. Mater. Biol. Appl.* **111**, 110801 (2020).
163. Price, D. N., Stromberg, L. R., Kunda, N. K. & Muttil, P. In vivo pulmonary delivery and magnetic-targeting of dry powder nano-in-microparticles. *Mol. Pharm.* **14**, 4741–4750 (2017).
164. Silva, L. H. A. et al. Magnetic targeting increases mesenchymal stromal cell retention in lungs and enhances beneficial effects on pulmonary damage in experimental silicosis. *Stem Cells Transl. Med.* **9**, 1244–1256 (2020).

165. Sanwal, R. et al. Ultrasound and microbubbles for targeted drug delivery to the lung endothelium in ARDS: cellular mechanisms and therapeutic opportunities. *Biomedicines* **9**, 803 (2021).
166. Li, M. et al. Ultrasound-responsive nanocarriers with siRNA and Fe(3)O(4) regulate macrophage polarization and phagocytosis for augmented non-small cell lung cancer immunotherapy. *J. Nanobiotechnol.* **22**, 605 (2024).
167. Weers, J. Inhaled antimicrobial therapy—barriers to effective treatment. *Adv. Drug Deliv. Rev.* **85**, 24–43 (2015).
168. Cipolla, D., Blanchard, J. & Gonda, I. Development of liposomal ciprofloxacin to treat lung infections. *Pharmaceutics* **8**, 6 (2016).
169. Wang, B. et al. Pulmonary inhalation for disease treatment: basic research and clinical translations. *Mater. Today Bio.* **25**, 100966 (2024).
170. Garcia-Mouton, C., Hidalgo, A., Cruz, A. & Perez-Gil, J. The lord of the lungs: the essential role of pulmonary surfactant upon inhalation of nanoparticles. *Eur. J. Pharm. Biopharm.* **144**, 230–243 (2019).
171. Darquenne, C. Deposition mechanisms. *J. Aerosol. Med. Pulm. Drug Deliv.* **33**, 181–185 (2020).
172. Jabbal, S., Poli, G. & Lipworth, B. Does size really matter?: Relationship of particle size to lung deposition and exhaled fraction. *J. Allergy Clin. Immunol.* **139**, 2013–2014 e2011 (2017).
173. Yu, Y. et al. Increasing stiffness promotes pulmonary retention of ligand-directed dexamethasone-loaded nanoparticle for enhanced acute lung inflammation therapy. *Bioact. Mater.* **20**, 539–547 (2023).
174. Alwani, S., Wasan, E. K. & Badea, I. Solid lipid nanoparticles for pulmonary delivery of biopharmaceuticals: a review of opportunities, challenges, and delivery applications. *Mol. Pharm.* **21**, 3084–3102 (2024).
175. Abrami, M. et al. Mucus structure, viscoelastic properties, and composition in chronic respiratory diseases. *Int. J. Mol. Sci.* **25**, 1933 (2024).
176. Murgia, X. et al. The role of mucus on drug transport and its potential to affect therapeutic outcomes. *Adv. Drug Deliv. Rev.* **124**, 82–97 (2018).
177. Chen, D., Liu, J., Wu, J. & Suk, J. S. Enhancing nanoparticle penetration through airway mucus to improve drug delivery efficacy in the lung. *Expert Opin. Drug Deliv.* **18**, 595–606 (2021).
178. Pearson, J. P., Chater, P. I. & Wilcox, M. D. The properties of the mucus barrier, a unique gel–how can nanoparticles cross it? *Ther. Deliv.* **7**, 229–244 (2016).
179. Murgia, X. et al. Size-limited penetration of nanoparticles into porcine respiratory mucus after aerosol deposition. *Biomacromolecules* **17**, 1536–1542 (2016).
180. Dickey, B. F. & Evans, C. M. Towards a better mucolytic. *Eur. Respir. J.* **61**, 2300619 (2023).
181. Guo, Y. et al. Mucus penetration of surface-engineered nanoparticles in various pH microenvironments. *ACS Nano.* **17**, 2813–2828 (2023).
182. Huckaby, J. T. & Lai, S. K. PEGylation for enhancing nanoparticle diffusion in mucus. *Adv. Drug Deliv. Rev.* **124**, 125–139 (2018).
183. Xu, Q. et al. Impact of surface polyethylene glycol (PEG) density on biodegradable nanoparticle transport in mucus ex vivo and distribution in vivo. *ACS Nano.* **9**, 9217–9227 (2015).
184. Craparo, E. F. et al. Pegylated polyaspartamide-polylactide-based nanoparticles penetrating cystic fibrosis artificial mucus. *Biomacromolecules* **17**, 767–777 (2016).
185. Yu, X. et al. Poly(ethyl ethylene phosphate): overcoming the "polyethylene glycol dilemma" for cancer immunotherapy and mRNA vaccination. *ACS Nano.* **17**, 23814–23828 (2023).
186. Carlson, T. L., Lock, J. Y. & Carrier, R. L. Engineering the mucus barrier. *Annu Rev. Biomed. Eng.* **20**, 197–220 (2018).
187. Park, K. et al. Nitric oxide-assisted mucus-walking zwitterionic nanocomplexes for synergistic treatment of severe respiratory infectious disease. *Mater. Today* **72**, 57–70 (2024).
188. Jiang, A. Y. et al. Zwitterionic polymer-functionalized lipid nanoparticles for the nebulized delivery of mRNA. *J. Am. Chem. Soc.* **146**, 32567–32574 (2024).
189. Liu, Q. et al. The influence of a biomimetic pulmonary surfactant modification on the in vivo fate of nanoparticles in the lung. *Acta Biomater.* **147**, 391–402 (2022).
190. Kumari, A. et al. Surface-engineered mucus penetrating nucleic acid delivery systems with cell penetrating peptides for the lungs. *Mol. Pharm.* **19**, 1309–1324 (2022).
191. Yang, M. Y. et al. Dual barrier-penetrating inhaled nanoparticles for enhanced idiopathic pulmonary fibrosis therapy. *Adv. Funct. Mater.* **34**, 2315128 (2024).
192. Liu, C. et al. Hydrogen-induced disruption of the airway mucus barrier enhances nebulized RNA delivery to reverse pulmonary fibrosis. *Sci. Adv.* **11**, eadt2752 (2025).
193. Liu, Q. et al. Physicochemical properties affecting the fate of nanoparticles in pulmonary drug delivery. *Drug Discov. Today* **25**, 150–159 (2020).
194. Seydoux, E. et al. Pulmonary delivery of cationic gold nanoparticles boost antigen-specific CD4(+) T cell proliferation. *Nanomedicine* **12**, 1815–1826 (2016).
195. Fromen, C. A. et al. Nanoparticle surface charge impacts distribution, uptake and lymph node trafficking by pulmonary antigen-presenting cells. *Nanomedicine* **12**, 677–687 (2016).
196. Lee, W. T. et al. Alveolar macrophage phagocytosis-evading inhaled microgels incorporating nintedanib-PLGA nanoparticles and pirfenidone-liposomes for improved treatment of pulmonary fibrosis. *Bioact. Mater.* **33**, 262–278 (2024).
197. Guichard, M. J., Leal, T. & Vanbever, R. PEGylation, an approach for improving the pulmonary delivery of biopharmaceuticals. *Curr. Opin. Colloid Interface Sci.* **31**, 43–50 (2017).
198. Murata, M., Tahara, K. & Takeuchi, H. Real-time in vivo imaging of surface-modified liposomes to evaluate their behavior after pulmonary administration. *Eur. J. Pharm. Biopharm.* **86**, 115–119 (2014).
199. Jung, S. et al. Zwitterionic inhaler with synergistic therapeutics for reprogramming of M2 macrophage to pro-inflammatory phenotype. *Adv. Health. Mater.* **12**, e2300226 (2023).
200. Wang, Q. et al. Surfactant proteins A/D-CD14 on alveolar macrophages is a common pathway associated with phagocytosis of nanomaterials and cytokine production. *Front. Immunol.* **12**, 758941 (2021).
201. Zhang, J. et al. Amikacin liposome inhalation suspension (ALIS) penetrates non-tuberculous mycobacterial biofilms and enhances amikacin uptake into macrophages. *Front. Microbiol.* **9**, 915 (2018).
202. Carregal-Romero, S. et al. Delayed alveolar clearance of nanoparticles through control of coating composition and interaction with lung surfactant protein A. *Biomater. Adv.* **134**, 112551 (2022).
203. Liu, C. et al. An inhalable hybrid biomimetic nanoplatform for sequential drug release and remodeling lung immune homeostasis in acute lung injury treatment. *ACS Nano.* **17**, 11626–11644 (2023).
204. Kamerkar, S. et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* **546**, 498–503 (2017).
205. Gou, J. et al. Inhalable exosomes in respiratory therapies with the transformative potential. *Int. J. Nanomed.* **20**, 14219–14252 (2025).
206. Lee, J. W. et al. Inhalation-based nanoparticle drug delivery targeting the diseased lower airways in idiopathic pulmonary fibrosis. *Pharmaceutics* **18**, 168 (2026).
207. Mahmoud, D. E. et al. Inhalable nanotechnology-based drug delivery systems for the treatment of inflammatory lung diseases. *Pharmaceutics* **17**, 893 (2025).
208. Kumar, R. et al. Nanotechnology-assisted metered-dose inhalers (MDIs) for high-performance pulmonary drug delivery applications. *Pharm. Res.* **39**, 2831–2855 (2022).
209. Mansour, H. M., Muralidharan, P. & Hayes, D. Jr. Inhaled nanoparticulate systems: composition, manufacture and aerosol delivery. *J. Aerosol. Med. Pulm. Drug Deliv.* **37**, 202–218 (2024).
210. Li, N. et al. Surfactant protein-A nanobody-conjugated liposomes loaded with methylprednisolone increase lung-targeting specificity and therapeutic effect for acute lung injury. *Drug Deliv.* **24**, 1770–1781 (2017).
211. Nowak-Jary, J. & Machnicka, B. In vivo biodistribution and clearance of magnetic iron oxide nanoparticles for medical applications. *Int. J. Nanomed.* **18**, 4067–4100 (2023).
212. Zelepukin, I. V., Shevchenko, K. G. & Deyev, S. M. Rediscovery of mononuclear phagocyte system blockade for nanoparticle drug delivery. *Nat. Commun.* **15**, 4366 (2024).
213. Pustulka, S. M., Ling, K., Pish, S. L. & Champion, J. A. Protein nanoparticle charge and hydrophobicity govern protein corona and macrophage uptake. *ACS Appl. Mater. Interfaces* **12**, 48284–48295 (2020).
214. Zou, Y. et al. Polyglycerol grafting shields nanoparticles from protein corona formation to avoid macrophage uptake. *ACS Nano* **14**, 7216–7226 (2020).
215. Chen, Y., Su, Y.-C. & Roffler, S. R. Polyethylene glycol immunogenicity in nanomedicine. *Nat. Rev. Bioeng.* **3**, 742–760 (2025).
216. Peng, S. et al. Biodegradable zwitterionic polymer membrane coating endowing nanoparticles with ultra-long circulation and enhanced tumor photothermal therapy. *Biomaterials* **231**, 119680 (2020).
217. Tavano, R. et al. Species differences in opsonization and phagocyte recognition of preclinical poly-2-alkyl-2-oxazoline-coated nanoparticles. *Nat. Commun.* **16**, 2642 (2025).
218. Fam, S. Y. et al. Stealth coating of nanoparticles in drug-delivery systems. *Nanomaterials* **10**, 787 (2020).
219. Fang, R. H., Kroll, A. V., Gao, W. & Zhang, L. Cell membrane coating nanotechnology. *Adv. Mater.* **30**, e1706759 (2018).
220. Kusumoto, K. et al. Lipid envelope-type nanoparticle incorporating a multifunctional peptide for systemic siRNA delivery to the pulmonary endothelium. *ACS Nano.* **7**, 7534–7541 (2013).
221. Abd Elwakil, M. M. et al. Lung-endothelium-targeted nanoparticles based on a ph-sensitive lipid and the GALA Peptide enable robust gene silencing and the regression of metastatic lung cancer. *Adv. Funct. Mater.* **29**, 1807677 (2019).

222. Cheng, Q. et al. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat. Nanotechnol.* **15**, 313–320 (2020).
223. Deng, Z. et al. Fluorinated amphiphilic Poly(beta-Amino ester) nanoparticle for highly efficient and specific delivery of nucleic acids to the lung capillary endothelium. *Bioact. Mater.* **31**, 1–17 (2024).
224. Yang, D. et al. Transferrin functionization elevates transcytosis of nanogranules across epithelium by triggering polarity-associated transport flow and positive cellular feedback loop. *ACS Nano.* **13**, 5058–5076 (2019).
225. Chen, Z. et al. Inhalable myofibroblast targeting nanoparticles for synergistic treatment of pulmonary fibrosis. *Sci. Adv.* **11**, eadv9571 (2025).
226. May, J., Mitchell, J. A. & Jenkins, R. G. Beyond epithelial damage: vascular and endothelial contributions to idiopathic pulmonary fibrosis. *J. Clin. Invest.* **133**, e172058 (2023).
227. Park, J. H. et al. Genetically engineered cell membrane-coated nanoparticles for targeted delivery of dexamethasone to inflamed lungs. *Sci. Adv.* **7**, eabf7820 (2021).
228. Akhter, M. H. et al. Impact of protein corona on the biological identity of nanomedicine: understanding the fate of nanomaterials in the biological milieu. *Biomedicines* **9**, 1496 (2021).
229. Zheng, Q. et al. Mortality and survival in idiopathic pulmonary fibrosis: a systematic review and meta-analysis. *ERJ Open Res.* **8**, 00591–2021 (2022).
230. Glass, D. S. et al. Idiopathic pulmonary fibrosis: current and future treatment. *Clin. Respir. J.* **16**, 84–96 (2022).
231. Mei, Q. et al. Idiopathic pulmonary fibrosis: an update on pathogenesis. *Front. Pharmacol.* **12**, 797292 (2021).
232. Todd, N. W., Luzina, I. G. & Atamas, S. P. Molecular and cellular mechanisms of pulmonary fibrosis. *Fibrogenes. Tissue Repair.* **5**, 11 (2012).
233. Henderson, N. C., Rieder, F. & Wynn, T. A. Fibrosis: from mechanisms to medicines. *Nature* **587**, 555–566 (2020).
234. Kendall, R. T. & Feghali-Bostwick, C. A. Fibroblasts in fibrosis: novel roles and mediators. *Front. Pharmacol.* **5**, 123 (2014).
235. Ju, X. et al. Regulation of myofibroblast dedifferentiation in pulmonary fibrosis. *Respir. Res.* **25**, 284 (2024).
236. Kheirollahi, V. et al. Metformin induces lipogenic differentiation in myofibroblasts to reverse lung fibrosis. *Nat. Commun.* **10**, 2987 (2019).
237. Huang, E. et al. The roles of immune cells in the pathogenesis of fibrosis. *Int. J. Mol. Sci.* **21**, 5203 (2020).
238. Gieseck, R. L. 3rd, Wilson, M. S. & Wynn, T. A. Type 2 immunity in tissue repair and fibrosis. *Nat. Rev. Immunol.* **18**, 62–76 (2018).
239. Zhao, M. et al. Targeting fibrosis, mechanisms and cilinical trials. *Signal Transduct. Target Ther.* **7**, 206 (2022).
240. Perrot, C. Y., Karampitsakos, T. & Herazo-Maya, J. D. Monocytes and macrophages: emerging mechanisms and novel therapeutic targets in pulmonary fibrosis. *Am. J. Physiol. Cell Physiol.* **325**, C1046–C1057 (2023).
241. Ortiz-Zapater, E., Signes-Costa, J., Montero, P. & Roger, I. Lung fibrosis and fibrosis in the lungs: is it all about myofibroblasts? *Biomedicines* **10**, 1423 (2022).
242. Zhao, W. et al. Injured endothelial cell: a risk factor for pulmonary fibrosis. *Int. J. Mol. Sci.* **24**, 8749 (2023).
243. Yanagihara, T. et al. Connective-tissue growth factor contributes to TGF-beta1-induced lung fibrosis. *Am. J. Respir. Cell Mol. Biol.* **66**, 260–270 (2022).
244. Armulik, A., Genove, G. & Betsholtz, C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev. Cell.* **21**, 193–215 (2011).
245. Leonard-Duke, J., Hung, C., Sharma, A. & Peirce, S. M. Multi-scale computational model of endothelial cell-pericyte coupling in idiopathic pulmonary fibrosis. *Faseb Journal.* **36**, (2022).
246. Hung, C. & Altemeier, W. Angiogenic role of lung pericytes in experimental viral lung injury. *FASEB J.* **36**, (2022).
247. Garrison, A. T., Bignold, R. E., Wu, X. & Johnson, J. R. Pericytes: the lung-forgotten cell type. *Front. Physiol.* **14**, 1150028 (2023).
248. GGao, W. et al. Emergence of fibrotic pericytes and their transcriptional regulation in pulmonary fibrosis. *Nat. Commun.* **17**, 5142 (2026).
249. Noble, P. W., Barkauskas, C. E. & Jiang, D. Pulmonary fibrosis: patterns and perpetrators. *J. Clin. Invest.* **122**, 2756–2762 (2012).
250. Selman, M. & Pardo, A. The leading role of epithelial cells in the pathogenesis of idiopathic pulmonary fibrosis. *Cell Signal.* **66**, 109482 (2020).
251. Salton, F., Volpe, M. C. & Confalonieri, M. Epithelial(-)mesenchymal transition in the pathogenesis of idiopathic pulmonary fibrosis. *Medicina* **55**, 83 (2019).
252. Yang, M.-Y. et al. Dual barrier-penetrating inhaled nanoparticles for enhanced idiopathic pulmonary fibrosis therapy. *Adv. Funct. Mater.* **34**, 2315128 (2024).
253. Massaro, M. et al. Lipid nanoparticle-mediated mRNA delivery in lung fibrosis. *Eur. J. Pharm. Sci.* **183**, 106370 (2023).
254. Bai, X. et al. Inhaled siRNA nanoparticles targeting IL11 inhibit lung fibrosis and improve pulmonary function post-bleomycin challenge. *Sci. Adv.* **8**, eabn7162 (2022).
255. Kim, J. et al. Lung-targeted delivery of TGF-beta antisense oligonucleotides to treat pulmonary fibrosis. *J. Control Release* **322**, 108–121 (2020).
256. Hou, J. et al. Co-delivery of siPTPN13 and siNOX4 via (myo)fibroblast-targeting polymeric micelles for idiopathic pulmonary fibrosis therapy. *Theranostics* **11**, 3244–3261 (2021).
257. Singh, A. et al. Nanoparticle targeting of de novo profibrotic macrophages mitigates lung fibrosis. *Proc. Natl. Acad. Sci. Usa.* **119**, e2121098119 (2022).
258. Wang, Y. et al. Realveolarization with inhalable mucus-penetrating lipid nanoparticles for the treatment of pulmonary fibrosis in mice. *Sci. Adv.* **10**, eado4791 (2024).
259. Jin, H. et al. Engineered lipid nanoparticles for the treatment of pulmonary fibrosis by regulating epithelial-mesenchymal transition in the lungs. *Adv. Funct. Mater.* **33**, 2209432 (2023).
260. Li, X. et al. ROS-responsive Janus Au/mesoporous silica core/shell nanoparticles for drug delivery and long-term CT imaging tracking of MSCs in pulmonary fibrosis treatment. *ACS Nano.* **17**, 6387–6399 (2023).
261. Knipe, R. S. et al. Myofibroblast-specific inhibition of the Rho kinase-MRTF-SRF pathway using nanotechnology for the prevention of pulmonary fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **324**, L190–L198 (2023).
262. Li, J. et al. Pulmonary delivery of specialized pro-resolving mediators-based nanotherapeutics attenuates pulmonary fibrosis in preclinical animal models. *ACS Nano.* **17**, 15354–15370 (2023).
263. Hou, J. et al. Spatial targeting of fibrosis-promoting macrophages with nanoscale metal-organic frameworks for idiopathic pulmonary fibrosis therapy. *Acta Biomater.* **174**, 372–385 (2024).
264. Chang, X. et al. Monocyte-derived multipotent cell delivered programmed therapeutics to reverse idiopathic pulmonary fibrosis. *Sci. Adv.* **6**, eaba3167 (2020).
265. Wang, X. et al. Magnetic liposome as a dual-targeting delivery system for idiopathic pulmonary fibrosis treatment. *J. Colloid Interface Sci.* **636**, 388–400 (2023).
266. Chang, X. et al. Nanoengineered immunosuppressive therapeutics modulating M1/M2 macrophages into the balanced status for enhanced idiopathic pulmonary fibrosis therapy. *Nanoscale* **12**, 8664–8678 (2020).
267. Cao, X. et al. Co-delivery of astaxanthin and siTGF-β1 via ionizable liposome nanoparticles for improved idiopathic pulmonary fibrosis therapy. *ACS Appl. Mater. Interfaces* **16**, 20260–20272 (2024).
268. Infections, G. B. D. L. R. & Antimicrobial Resistance, C. Global, regional, and national incidence and mortality burden of non-COVID-19 lower respiratory infections and aetiologies, 1990-2021: a systematic analysis from the Global Burden of Disease Study 2021. *Lancet Infect. Dis.* **24**, 974–1002 (2024).
269. Xiao, K. et al. A pan-immune panorama of bacterial pneumonia revealed by a large-scale single-cell transcriptome atlas. *Signal Transduct. Target Ther.* **10**, 5 (2025).
270. Muhammad, M. H. et al. Beyond risk: bacterial biofilms and their regulating approaches. *Front. Microbiol.* **11**, 928 (2020).
271. Gao, S. et al. Bacterial capsules: occurrence, mechanism, and function. *NPJ Biofilms Microbiomes* **10**, 21 (2024).
272. Grousd, J. A., Rich, H. E. & Alcorn, J. F. Host-pathogen interactions in gram-positive bacterial pneumonia. *Clin. Microbiol. Rev.* **32**, e00107–18 (2019).
273. Talapko, J. et al. *Candida albicans*-the virulence factors and clinical manifestations of infection. *J. Fungi* **7**, 79 (2021).
274. Santana, D. J. et al. A Candida auris-specific adhesin, Scf1, governs surface association, colonization, and virulence. *Science* **381**, 1461–1467 (2023).
275. Balducci, E., Papi, F., Capialbi, D. E. & Del Bino, L. Polysaccharides' structures and functions in biofilm architecture of antimicrobial-resistant (AMR) pathogens. *Int. J. Mol. Sci.* **24**, 4030 (2023).
276. Greenwood, D. J. et al. Subcellular antibiotic visualization reveals a dynamic drug reservoir in infected macrophages. *Science* **364**, 1279–1282 (2019).
277. Jia, L. J. et al. Aspergillus fumigatus hijacks human p11 to redirect fungal-containing phagosomes to non-degradative pathway. *Cell Host Microbe* **31**, 373–388 e310 (2023).
278. Clementi, N. et al. Viral respiratory pathogens and lung injury. *Clin. Microbiol. Rev.* **34**, e00103–20 (2021).
279. Omidian, H. & Wilson, R. L. PLGA-based strategies for intranasal and pulmonary applications. *Pharmaceutics* **17**, 207 (2025).
280. Guo, X. et al. PLGA-based micro/nanoparticles: an overview of their applications in respiratory diseases. *Int. J. Mol. Sci.* **24**, 4333 (2023).
281. Gao, W. et al. Nanoparticle-based local antimicrobial drug delivery. *Adv. Drug Deliv. Rev.* **127**, 46–57 (2018).
282. Vu, M. N., Kelly, H. G., Kent, S. J. & Wheatley, A. K. Current and future nanoparticle vaccines for COVID-19. *EBioMedicine* **74**, 103699 (2021).
283. Ren, H.-M. et al. Inhalable responsive polysaccharide-based antibiotic delivery nanoparticles to overcome mucus barrier for lung infection treatment. *Nano Today* **44**, 101489 (2022)

284. Pan, X. T. et al. Inhalable MOF-derived nanoparticles for sonodynamic therapy of bacterial pneumonia. *Adv. Funct. Mater.* **32**, 2205123 (2022).
285. Song, Y. et al. Recent advances of cell membrane-coated nanoparticles for therapy of bacterial infection. *Front. Microbiol.* **14**, 1083007 (2023).
286. Li, B. et al. Photothermal therapy of tuberculosis using targeting pre-activated macrophage membrane-coated nanoparticles. *Nat. Nanotechnol.* **19**, 834–845 (2024).
287. Li, Y. et al. Inflammation and microbiota regulation potentiate pneumonia therapy by biomimetic bacteria and macrophage membrane nanosystem. *Research* **6**, 0096 (2023).
288. Peng, X. et al. Biofunctional lipid nanoparticles for precision treatment and prophylaxis of bacterial infections. *Sci. Adv.* **10**, eadk9754 (2024).
289. Zhang, F. et al. Nanoparticle-modified microrobots for in vivo antibiotic delivery to treat acute bacterial pneumonia. *Nat. Mater.* **21**, 1324–1332 (2022).
290. Sung, J. C. et al. Formulation and pharmacokinetics of self-assembled rifampicin nanoparticle systems for pulmonary delivery. *Pharm. Res.* **26**, 1847–1855 (2009).
291. Maloney Norcross, S. E., Levin, L. P. K., Hickey, A. J. & Hill, D. B. Biopolymeric inhalable dry powders for pulmonary drug delivery. *Pharmaceuticals* **17**, 1628 (2024).
292. Durham, P. G. et al. Spray dried aerosol particles of pyrazinoic acid salts for tuberculosis therapy. [Corrected]. *Mol. Pharm.* **12**, 2574–2581 (2015).
293. Peppe, S. et al. Nanoparticle-mediated delivery of deferasirox: a promising strategy against invasive aspergillosis. *Bioeng.* **11**, 1115 (2024).
294. Fayyaz, H. A. et al. Targeted DPPC/DMPG surface-modified voriconazole lipid nanoparticles control invasive pulmonary aspergillosis in immunocompromised population: in-vitro and in-vivo assessment. *Int. J. Pharm.* **649**, 123663 (2024).
295. Ali, E. M. & Abdallah, B. M. Effective inhibition of invasive pulmonary aspergillosis by silver nanoparticles biosynthesized with artemisia sieberi leaf extract. *Nanomaterials* **12**, 51 (2021).
296. Yu, Y. et al. Cascade-targeting polymeric particles eliminate intracellular C. neoformans in fungal infection therapy. *J. Control Release* **373**, 399–409 (2024).
297. Wang, Y. et al. mRNA vaccine: a potential therapeutic strategy. *Mol. Cancer* **20**, 33 (2021).
298. Wilson, B. & Geetha, K. M. Lipid nanoparticles in the development of mRNA vaccines for COVID-19. *J. Drug Deliv. Sci. Technol.* **74**, 103553 (2022).
299. Zhang, L. et al. Effect of mRNA-LNP components of two globally-marketed COVID-19 vaccines on efficacy and stability. *NPJ Vaccines* **8**, 156 (2023).
300. Alu, A. et al. Intranasal COVID-19 vaccines: from bench to bed. *EBioMedicine* **76**, 103841 (2022).
301. Lei, H. et al. Cationic crosslinked carbon dots-adjuvanted intranasal vaccine induces protective immunity against Omicron-included SARS-CoV-2 variants. *Nat. Commun.* **14**, 2678 (2023).
302. Igyarto, B. Z. & Qin, Z. The mRNA-LNP vaccines—the good, the bad and the ugly? *Front. Immunol.* **15**, 1336906 (2024).
303. Idris, A. et al. A SARS-CoV-2 targeted siRNA-nanoparticle therapy for COVID-19. *Mol. Ther.* **29**, 2219–2226 (2021).
304. Zhao, G. et al. Precision treatment of viral pneumonia through macrophage-targeted lipid nanoparticle delivery. *Proc. Natl. Acad. Sci. USA* **121**, e2314747121 (2024).
305. Li, Z. et al. Cell-mimicking nanodecoys neutralize SARS-CoV-2 and mitigate lung injury in a non-human primate model of COVID-19. *Nat. Nanotechnol.* **16**, 942–951 (2021).
306. Martin-Faivre, L. et al. Pulmonary delivery of silver nanoparticles prevents influenza infection by recruiting and activating lymphoid cells. *Biomaterials* **312**, 122721 (2025).
307. De Oca, M. M. et al. The global burden of COPD: epidemiology and effect of prevention strategies. *Lancet Respir. Med.* **13**, 709–724 (2025).
308. Fabbri, L. M. Smoking, not COPD, as the disease. *N. Engl. J. Med.* **374**, 1885–1886 (2016).
309. Miravitlles, M. et al. Disease burden associated with alpha-1 antitrypsin deficiency: systematic and structured literature reviews. *Eur. Respir. Rev.* **31**, 210262 (2022).
310. Barnes, P. J., Ito, K. & Adcock, I. M. Corticosteroid resistance in chronic obstructive pulmonary disease: inactivation of histone deacetylase. *Lancet* **363**, 731–733 (2004).
311. Xu, J. et al. Inflammation mechanism and research progress of COPD. *Front. Immunol.* **15**, 1404615 (2024).
312. Brightling, C. & Greening, N. Airway inflammation in COPD: progress to precision medicine. *Eur. Respir. J.* **54**, 1900651 (2019).
313. Irusen, E. M. & Malange, T. Pharmacotherapy of chronic obstructive pulmonary disease: therapeutic considerations with a focus on inhaled corticosteroids. *S. Afr. Fam. Pract.* **62**, e1–e6 (2020).
314. Yang, I. A. et al. Inhaled corticosteroids versus placebo for stable chronic obstructive pulmonary disease. *Cochrane Database Syst. Rev.* **3**, CD002991 (2023).
315. Chong, J., Leung, B. & Poole, P. Phosphodiesterase 4 inhibitors for chronic obstructive pulmonary disease. *Cochrane Database Syst. Rev.* **9**, CD002309 (2017).
316. Beeh, K. M. The role of bronchodilators in preventing exacerbations of chronic obstructive pulmonary disease. *Tuberc. Respir. Dis.* **79**, 241–247 (2016).
317. Vanfleteren, L. et al. Triple therapy (ICS/LABA/LAMA) in COPD: time for a reappraisal. *Int. J. Chron. Obstruct. Pulmon. Dis.* **13**, 3971–3981 (2018).
318. Chen, W. H., Chen, C. H. & Chang, M. H. Exploring nanoparticles in lungs under COPD conditions for nanospray drug flow and deposition: CFD simulations and AI predictions. *Int. J. Pharm.* **678**, 125740 (2025).
319. Aghapour, M. et al. Airway epithelial barrier dysfunction in chronic obstructive pulmonary disease: role of cigarette smoke exposure. *Am. J. Respir. Cell Mol. Biol.* **58**, 157–169 (2018).
320. Baker, J. R. & Donnelly, L. E. Leukocyte function in COPD: clinical relevance and potential for drug therapy. *Int. J. Chron. Obstruct. Pulmon. Dis.* **16**, 2227–2242 (2021).
321. De Leo, V. et al. Preparation of drug-loaded small unilamellar liposomes and evaluation of their potential for the treatment of chronic respiratory diseases. *Int. J. Pharm.* **545**, 378–388 (2018).
322. Vij, N. et al. Neutrophil targeted nano-drug delivery system for chronic obstructive lung diseases. *Nanomedicine* **12**, 2415–2427 (2016).
323. Mohamed, A. et al. Pulmonary delivery of nanocomposite microparticles (NCMPs) incorporating miR-146a for treatment of COPD. *Int. J. Pharm.* **569**, 118524 (2019).
324. Hayashi, G. et al. Dimethyl fumarate mediates Nrf2-dependent mitochondrial biogenesis in mice and humans. *Hum. Mol. Genet.* **26**, 2864–2873 (2017).
325. Muralidharan, P., Hayes, D. Jr., Black, S. M. & Mansour, H. M. Microparticulate/nanoparticulate powders of a novel Nrf2 activator and an aerosol performance enhancer for pulmonary delivery targeting the lung Nrf2/Keap-1 pathway. *Mol. Syst. Des. Eng.* **1**, 48–65 (2016).
326. De Rubis, G. et al. Agarwood oil nanoemulsion attenuates cigarette smoke-induced inflammation and oxidative stress markers in BCi-NS1.1 airway epithelial cells. *Nutrients* **15**, 1019 (2023).
327. Paudel, K. R. et al. Attenuation of cigarette-smoke-induced oxidative stress, senescence, and inflammation by berberine-loaded liquid crystalline nanoparticles: in vitro study in 16HBE and RAW264.7 cells. *Antioxidants* **11**, 873 (2022).
328. Chikuma, K. et al. The potential of lipid-polymer nanoparticles as epigenetic and ROS control approaches for COPD. *Free Radic. Res.* **54**, 829–840 (2020).
329. Zhu, J. et al. Inhaled immunoantimicrobials for the treatment of chronic obstructive pulmonary disease. *Sci. Adv.* **10**, eabd7904 (2024).
330. Li, Z. et al. Mediated drug release from nanovehicles by black phosphorus quantum dots for efficient therapy of chronic obstructive pulmonary disease. *Angew. Chem. Int. Ed. Engl.* **59**, 20568–20576 (2020).
331. Yu, Q. et al. Inhalable neutrophil-mimicking nanoparticles for chronic obstructive pulmonary disease treatment. *J. Control Release* **381**, 113648 (2025).
332. Kent, B. D. Pulmonary vascular disease for the general respiratory clinician. *Breathe* **18**, 220261 (2022).
333. Maron, B. A. Revised definition of pulmonary hypertension and approach to management: a clinical primer. *J. Am. Heart Assoc.* **12**, e029024 (2023).
334. Kovacs, G. et al. Definition, classification and diagnosis of pulmonary hypertension. *Eur. Respir. J.* **64**, 2401324 (2024).
335. Luijten, D. et al. Incidence of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: an updated systematic review and meta-analysis. *Eur. Respir. J.* **62**, 2300449 (2023).
336. Lechartier, B. et al. Pulmonary veno-occlusive disease: illustrative cases and literature review. *Eur. Respir. Rev.* **33**, 230156 (2024).
337. Davis, M. J., Earley, S., Li, Y. S. & Chien, S. Vascular mechanotransduction. *Physiol. Rev.* **103**, 1247–1421 (2023).
338. Hoeper, M. M. et al. Phase 3 trial of sotatercept for treatment of pulmonary arterial hypertension. *N. Engl. J. Med.* **388**, 1478–1490 (2023).
339. Wang, X. & He, B. Endothelial dysfunction: molecular mechanisms and clinical implications. *MedComm* **5**, e651 (2024).
340. Gorelova, A., Berman, M. & Al Ghouleh, I. Endothelial-to-mesenchymal transition in pulmonary arterial hypertension. *Antioxid. Redox Signal.* **34**, 891–914 (2021).
341. Aldred, M. A., Morrell, N. W. & Guignabert, C. New mutations and pathogenesis of pulmonary hypertension: progress and puzzles in disease pathogenesis. *Circ. Res.* **130**, 1365–1381 (2022).
342. Zou, X. et al. SOX17 prevents endothelial-mesenchymal transition of pulmonary arterial endothelial cells in pulmonary hypertension through mediating TGF-beta/Smad2/3 signaling. *Am. J. Respir. Cell Mol. Biol.* **72**, 364–379 (2025).
343. Sun, F. et al. Nanoparticle delivery of STAT3 alleviates pulmonary hypertension in a mouse model of alveolar capillary dysplasia. *Circulation* **144**, 539–555 (2021).

344. Isobe, S. et al. Reduced FOXF1 links unrepaired DNA damage to pulmonary arterial hypertension. *Nat. Commun.* **14**, 7578 (2023).
345. Dharmadhikari, A. V., Szafranski, P., Kalinichenko, V. V. & Stankiewicz, P. Genomic and epigenetic complexity of the FOXF1 locus in 16q24.1: implications for development and disease. *Curr. Genomics.* **16**, 107–116 (2015).
346. Ren, X. et al. Postnatal alveologenesis depends on FOXF1 signaling in c-KIT(+) endothelial progenitor cells. *Am. J. Respir. Crit. Care Med.* **200**, 1164–1176 (2019).
347. Malin, D. et al. Forkhead box F1 is essential for migration of mesenchymal cells and directly induces integrin-beta3 expression. *Mol. Cell Biol.* **27**, 2486–2498 (2007).
348. Shen, H. et al. BRCC3 regulation of ALK2 in vascular smooth muscle cells: implication in pulmonary hypertension. *Circulation* **150**, 132–150 (2024).
349. Liu, R. et al. A non-metallic nanozyme ameliorates pulmonary hypertension through inhibiting ROS/TGF-beta1 signaling. *Adv. Health. Mater.* **13**, e2401909 (2024).
350. Zhao, H. et al. Hydrogen-generated metal-organic framework nanoparticles for ameliorated pulmonary hypertension by ROS scavenging, hydrogen anti-inflammation, and reducing DNA damage. *Mater. Today Nano* **31**, 100652 (2025).
351. Cao, Y. et al. Targeted delivery of BMPR2 mRNA attenuates pulmonary arterial hypertension by reversing pulmonary vascular remodeling. *Acta Pharm Sin. B* **15**, 5416–5430 (2025).
352. Cai, D. & Chen, S. Y. Nanoparticle endothelial delivery of PGC-1alpha attenuates hypoxia-induced pulmonary hypertension by attenuating EndoMT-caused vascular wall remodeling. *Redox Biol.* **58**, 102524 (2022).
353. Li, M. et al. Active anchoring stimuli-responsive nano-craft to relieve pulmonary vasoconstriction by targeting smooth muscle cell for hypoxic pulmonary hypertension treatment. *Adv. Health. Mater.* **13**, e2400113 (2024).
354. Yuan, R. et al. Biomimetic nanoparticle-mediated target delivery of hypoxia-responsive plasmid of angiotensin-converting enzyme 2 to reverse hypoxic pulmonary hypertension. *ACS Nano.* **17**, 8204–8222 (2023).
355. Ning, L. et al. Targeted rapamycin delivery via magnetic nanoparticles to address stenosis in a 3D bioprinted in vitro model of pulmonary veins. *Adv. Sci.* **11**, e2400476 (2024).
356. Plaunt, A. J. et al. Strategies to overcome biological barriers associated with pulmonary drug delivery. *Pharmaceutics* **14**, 302 (2022).
357. Ismat, F. A. et al. Safety, tolerability, and pharmacokinetics of treprostinil palmitil inhalation powder for pulmonary hypertension: a phase 1, randomized, double-blind, single- and multiple-dose study. *Adv. Ther.* **39**, 5144–5157 (2022).
358. Chen, Q., Shen, Y. & Zheng, J. A review of cystic fibrosis: basic and clinical aspects. *Anim. Model Exp. Med.* **4**, 220–232 (2021).
359. Fiedorczuk, K. & Chen, J. Molecular structures reveal synergistic rescue of Delta508 CFTR by Trikafta modulators. *Science* **378**, 284–290 (2022).
360. Endres, T. M. & Konstan, M. W. What is cystic fibrosis? *JAMA* **327**, 191 (2022).
361. Graeber, S. Y. & Mall, M. A. The future of cystic fibrosis treatment: from disease mechanisms to novel therapeutic approaches. *Lancet* **402**, 1185–1198 (2023).
362. Allaire, N. E. et al. Gene, RNA, and ASO-based therapeutic approaches in cystic fibrosis. *J. Cyst. Fibros.* **22**, S39–S44 (2023).
363. McLachlan, G. et al. Progress in respiratory gene therapy. *Hum. Gene Ther.* **33**, 893–912 (2022).
364. Robinson, E. et al. Lipid nanoparticle-delivered chemically modified mRNA restores chloride secretion in cystic fibrosis. *Mol. Ther.* **26**, 2034–2046 (2018).
365. Tafech, B. et al. Exploring mechanisms of lipid nanoparticle-mucus interactions in healthy and cystic fibrosis conditions. *Adv. Health. Mater.* **13**, e2304525 (2024).
366. Kim, J. et al. Engineering lipid nanoparticles for enhanced intracellular delivery of mRNA through inhalation. *ACS Nano.* **16**, 14792–14806 (2022).
367. Conte, G. et al. Hybrid lipid/polymer nanoparticles to tackle the cystic fibrosis mucus barrier in sirna delivery to the lungs: does PEGylation make the difference? *ACS Appl Mater. Interfaces* **14**, 7565–7578 (2022).
368. Anderson, S. et al. Inhaled medicines: past, present, and future. *Pharm. Rev.* **74**, 48–118 (2022).
369. Guan, S. et al. Self-assembled peptide-poloxamine nanoparticles enable in vitro and in vivo genome restoration for cystic fibrosis. *Nat. Nanotechnol.* **14**, 287–297 (2019).
370. Piotrowski-Daspit, A. S. et al. In vivo correction of cystic fibrosis mediated by PNA nanoparticles. *Sci. Adv.* **8**, eabo0522 (2022).
371. Leon, B. & Ballesteros-Tato, A. Modulating Th2 cell immunity for the treatment of asthma. *Front. Immunol.* **12**, 637948 (2021).
372. Ji, T. & Li, H. T-helper cells and their cytokines in pathogenesis and treatment of asthma. *Front. Immunol.* **14**, 1149203 (2023).
373. Harker, J. A. & Lloyd, C. M. T helper 2 cells in asthma. *J. Exp. Med.* **220**, e20221094 (2023).
374. Britt, R. D. Jr., Ruwanpathirana, A., Ford, M. L. & Lewis, B. W. Macrophages orchestrate airway inflammation, remodeling, and resolution in asthma. *Int. J. Mol. Sci.* **24**, 10451 (2023).
375. Murphy, R. C. & Hallstrand, T. S. Exploring the origin and regulatory role of mast cells in asthma. *Curr. Opin. Allergy Clin. Immunol.* **21**, 71–78 (2021).
376. Gao, H., Ying, S. & Dai, Y. Pathological roles of neutrophil-mediated inflammation in asthma and its potential for therapy as a target. *J. Immunol. Res.* **2017**, 3743048 (2017).
377. Patel, V. H. et al. Current limitations and recent advances in the management of asthma. *Dis. Mon.* **69**, 101483 (2023).
378. Wadhwa, R. et al. Cellular mechanisms underlying steroid-resistant asthma. *Eur. Respir. Rev.* **28**, 190096 (2019).
379. An, E. K. et al. Tolerogenic dendritic cell- and M2 macrophage polarization-inducible hybrid nanoparticles alleviate asthma in mice. *ACS Nano.* **19**, 23606–23619 (2025).
380. Pei, W. et al. Exosome membrane-modified M2 macrophages targeted nanomedicine: treatment for allergic asthma. *J. Control Release* **338**, 253–267 (2021).
381. Zhang, M. et al. Airway epithelial cell-specific delivery of lipid nanoparticles loading siRNA for asthma treatment. *J. Control Release* **352**, 422–437 (2022).
382. Kang, S. H. et al. Interactions of nanoparticles with macrophages and feasibility of drug delivery for asthma. *Int. J. Mol. Sci.* **23**, 1622 (2022).
383. da Silva, A. L. et al. Nanoparticle-based thymulin gene therapy therapeutically reverses key pathology of experimental allergic asthma. *Sci. Adv.* **6**, eaay7973 (2020).
384. Yu, Y., Ni, M., Zheng, Y. & Huang, Y. Airway epithelial-targeted nanoparticle reverses asthma in inhalation therapy. *J. Control Release* **367**, 223–234 (2024).
385. Frankell, A. M. et al. The evolution of lung cancer and impact of subclonal selection in TRACERx. *Nature* **616**, 525–533 (2023).
386. Smolarz, B. et al. Lung cancer-epidemiology, pathogenesis, treatment and molecular aspect (review of literature). *Int. J. Mol. Sci.* **26**, 2049 (2025).
387. Cheng, E. S., Weber, M., Steinberg, J. & Yu, X. Q. Lung cancer risk in never-smokers: an overview of environmental and genetic factors. *Chin. J. Cancer Res.* **33**, 548–562 (2021).
388. Ciabattini, M. et al. Systematic review and meta-analysis of recent high-quality studies on exposure to particulate matter and risk of lung cancer. *Environ. Res.* **196**, 110440 (2021).
389. Tan, A. C. & Tan, D. S. W. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J. Clin. Oncol.* **40**, 611–625 (2022).
390. Jin, Y. et al. Activation of PI3K/AKT pathway is a potential mechanism of treatment resistance in small cell lung cancer. *Clin. Cancer Res.* **28**, 526–539 (2022).
391. Zhang, L. et al. PTEN loss expands the histopathologic diversity and lineage plasticity of lung cancers initiated by Rb1/Trp53 deletion. *J. Thorac. Oncol.* **18**, 324–338 (2023).
392. Redig, A. J. et al. Clinical and molecular characteristics of NF1-mutant lung cancer. *Clin. Cancer Res.* **22**, 3148–3156 (2016).
393. Edmonds, M. D. et al. MicroRNA-31 initiates lung tumorigenesis and promotes mutant KRAS-driven lung cancer. *J. Clin. Invest.* **126**, 349–364 (2016).
394. Liu, D., Lu, X., Huang, W. & Zhuang, W. Long non-coding RNAs in non-small cell lung cancer: implications for EGFR-TKI resistance. *Front. Genet.* **14**, 1222059 (2023).
395. Kitajima, S. & Barbie, D. A. RASA1/NF1-mutant lung cancer: racing to the clinic? *Clin. Cancer Res.* **24**, 1243–1245 (2018).
396. George, J. et al. Comprehensive genomic profiles of small cell lung cancer. *Nature* **524**, 47–53 (2015).
397. Itchins, M. & Tan, A. C. Defining the pathway to precision therapies for squamous lung cancers. *J. Thorac. Oncol.* **19**, 1591–1593 (2024).
398. Heiden, B. T. et al. Association between surgical quality metric adherence and overall survival among us veterans with early-stage non-small cell lung cancer. *JAMA Surg.* **158**, 293–301 (2023).
399. Chaft, J. E. et al. Evolution of systemic therapy for stages I-III non-metastatic non-small-cell lung cancer. *Nat. Rev. Clin. Oncol.* **18**, 547–557 (2021).
400. Planchard, D. et al. Osimertinib with or without chemotherapy in EGFR-mutated advanced NSCLC. *N. Engl. J. Med.* **389**, 1935–1948 (2023).
401. Sun, A. et al. Initial management of small-cell lung cancer (limited- and extensive-stage) and the role of thoracic radiotherapy and first-line chemotherapy: a systematic review. *Curr. Oncol.* **26**, e372–e384 (2019).
402. Brahmer, J. R. et al. Five-year survival outcomes with nivolumab plus ipilimumab versus chemotherapy as first-line treatment for metastatic non-small-cell lung cancer in CheckMate 227. *J. Clin. Oncol.* **41**, 1200–1212 (2023).
403. Herzog, B. H., Devarakonda, S. & Govindan, R. Overcoming chemotherapy resistance in SCLC. *J. Thorac. Oncol.* **16**, 2002–2015 (2021).
404. Deng, Z. et al. Nanoparticle-based delivery systems for synergistic therapy in lung cancers. *Bioengineering* **12**, 968 (2025).
405. Reda, M. et al. Development of a nanoparticle-based immunotherapy targeting PD-L1 and PLK1 for lung cancer treatment. *Nat. Commun.* **13**, 4261 (2022).
406. Saha, T. et al. Antibody nanoparticle conjugate-based targeted immunotherapy for non-small cell lung cancer. *Sci. Adv.* **10**, eadi2046 (2024).

407. Jin, K. et al. RNA-micelles as self-assembling structures for efficient co-delivery of synergistic siRNA and nucleoside analogues to treat CRC lung metastasis. *Adv. Funct. Mater.* e21863 (2026).
408. Gonzalez, T., Muminovic, M., Nano, O. & Vulfovich, M. Folate receptor alpha-A novel approach to cancer therapy. *Int. J. Mol. Sci.* **25**, 1046 (2024).
409. Parashar, P. et al. A synergistic approach for management of lung carcinoma through folic acid functionalized co-therapy of capsaicin and gefitinib nanoparticles: enhanced apoptosis and metalloproteinase-9 down-regulation. *Phytomedicine* **53**, 107–123 (2019).
410. Gai, C. et al. MT1DP loaded by folate-modified liposomes sensitizes erastin-induced ferroptosis via regulating miR-365a-3p/NRF2 axis in non-small cell lung cancer cells. *Cell Death Dis.* **11**, 751 (2020).
411. Wang, X., Cheng, R. & Zhong, Z. Facile fabrication of robust, hyaluronic acid-surfaced and disulfide-crosslinked PLGA nanoparticles for tumor-targeted and reduction-triggered release of docetaxel. *Acta Biomater.* **125**, 280–289 (2021).
412. Tsai, T. et al. CD44-hyaluronan mediating endocytosis of iron-platinum alloy nanoparticles induces ferroptotic cell death in mesenchymal-state lung cancer cells with tyrosine kinase inhibitor resistance. *Acta Biomater.* **186**, 396–410 (2024).
413. Wang, H. et al. Chondroitin sulfate nanoparticles based on co-delivery dual drug induced ferroptosis in lung cancer cells by disrupting mitochondrial oxidative homeostasis. *Mater. Today Bio.* **31**, 101632 (2025).
414. Li, H. et al. Chondroitin sulfate-linked prodrug nanoparticles target the golgi apparatus for cancer metastasis treatment. *ACS Nano.* **13**, 9386–9396 (2019).
415. Garbuzenko, O. B. et al. Strategy to enhance lung cancer treatment by five essential elements: inhalation delivery, nanotechnology, tumor-receptor targeting, chemo- and gene therapy. *Theranostics* **9**, 8362–8376 (2019).
416. Fu, X. et al. Inhalable liposomal delivery of osimertinib and DNA for treating primary and metastasis lung cancer. *Nat. Commun.* **16**, 3336 (2025).
417. Zhao, G. et al. Inhalable siRNA nanoparticles for enhanced tumor-targeting treatment of KRAS-mutant non-small-cell lung cancer. *ACS Appl Mater. Interfaces* **15**, 31273–31284 (2023).
418. Jeong, Y., Shim, Y. S., Jo, Y. K. & Cha, H. J. Redox-activatable inhalable mucoadhesive proteinic nanotherapeutics for targeted treatment of lung cancer. *Biomaterials* **316**, 123004 (2025).
419. Ge, D. et al. Pulmonary delivery of dual-targeted nanoparticles improves tumor accumulation and cancer cell targeting by restricting macrophage interception in orthotopic lung tumors. *Biomaterials* **315**, 122955 (2025).
420. Cheng, Y. et al. Tumor associated macrophages and TAMs-based anti-tumor nanomedicines. *Adv. Health. Mater.* **10**, e2100590 (2021).
421. Novoselova, M. V. et al. Biodegradable polymeric multilayer capsules for therapy of lung cancer. *ACS Appl Mater. Interfaces* **12**, 5610–5623 (2020).
422. Tie, Y. et al. Targeting folate receptor beta positive tumor-associated macrophages in lung cancer with a folate-modified liposomal complex. *Signal Transduct. Target Ther.* **5**, 6 (2020).
423. Park, Y. I. et al. pH-sensitive multi-drug liposomes targeting folate receptor beta for efficient treatment of non-small cell lung cancer. *J. Control Release* **330**, 1–14 (2021).
424. Rong, A. et al. Respiratory delivery of single low-dose nebulized PFCE-C25 NEs for lymphatic transport and durable stimulation of antitumor immunity in lung cancer. *Sci. Adv.* **10**, eadp7561 (2024).
425. Zhang, R. et al. Low-dose inhalable amino acid-modified multilamellar nanoparticles activate STING for safe and effective regression of lung metastases. *J. Control Release* **386**, 114061 (2025).
426. Bian, F. et al. FOXF1 promotes tumor vessel normalization and prevents lung cancer progression through FZD4. *EMBO Mol. Med.* **16**, 1063–1090 (2024).
427. Wei, D., Sun, Y., Zhu, H. & Fu, Q. Stimuli-responsive polymer-based nanosystems for cancer theranostics. *ACS Nano.* **17**, 23223–23261 (2023).
428. Jing, X. et al. The intracellular and extracellular microenvironment of tumor site: the trigger of stimuli-responsive drug delivery systems. *Small Methods* **6**, e2101437 (2022).
429. Chen, Q. et al. Tumor microenvironment-responsive nano-immunomodulators for enhancing chimeric antigen receptor-T cell therapy in lung cancer. *ACS Nano.* **19**, 8212–8226 (2025).
430. Huang, Q. et al. An "AND" logic gate-based supramolecular therapeutic nanoplatform for combatting drug-resistant non-small cell lung cancer. *Sci. Adv.* **10**, eadp9071 (2024).
431. Sainz de Aja, J., Dost, A. F. M. & Kim, C. F. Alveolar progenitor cells and the origin of lung cancer. *J. Intern. Med.* **289**, 629–635 (2021).
432. Siegel, R. L., Miller, K. D., Fuchs, H. E. & Jemal, A. Cancer statistics, 2021. *CA Cancer J. Clin.* **71**, 7–33 (2021).
433. Xiao, S. et al. Accurate and convenient lung cancer diagnosis through detection of extracellular vesicle membrane proteins via forster resonance energy transfer. *Nano Lett.* **23**, 8115–8125 (2023).
434. Deng, Z., Wu, S., Wang, Y. & Shi, D. Circulating tumor cell isolation for cancer diagnosis and prognosis. *EBioMedicine* **83**, 104237 (2022).
435. Benariba, M. A. et al. A liposome-based assay for cancer biomarker detection: exploring the correlation between platelet-derived microvesicles and NSCLC-associated miRNAs. *Nanoscale* **16**, 22037–22046 (2024).
436. Zhong, Q. et al. Inhalable point-of-care urinary diagnostic platform. *Sci. Adv.* **10**, eadj9591 (2024).
437. Shankar, S. *et al.* Nanoengineered multiwalled carbon nanotube for lung cancer diagnosis. *J. Mol. Struct.* **1320**, 139629 (2025).
438. Tran, A. T. T. et al. Graphene and metal-organic framework hybrids for high-performance sensors for lung cancer biomarker detection supported by machine learning augmentation. *Nanoscale* **16**, 9084–9095 (2024).
439. Xu, X. et al. A comprehensive review on synergy of multi-modal data and AI technologies in medical diagnosis. *Bioengineering* **11**, 219 (2024).
440. Collins, G. S. et al. TRIPOD+AI statement: updated guidance for reporting clinical prediction models that use regression or machine learning methods. *BMJ* **385**, e078378 (2024).
441. Wedgwood, S. & Steinhorn, R. H. Role of reactive oxygen species in neonatal pulmonary vascular disease. *Antioxid. Redox Signal.* **21**, 1926–1942 (2014).
442. Pradhan, A. et al. Novel FOXF1-stabilizing compound tanfe stimulates lung angiogenesis in alveolar capillary dysplasia. *Am. J. Respir. Crit. Care Med.* **207**, 1042–1054 (2023).
443. Katz, T. A. et al. Towards a harmonized bronchopulmonary dysplasia definition: a study protocol for an international delphi procedure. *BMJ Paediatr. Open.* **7**, e002112 (2023).
444. Abiramalatha, T. et al. Interventions to prevent bronchopulmonary dysplasia in preterm neonates: an umbrella review of systematic reviews and meta-analyses. *JAMA Pediatr.* **176**, 502–516 (2022).
445. Bolte, C., Whitsett, J. A., Kalin, T. V. & Kalinichenko, V. V. Transcription factors regulating embryonic development of pulmonary vasculature. *Adv. Anat. Embryol. Cell Biol.* **228**, 1–20 (2018).
446. Kalinichenko, V. V. et al. Foxf1 haploinsufficiency reduces Notch-2 signaling during mouse lung development. *Am. J. Physiol. Lung Cell Mol. Physiol.* **286**, L521–530 (2004).
447. Kalinichenko, V. V. et al. Wild-type levels of the mouse Forkhead Box f1 gene are essential for lung repair. *Am. J. Physiol. Lung Cell Mol. Physiol.* **282**, L1253–1265 (2002).
448. Kalinichenko, V. V., Gusarova, G. A., Shin, B. & Costa, R. H. The forkhead box F1 transcription factor is expressed in brain and head mesenchyme during mouse embryonic development. *Gene Expr. Patterns* **3**, 153–158 (2003).
449. Kim, I. M. et al. Functional characterization of evolutionarily conserved DNA regions in forkhead box f1 gene locus. *J. Biol. Chem.* **280**, 37908–37916 (2005).
450. Ustiyan, V. et al. FOXF1 transcription factor promotes lung morphogenesis by inducing cellular proliferation in fetal lung mesenchyme. *Dev. Biol.* **443**, 50–63 (2018).
451. Shirazi, S. P. et al. Bronchopulmonary dysplasia with pulmonary hypertension associates with semaphorin signaling loss and functionally decreased FOXF1 expression. *Nat. Commun.* **16**, 5004 (2025).
452. Xia, H. et al. Foxm1 regulates resolution of hyperoxic lung injury in newborns. *Am. J. Respir. Cell Mol. Biol.* **52**, 611–621 (2015).
453. Wang, G. et al. Generation of pulmonary endothelial progenitor cells for cell-based therapy using interspecies mouse-rat chimeras. *Am. J. Respir. Crit. Care Med.* **204**, 326–338 (2021).
454. Pradhan, A. et al. The S52F FOXF1 mutation inhibits STAT3 signaling and causes alveolar capillary dysplasia. *Am. J. Respir. Crit. Care Med.* **200**, 1045–1056 (2019).
455. Guo, M. et al. Single cell multiomics identifies cells and genetic networks underlying alveolar capillary dysplasia. *Am. J. Respir. Crit. Care Med.* **208**, 709–725 (2023).
456. Vincent, M. et al. Clinical, histopathological, and molecular diagnostics in lethal lung developmental disorders. *Am. J. Respir. Crit. Care Med.* **200**, 1093–1101 (2019).
457. Edel, G. G. et al. The molecular consequences of FOXF1 missense mutations associated with alveolar capillary dysplasia with misalignment of pulmonary veins. *J. Biomed. Sci.* **31**, 100 (2024).
458. Wang, G. et al. Endothelial progenitor cells stimulate neonatal lung angiogenesis through FOXF1-mediated activation of BMP9/ACVRL1 signaling. *Nat. Commun.* **13**, 2080 (2022).
459. Sharma, A. & Niethamer, T. K. Specialized pulmonary vascular cells in development and disease. *Annu. Rev. Physiol.* **87**, 229–255 (2025).
460. Wang, I. C. et al. Foxm1 mediates cross talk between Kras/mitogen-activated protein kinase and canonical Wnt pathways during development of respiratory epithelium. *Mol. Cell Biol.* **32**, 3838–3850 (2012).
461. Kalin, T. V. et al. Pulmonary mastocytosis and enhanced lung inflammation in mice heterozygous null for the Foxf1 gene. *Am. J. Respir. Cell Mol. Biol.* **39**, 390–399 (2008).

462. Ustiyan, V. et al. Foxm1 transcription factor is critical for proliferation and differentiation of Clara cells during development of conducting airways. *Dev. Biol.* **370**, 198–212 (2012).
463. Wen, B. et al. In vivo generation of lung and thyroid tissues from embryonic stem cells using blastocyst complementation. *Am. J. Respir. Crit. Care Med.* **203**, 471–483 (2021).
464. Li, X. et al. Endothelial Cpt1a inhibits neonatal hyperoxia-induced pulmonary vascular remodeling by repressing endothelial-mesenchymal transition. *Adv. Sci.* **12**, e2415824 (2025).
465. Zhao, Z. et al. Boosting RNA nanotherapeutics with V-ATPase activating non-inflammatory lipid nanoparticles to treat chronic lung injury. *Nat. Commun.* **16**, 6477 (2025).
466. Loukogeorgakis, S. P. et al. Prenatal VEGF nanodelivery reverses congenital diaphragmatic hernia-associated pulmonary abnormalities. *Am. J. Respir. Crit. Care Med.* **211**, 992–1006 (2025).
467. Domingues, C. et al. Where is nano today and where is it headed? A review of nanomedicine and the dilemma of nanotoxicology. *ACS Nano.* **16**, 9994–10041 (2022).
468. Yan, L. et al. A safe-by-design strategy towards safer nanomaterials in nanomedicines. *Adv. Mater.* **31**, e1805391 (2019).
469. Ettlinger, R. et al. Toxicity of metal-organic framework nanoparticles: from essential analyses to potential applications. *Chem. Soc. Rev.* **51**, 464–484 (2022).
470. Herdiana, Y. Bridging the gap: the role of advanced formulation strategies in the clinical translation of nanoparticle-based drug delivery systems. *Int. J. Nanomed.* **20**, 13039–13053 (2025).
471. Valcourt, D. M. et al. Best practices for preclinical in vivo testing of cancer nanomedicines. *Adv. Health. Mater.* **9**, e2000110 (2020).
472. Griffith, D. E. et al. Amikacin liposome inhalation suspension for treatment-refractory lung disease caused by mycobacterium avium complex (CONVERT). A prospective, open-label, randomized study. *Am. J. Respir. Crit. Care Med.* **198**, 1559–1569 (2018).
473. Haworth, C. S. et al. Inhaled liposomal ciprofloxacin in patients with non-cystic fibrosis bronchiectasis and chronic lung infection with Pseudomonas aeruginosa (ORBIT-3 and ORBIT-4): two phase 3, randomised controlled trials. *Lancet Respir. Med.* **7**, 213–226 (2019).
474. Chalmers, J. D. et al. Changes in respiratory symptoms during 48-week treatment with ARD-3150 (inhaled liposomal ciprofloxacin) in bronchiectasis: results from the ORBIT-3 and -4 studies. *Eur. Respir. J.* **56**, 2000110 (2020).
475. Stucchi, F., Li, M., Castellano, G. & Cellesi, F. Regulatory framework for polymer-based nanotherapeutics in clinical translation. *Front. Bioeng. Biotechnol.* **14**, 1735885 (2026).
476. Polack, F. P. et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).
477. Baden, L. R. et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* **384**, 403–416 (2021).
478. Gilbert, P. B. et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* **375**, 43–50 (2022).
479. US Food and Drug Administration. *Drug Products, Including Biological Products, that Contain Nanomaterials* https://www.fda.gov/media/157812/download (2022).
480. Joyce, P. et al. A translational framework to DELIVER nanomedicines to the clinic. *Nat. Nanotechnol.* **19**, 1597–1611 (2024).
481. Kneyber, M. C. J. et al. Understanding clinical and biological heterogeneity to advance precision medicine in paediatric acute respiratory distress syndrome. *Lancet Respir. Med.* **11**, 197–212 (2023).
482. Khemani, R. G. et al. Paediatric acute respiratory distress syndrome incidence and epidemiology (PARDIE): an international, observational study. *Lancet Respir. Med.* **7**, 115–128 (2019).
483. Patel, B. M. et al. Association between age and mortality in pediatric and adult acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* **209**, 871–878 (2024).
484. Liu, L. et al. The perinatal period should be considered in neonatal acute respiratory distress syndrome: comparison of the Montreux definition vs. the second pediatric acute lung injury consensus conference definition. *Front. Pediatr.* **11**, 1216073 (2023).
485. Rudolph, M. et al. Performance of acute respiratory distress syndrome definitions in a high acuity paediatric intensive care unit. *Respir. Res.* **22**, 256 (2021).
486. Shah, K. A. et al. Current trends in inhaled pharmaceuticals: challenges and opportunities in respiratory infections treatment. *J. Pharm. Investig.* **55**, 191–225 (2025).
487. Yang, M. S., Kang, J. H., Kim, D. W. & Park, C. W. Recent developments in dry powder inhalation (DPI) formulations for lung-targeted drug delivery. *J. Pharm. Investig.* **54**, 113–130 (2024).
488. Seo, S. H. & Song, M. K. Advancements and challenges in next-generation mRNA vaccine manufacturing systems. *Clin. Exp. Vaccin. Res.* **14**, 299–307 (2025).
489. Huang, Y. et al. Aerosol inhalation of gene delivery therapy for pulmonary diseases. *Biomolecules* **14**, 904 (2024).
490. Chow, M. Y. T., Chang, R. Y. K. & Chan, H. K. Inhalation delivery technology for genome-editing of respiratory diseases. *Adv. Drug Deliv. Rev.* **168**, 217–228 (2021).
491. Li, B. et al. Combinatorial design of nanoparticles for pulmonary mRNA delivery and genome editing. *Nat. Biotechnol.* **41**, 1410–1415 (2023).
492. Hou, Y. et al. Acute lung injury: from molecular circuits to system-level therapeutics. *MedComm* **7**, e70778 (2026).
493. Svenningsen, S. & Nair, P. Asthma endotypes and an overview of targeted therapy for asthma. *Front. Med.* **4**, 158 (2017).
494. Hosseinalizadeh, H. et al. Nanovaccines for lung cancer: platforms, mechanistic insights, and translational challenges. *Chin. Med. J. Pulm. Crit. Care Med.* **4**, 1–18 (2026).
495. Witten, J. et al. Artificial intelligence-guided design of lipid nanoparticles for pulmonary gene therapy. *Nat. Biotechnol.* **43**, 122–134 (2025).