Nanomedicine: Nanotechnology, Biology, and Medicine 69 (2025) 102855

Contents lists available at ScienceDirect

Nanomedicine: Nanotechnology, Biology, and Medicine

journal homepage: www.sciencedirect.com/journal/nanomedicine-nanotechnology-biology-and-medicine

Review Article

Liposomal drug delivery system for lung diseases: Recent advancement and future perspectives

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ARTICLE INFO

Keywords:
Liposomes
Drug delivery
Asthma
Emphysema
Lung Cancer

ABSTRACT

Lung diseases such as asthma, chronic respiratory diseases, and lung cancer are among the most prevalent and vulnerable health conditions. Various therapeutic approaches, including nucleic acids, peptides, and small molecules have been developed along with different delivery strategies for their treatment. Liposomes have been recognized as promising delivery candidates attributed to their biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and hydrophobic drugs effectively. Their composition closely resembles pulmonary surfactants and can facilitate targeting deep lung tissue while also allowing localized drug delivery with less systemic exposure. The most rigorously explored route for delivering liposomal formulations to the lungs is inhalation. Additionally, liposomes help combat antimicrobial resistance and enhance targeted drug delivery by utilizing stimuli-responsive liposomes or modifying their physicochemical properties. Herein, we explore the liposomal delivery of different classes of drugs, their therapeutic potential in the treatment of lung disease, and discuss perspectives on clinical translation.

Introduction

Worldwide, millions of people are suffered from asthma, chronic respiratory diseases including chronic obstructive pulmonary disease (COPD), pulmonary tuberculosis, lung cancers, cystic fibrosis(*CF*), idiopathic pulmonary fibrosis (IPF), coronavirus diseases (COVID), Middle east respiratory syndrome (MERS), pneumonia and acute lung injury (ALI).[1,2] Diverse pharmacotherapeutic agents, including β_2-agonists, corticosteroids, anticholinergics, antimicrobials, chemotherapeutics, therapeutic peptides, antibodies, and nucleotides have been used as lung therapeutics.[1,3] The integration of pharmacotherapy and non-pharmacotherapy approaches has proven effective in managing chronic conditions.[4] Conventional treatment options also have several limitations, including low diffusion and suboptimal pharmacokinetics, which reduce the efficacy of the therapy.[5] Therapeutic approaches for chronic and non-communicable diseases require the safest and most

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https://doi.org/10.1016/j.nano.2025.102855
Received in revised form 3 August 2025;
Available online 29 August 2025

novel therapeutic strategies or options that can improve treatment efficacy, overcome limitations, and enhance patient's overall health and well-being. To combat with the unmet need of treatment for lung diseases, various alternative approaches are being investigated, such as nanotechnology-assisted novel drug delivery strategy and drug repurposing to overcome the limitations of existing drugs.[6–9] Recently, nano drug delivery strategies have gained considerable interest among researchers and drug delivery scientists due to their potential to improve pharmacotherapy by offering enhanced and favorable physicochemical characteristics.[10,11] Nanoparticles are discrete nano-object, less than 100 nm size. Their favorable features include optimized physiochemical and mechanical characteristics, enhanced cellular uptake and improved therapeutic effectiveness.[1,12] For the treatment of airway diseases, several nanoparticle systems have been studied, including lipid nanoparticles, polymeric nanoparticles, solid-lipid nanoparticles, microspheres, inorganic nanoparticles, nanocrystals, dendrimers, and respiratory-triggered magnetic nanoparticles.[13–15] Liposomal formulations are also among the most widely studied and clinically translatable formulations for the treatment of lung diseases. Existing reviews on liposomal drug delivery targeting lung diseases have primarily focused on formulation design and both preclinical and clinical investigations. The exact mechanism and interaction of liposomes in the lung environment remain poorly understood. There is still a significant gap in the research and reviews on lung-targeted liposomal delivery of nucleic acids or macromolecules.

This review intends to describe and summarize the emerging advancements in liposomal formulations of small and macromolecular therapeutics targeting lung diseases, interactions of liposomes with the lung environment, tissue-targeting approaches, use of liposomes as adjuvants in tuberculosis vaccine, and translation approaches toward clinical application. This review is also intended to guide researchers in designing and engineering liposomal formulations with improved efficacy, while also providing the recent updates on the progression of liposomal formulations for the treatment of several lung diseases.

Liposomes

Liposomes, which marked the advent of lipid nanoparticle development, originating in 1960s. The wider concept "lipid nanoparticles" appeared in the 1990s as nanoscience and technology continue to evolve. Liposomes are closed lipid bilayer vesicles, typically nanosized, and are recognized as the initial form of lipid nanoparticles.[16] In the 1960s, Alec Bangham originally witnessed that liposomes instantaneously develop upon dispersing phospholipids in water while experimenting on their effect on blood coagulation.[17,18] Liposomes are generally in spherical shape and sizes vary between 50 and 500 nm. Basically, liposomes are assembled in aqueous media due to their amphiphilic nature, where phosphate group containing hydrophilic heads face the exterior and hydrophobic fatty acid tails are oriented toward inside of the bilayer, where they align with each other to form regions free from water. The center core allows the incorporation of hydrophilic drugs and hydrophobic drugs inside their lipid bilayer.[19,20] Liposomes typically consist of phospholipids, including phosphatidylethanolamines, phosphatidylserines, phosphatidylcholines, and phosphatidylglycerols, and are often stabilized with cholesterol.[16] The hydrophilic nature of polyethylene glycol (PEG) has also been used to influence the pharmacokinetic profile of liposomes and is employed for its stealth properties.[21] Liposomes are one of the choices for encapsulating the therapeutic agents due to their biological compatibility, reduced toxicity, biodegradability, and lipid and water loving traits.[19] Liposomes were the initial drug delivery strategies successfully translated from bench to bedside applications. Several liposomal pharmaceutical preparations have received approval for cancer treatment and other diseases. The first approved liposomal drug was Doxil®, aimed at the management of ovarian cancer.[22]

Based on the number of bilayers, liposomes are categorized into unilamellar, multilamellar (MLV), and multivesicular vesicles (MVV). They are further distinguished according to their size, such as small unilamellar vesicles (SUV) with a diameter of 20–100 nm, large unilamellar vesicles (LUV) ranging from 100 to 1000 nm, and giant unilamellar vesicles (GUV) measuring 1000-2000 nm. SUV and smaller MLV are predominantly designed for drug delivery systems, while GUV are primarily used as models for cells.[16,23] The drug encapsulation and biodistribution can be primarily affected by the size of liposomes; the smaller liposomes have a chance to escape phagocytosis.[24,25] Liposomes with a diameter of less than 100 nm were generally accepted for pharmaceutical applications, especially in parenteral administration.[25,26] The surface charge of liposomes is primarily influenced by the chemical properties of lipid head groups, which can be positive, negative, or zwitterionic. The resulting surface charge density affects the surface potential of liposomes, a key factor in their stability, by regulating interactions between particles and the binding of counterions. The high charge containing particles prevents aggregation due to electrostatic repulsion, and uncharged particles are susceptible to aggregation over time. A valuable insight into the stability, biodistribution, cellular internalization, and biological interaction of nanoparticles can be gained by characterizing their surface charge using the zeta potential, which is the electrical potential at the hydrodynamic shear boundary of the particles. The ionic lipid fraction in liposomes is linearly related to zetapotential, and zeta potentials less than -30 mV and more than +30 mV are typically considered for preserving stability of the particle in suspension.[16,27,28]

Classical liposomes, pegylated (stealth) liposomes, cationic or anionic liposomes, bubble liposomes, actively targeted liposomes, and stimuli responsive liposomes are among the various types of liposomes based on liposome composition and intended application.[18] The composition of conventional liposomes generally consists of naturally derived or fabricated phospholipids, in the presence or absence of cholesterol. However, they are prone to rapid clearance by the reticuloendothelial system (RES), preventing efficient accumulation at the target site.[29–32] Stealth liposomes are liposomes that have been stabilized sterically by altering their surface using hydrophilic polymers such glycoproteins, polysaccharides, or PEG. These modifications enhance circulation time and enhance accumulation at the target site by reducing opsonization.[33,34] Charged liposomes are formulated using charge-containing lipids and have various applications. Cationic liposomes efficiently encapsulate nucleic acids through electrostatic forces between their positive charge and the negatively charged phosphate backbone of nucleotide sequences.[35] Conversely, transdermal drug delivery often employs anionic liposomes, as their negative charge enhances drug permeation across the skin more effectively than cationic liposomes.[36]

Bubble liposomes are small microbubbles with a core filled with gas, coated with lipids and other materials such as galactose, proteins, surfactants, or biocompatible polymers. They release their gaseous content upon ultrasound exposure through cavitation. Ultrasound application induces sonoporation, forming transient pores in the cell membrane, which facilitates the delivery of encapsulated materials into targeted cells.[37,38] Doxorubicin-loaded oxygen-containing nanobubbles liposomes have been shown to effectively supply oxygen to the lungs, paving the way for their use in mitigating hypoxia in tumors, which contributes to enhanced resistance to chemotherapy.[39] Liposomes are directed to tumors through passive targeting *via* enhanced permeability and retention (EPR) effect, but this mechanism does not selectively target the cancerous cells. In contrast, actively targeted liposomes selectively recognize tumor cells through receptor-ligand interactions, achieved by conjugating recognition moieties to the liposome surface. Antibodies, nucleic acids, carbohydrates, vitamins, or peptides can serve as recognition moieties for active targeting.[40,41]

A range of stimuli-responsive liposomes has been developed to facilitate the efficient delivery of therapeutic molecules to the specific target sites (Fig. 1). Liposomes can be engineered to release their

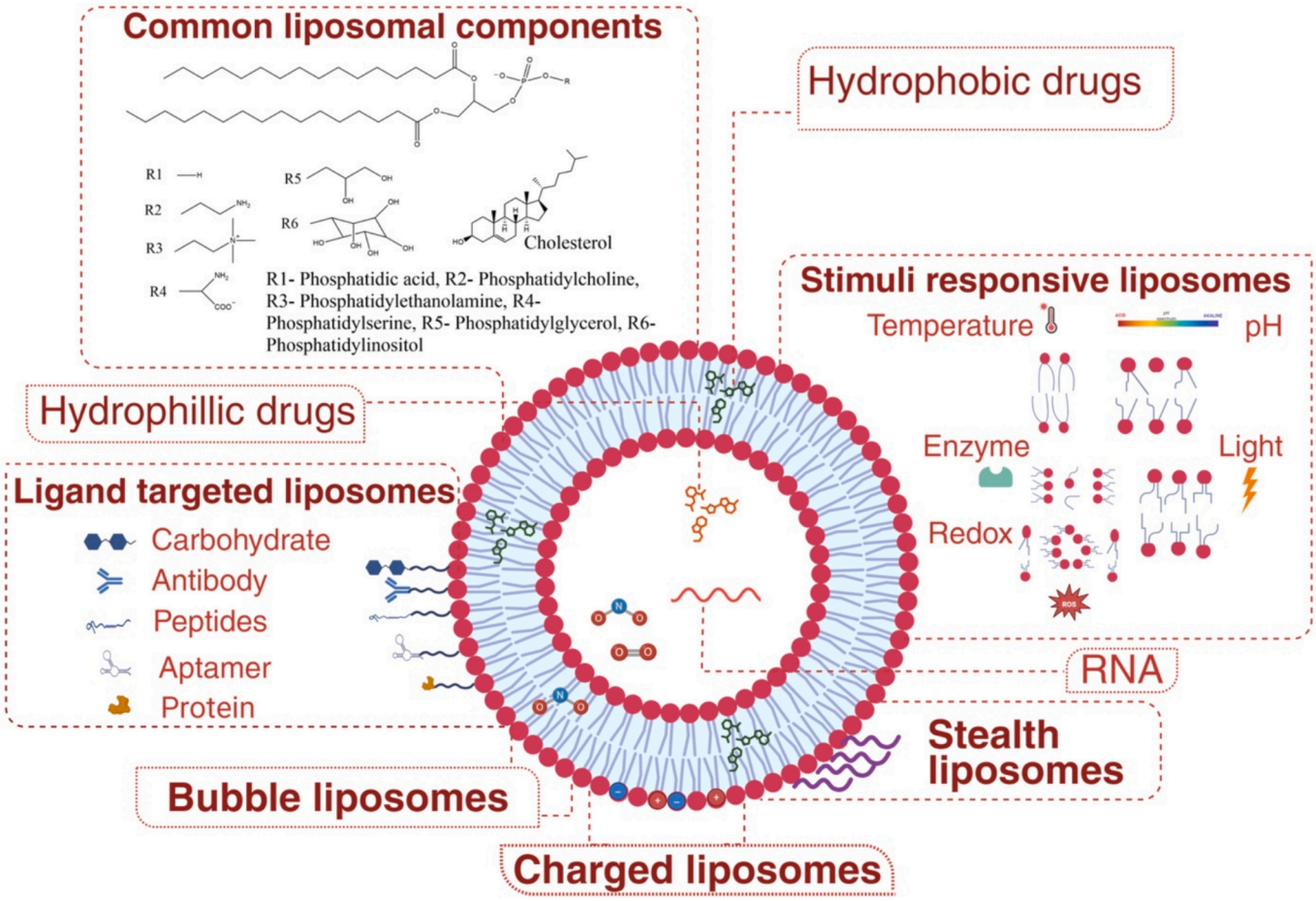

Fig. 1. Various types of liposomes

payload from liposomes in response to systemic or external stimuli such as changes in pH, enzymatic activity, redox reactions, light, heat, ultrasound, or magnetic fields.18 pH-responsive liposomes are triggered by changes in pH, where they become unstable in an acidic environment. The functional groups present in the liposomes are protonated, causing the liposomes to become unstable and release the drug.42 Redox-responsive liposomes containing disulfide bonds are reduced when exposed to the elevated level of glutathione, which cleaves the bonds and releases the drugs from the liposomes at the targeted site.43,44 Enzyme-responsive liposomes generally release the encapsulated drug in response to elevated enzyme expression, which is commonly found in diseased cells. Overexpressed enzymes, such as proteases and esterases, cleave amide or ester bonds, disrupting the liposomal lipid bilayer and causing drug release into target cells.45 Some liposomes are photosensitive and become unstable upon exposure to a light source, releasing the drug at the targeted site because of the availability of photoreactive functional groups in the phospholipid bilayer.46,47 Thermosensitive liposomes experience a transformation from a gel state to a crystalline liquid phase, increasing phospholipid bilayer permeation and facilitating drug release when triggered by mild hyperthermia. A paramagnetic substance, such as iron oxide, is added to magnetic field-responsive liposomes, a specific category of thermosensitive liposomes. An alternating electric current magnetic field generates magnetic hyperthermia, which facilitates the release of therapeutic agents.48,49

Interaction of liposome with the pulmonary environment

The interaction of liposomes varies depending on the route of administration. Liposomes are destabilized by gastric and pancreatic enzymes, gastric acid, bile salts, and intestinal surfactants during oral administration, resulting in the release of the drug. Many strategies have been implemented to improve liposomal stability in the gastric milieu, formulation with hydrogenated long-chain phospholipids, which have a greater phase transition temperature and help shield liposomes from bile salts.50 One of the most extensively studied routes for liposomal drug delivery is the transdermal route. Phospholipids in the stratum corneum can engage with liposomes, facilitating the successful delivery of the encapsulated drug. Transdermal delivery of liposomal formulations has shown promise in delivering anticancer drugs for the treatment of skin and breast cancers.51 Because of its high vascularity, the intranasal route is also effective for both systemic and local drug delivery. Additionally, it also avoids the enzymatic breakdown in the gastrointestinal tract and the first-pass effect.52

The pulmonary route is another area of significant interest for liposomal drug delivery of low-dose drugs, particularly for treating lung diseases. When liposomes are administered systematically, they aggregate in the reticuloendothelial system (RES), mainly in the bone marrow, lymph nodes, lungs, kidneys, liver, and spleen. This accumulation initiates clearance through macrophage-mediated phagocytosis through direct interaction between liposomes and macrophages. The high prevalence of RES in lungs, direct pulmonary delivery of liposomal drugs could be beneficial in the treatment of lung infections caused by intracellular pathogens.53 A thin cellular lining, high-air blood gas exchange area, by-passing first-pass metabolism, and exhibiting low enzymatic activity are some of the benefits of the pulmonary system.54,55 A proper understanding of the interaction between liposomes, the lung environment, and pulmonary surfactants is crucial for

designing targeted liposomal formulations for the treatment of lung disease.

Biochemical composition of pulmonary surfactants

Pulmonary surfactants are heterogeneous compositions of lipids and proteins produced by alveolar type II cells onto the air-liquid surface of the pulmonary epithelium. The pulmonary surface is stabilized during the compression and expansion cycles of breathing by surfactants that decrease surface tension at the air-liquid interface, contributing to the prevention of lung collapse.56 The lipid fraction, which accounts for more than 90 % of pulmonary surfactants, mainly consists of phosphatidylcholine derivatives (60–70 %), with dipalmitoylphosphatidylcholine (DPPC) comprising approximately 40 % of the total surfactant mass. Another major lipid fraction, phosphatidylglycerol, makes up about 10 %, while marginal phospholipids such as phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, and lysophosphatidylcholine are present in smaller amounts. Neutral lipids and cholesterol are also components of pulmonary surfactant membranes.57,58 The remaining 10 % of the surfactant mass consists of proteins, which are categorized into hydrophilic and lipophilic surfactant proteins (SP). Hydrophilic proteins include SP-A (3–5 %) and SP-D (0.5 %), both of which fall under the collectin family and play key roles in alveolar homeostasis, innate immunity, and inflammatory reaction. Lipophilic proteins, SP-B and SP-C (0.5–1 %), are membrane related and vital for the biophysical role of surfactants. Unlike SP-A and SP-B, SP-D is not normally linked to surfactant membranes.59,60

The composition of pulmonary surfactants can be altered in different pathological conditions. Changes in cytokine levels within alveolar spaces can affect surfactant synthesis, leading to abnormalities in surfactant protein levels.61 Insufficient production or low levels of pulmonary surfactants contribute to respiratory disorders such as neonatal respiratory distress syndrome (NRDS), acute respiratory distress syndrome (ARDS) and meconium aspiration syndrome.62,63 These conditions can be treated with pulmonary surfactant therapy. Surfaxin, a synthetic surfactant, has been FDA-approved for NRDS treatment, and several other synthetic lipids and surfactant substitutes are presently in preclinical trials.64

Interaction of liposomes with pulmonary phagocytes

Pulmonary surfactants are promising components for drug delivery, as they facilitate the solubilization of hydrophobic drugs and optimize their transport and distribution over the respiratory surface, and provides protection against extracellular barriers.65 The main goal of inhalable liposomal drugs is to enhance drug availability deep within the lungs. Various studies have demonstrated interactions between inhalable liposomal formulations and pulmonary tissues. Liposomal formulations must overcome the physiological barriers of the lungs to increase pulmonary retention.66 However, information on nanocarrier interactions within the lung environment after administration remains limited. It is commonly believed that alveolar macrophages rapidly absorb inhaled nanocarriers while those that remain in the alveoli experience size and stability changes because of interactions with surfactant proteins. Studies on the interactions between nanoparticle-antigen presenting cells in the airways and between nanocarrier and leukocyte interactions in the bloodstream support this assumption. Additionally, mucus and surfactant layers are thought to hinder transport of nanocarriers to the distal lung. However, studies suggest that surface modifications such as PEGylation, protein coating, or neutral/negative surface charge can facilitate passage through mucus and surfactant layers.67 Phosphatidylcholine, the major component of pulmonary surfactants, is also a key component of liposomes, which helps maintain alveolar patency and enable deep alveolar penetration without inducing pulmonary toxicity. Ferguson et al. studied the distribution mechanism of inhaled liposomes within specific lung compartments and cells using an inhalable liposome formulation of nintedanib, yielding several key findings. Surprisingly, they found that drug-encapsulated liposomes remained unchanged and free from aggregation for several hours after exposure to the cellular interior within the alveoli *in vivo*, even though several surfactants could rupture liposomes and nanoparticle aggregation commonly occurs in other bodily fluids. This indicates that liposomes are compatible with pulmonary surfactants, suggesting their potential use in treating lung diseases such as pulmonary fibrosis. Additionally, they observed that alveolar macrophages do not play a dominant role in the entry of inhaled liposomes. Their results further suggest that molecularly small drugs can give significant drug delivery over time when they gradually release from adjacent compartments and cell types, even in lung parenchymal cell types with limited uptake at first. The liposomal formulation of nintedanib increased lung exposure area under the curve (AUC) by 8000-fold compared to oral delivery, highlighting its potential clinical benefits. Additionally, they demonstrated that liposomes do not degrade over time in bronchoalveolar lavage fluid (BALF), supporting the theory that alveolar macrophages do not contribute much to extended drug release since they are not readily saturated. These important findings hold great promise for improving lung disease therapeutics and provide valuable guidance for the future engineering of inhalable drug formulations.67

The physiochemical properties of liposomes play a crucial role in their interaction with pulmonary barriers. Factors such as liposome size, membrane fluidity and surface charge have been rarely studied for their influence on pulmonary physiological interactions. The liposomes' size of approximately 200 nm demonstrated effective lung delivery, uniform distribution throughout the lungs, and enhanced penetration into biofilms and stimulated macrophages to react at infection sites.68,69 Macrophage phagocytosis and mucus penetration activity can be tuned by altering the membrane fluidity of liposomes. Membrane fluidity represents a key characteristic of liposomes that influence their stability and permeability. Jing Zhao et al. studied the interaction of a series of PEGylated DPPC inhalable liposomes with different membrane fluidities and pulmonary physiological barriers. Liposomes with reduced membrane fluidity enhanced mucus permeability, whereas increased membrane fluidity resulted in reduced macrophage uptake under *in vitro* conditions. In *in vivo* studies, liposomes possessing moderate membrane fluidity exhibited enhanced tracheal permeation, while no substantial change was observed in lung retention.70 Ryoya Ibuki et al. investigated the impact of lipid composition on the cellular uptake of dipalmitoylphosphatidylcholine (DPP) /cholesterol liposomes with the size of approximately 200 nm into THP-1-derived macrophages. DPPC/Cholesterol with an 80/20 mol% showed the highest membrane fluidity compared to other prepared liposomes. These liposomes were internalized through clathrin- and caveolae-mediated endocytosis and phagocytosis. Membrane fluidity significantly affects protein adsorption as well as the subsequent uptake efficiency of liposomes which is primarily influenced by the cholesterol ratio in the formulation.68

The interaction between inhalable nanovesicles and neutrophils are not well studied due to their short half-life and terminally differentiated and non-proliferating properties.71 The interaction of phagocytes can be specified by tuning the properties of nanovesicles. Chang Liu et al. constructed various surface charge containing liposomes such as neutral, anionic and cationic and studied the interaction of those inhaled liposomes with macrophages and neutrophils to evaluate the particle biofate and anti-inflammatory effect in acute lung inflammation. They designed the pH-responsive, charge-sensitive lipid that remains neutrally charged while passing through the airways and acquire a cationic surface once accumulated in the inflamed alveoli due to the potential limitations of cationic liposomes such as insufficient mucus permeation and chronic inhalation toxicity. Those liposomes showed the increased distribution compared to alveolar macrophages and infiltrating neutrophils, acted as potential carriers for inhaled liposomes, prolonging lung retention in acute lung injury models. Roflumilast was loaded in a liposome with a different charge and anti-inflammatory

response of those varied surface charges liposomes were evaluated. Roflumilast-loaded liposomes, preferentially delivered to macrophages or neutrophils, exhibited maximal anti-inflammatory effects, which were linked to either the reduced phagocytic capacity of alveolar macrophages or the extended circulation time of neutrophils. This study suggests that designing lung phagocyte-targeted pulmonary delivery systems and exploring potential pathways to precisely control lung phagocyte activity in inflammatory lung diseases, where these immune cells are pivotal.72 Jing Zhao et al., explored the influence of micron-sized liposomes that interact with the lungs in various manners depending on their microstructure and phospholipid composition. They prepared micron-sized MVL and Multilamellar liposomes with various types of phospholipids such as soya phosphatidylcholine (SPC), egg yolk phosphatidylcholine (EPC), and DPPC, and *in vivo* lung retention in the lungs and distribution across organs were investigated. The type of phospholipid greatly influenced the interaction of liposomes in the lungs. Both NR8383 and A549 bronchial epithelial cells showed higher cellular absorption of SPC-based liposomes than DPPC-based ones and exhibited enhanced lung retention. The microstructure of liposomes had no impact on pulmonary cellular uptake, *in vivo* retention, or organ distribution.73

Liposomes for nucleic acid delivery in the treatment of lung diseases

After the COVID-19 pandemic, mRNA therapy has gained intense interest in advancing nucleic acids and their delivery strategies.74 Nucleic acids regulate key signaling pathways in diseases, either directly or indirectly, through gene expression, gene silencing, and gene deletion, thereby disrupting the expression of their coded proteins.75,76 Nucleic acid therapy has shown effectiveness in tumor precision therapy, genetic disease treatment, and vaccination. However, effective delivery strategies remain a major challenge for their development and clinical application.74

For efficient delivery, the carrier material must safeguard nucleic acids from decomposition by nucleases and other enzymes while ensuring high encapsulation and transfection efficiency. Several methods of delivering nucleic acids have been investigated for the treatment of respiratory diseases, attracting growing interest from researchers in drug delivery formulations. Different administration routes have been investigated for pulmonary delivery, including inhalation methods. Inhalation offers high lung-targeted accumulation, improved pulmonary bioavailability, and is particularly suitable for local treatment of pulmonary diseases.74 Additionally, lung-targeted delivery of nucleic acids *via* parenteral administration is possible due to the tissue tropism effect of nanocarriers. Cationic charged nanoparticles can accumulate *via* protein opsonization, particularly involving proteins like vitronectin.77 Additionally, by means of the electrostatic attraction between negatively charged blood components and positively charged nanoparticles, passive organ tropism improves preferred lung deposition. The primary contributing factors of this impact are the pulmonary endothelium's large surface area and high local blood flow, which promote the distribution of drug or nucleic acid molecules into the lungs.78,79

The proper choice of inhalation devices and nanocarriers is crucial for effective nucleic acid inhalation therapy. Several biological barriers influence the bioavailability of inhaled nucleic acids by affecting deposition, clearance, mucus penetration, cellular uptake, and lysosomal escape.80 In pulmonary diseases, structural changes in the respiratory tract can impact the bioavailability of nanodrugs. Reduced respiratory function or low air velocity in the lungs may limit the deep deposition of nanoparticles, leading to lower bioavailability.81 In cystic fibrosis, the thick and sticky mucus layer can hinder the penetration of nucleic acid formulations.82 Similarly, pulmonary infections can also obstruct nucleic acid delivery by inducing airway inflammation, enhancing pulmonary clearance *via* macrophage phagocytosis, and restricting movement due to excessive mucus buildup.83

The carboxyl groups on mucin proteoglycans carry negative charges, allowing them to interact electrostatically with cationic nanomaterials. In contrast, nanoparticles with neutral or negative surface charges exhibit improved mucus penetration.84,85 Additionally, mucus strongly entraps particles with hydrophobic surface regions. Therefore, low-mucus-adhesive nanomaterials are the most suitable for inhaled nucleic acid delivery.86,87 Several nanocarriers with enhanced lung tropism, such as cationic polymers, cationic lipids, and hybrid polymer-lipid nanoparticles, have been designed for nucleic acid delivery. Among these, liposomes represent one of the most thoroughly researched delivery strategies.

Various research groups have used liposomes or lipid nanoparticles to deliver different types of genetic materials, specifically targeting lung diseases. Wang et al. demonstrated the role of the Srpx2 gene in the progression of pulmonary fibrosis, showing that downregulating Srpx2 could serve as a potential therapeutic approach. Srpx2 is upregulated through the canonical TGF-β1 signaling pathway during fibroblast-to-myofibroblast transition. The elevated levels of Srpx2 suppress AP1 expression, resulting in reduced SMAD7 expression. This forms a positive feedback loop that enhances TGF-β/SMAD signaling, ultimately promoting fibroblast-to-myofibroblast transition and worsening pulmonary fibrosis. The group also developed siRNA-loaded liposomes targeting Srpx2, and intratracheal administration of this formulation effectively silenced Srpx2 expression and improved bleomycin-induced pulmonary fibrosis.88 Similarly, Taetz et al. and their group targeted telomerase, which is considered a promising strategy for cells that overexpress this enzyme, as telomerase plays a key role in cell immortalization and cancer development. They developed cationic hyaluronic acid-modified DOTAP/DOPE liposomes for the targeted delivery of anti-telomerase siRNA to CD44-expressing lung cancer cells. The modification of liposomes with anionic hyaluronic acid did not affect the binding efficiency or siRNA protection, but it improved stability in high-salt cell culture media and reduced cytotoxicity.89 MicroRNAs (miRNAs) also play a role in the development of pulmonary fibrosis by supporting both fibroblast-to-myofibroblast transition and epithelial-mesenchymal transition. Intravenous injection of anti-miR-21-loaded cationic liposomes has shown promising therapeutic potential in a bleomycin-induced pulmonary fibrosis mouse model.90 Various ionizable polymers have also been used in combination with lipid components to deliver genetic material to the lung surface. Vlasova et al. and their group synthesized polyethyleneamine-based ionizable lipopolymers and prepared lipopolymer-hybrid nanoparticles using the lipopolymer, DSPG, soyPC, cholesterol, and DMG-PEG for the delivery of mRNA into lung endothelial and immune cells. Using this formulation, they delivered various sizes of mRNA, including IL-12 mRNA, which slowed the progression of Lewis lung carcinoma, CFTR mRNA, and CRISPR-Cas9 mRNA for gene editing in lung tissue.91 Le et al. also synthesized derivatives of poly(β-aminoesters) and prepared a formulation using the polymer, DOPE, and PEG-lipid, which effectively transfected bevacizumab-encoded mRNA and inhibited VEGF expression in lung tissues. This study also demonstrated lung tissue-targeting by nanoparticles. Tables 1 and 2 summarize various nucleic acid-loaded liposomal formulations for lung diseases that are currently in preclinical and clinical stages.

Liposomes for the delivery of peptides and proteins in lung diseases

Therapeutic peptides have been developed for the management of different types of lung diseases, such as lung infection, fibrosis, and cancer. Most of the peptides that are synthesized from natural sources are considered antimicrobial peptides (AMPs).93 WHO suggests the *S. pneumoniae*, *P. aeruginosa*, *H. influenzae* and *S. aureus* are considered highly resistant to antibiotics prioritized for lung-related harm, with both acute and chronic infections being the vulnerable disease affecting

Table 1
Various nucleic acid-loaded liposomal formulations for lung diseases that are currently in preclinical stages.

Nucleic acid	Composition	Target	model/target cell	Outcomes	Ref
siRNA	C12–200, Cholesterol, DSPC, and mPEG-DMG	Srpx2	BLM-induced pulmonary fibrosis mice	Intratracheal administration of Srpx2 siRNA loaded liposomes effectively inhibited the Srpx2 expression that suppressed the fibroblast to myofibroblast transition which was effective in the treatment of PF in BLM induced PF mice.	88
siRNA	Cationic Hyaluronic acid modified DOTAP/ DOPE	Catalytic domain of human telomerase (hTERT).	CD44-expressing A549 lung cancer cell line	Hyaluronic acid modified DOTAP/DOPE liposomes effectively transfected the siRNA into CD44-expressing A549 cell lines showing those strategies can be utilized in the treatment of Lung cancer.	89
miRNA	DOTMA, Cholesterol, PEG	Anti-miRNA-21	Fibrosis induced NHLF cells, Bleomycin induced lung fibrosis mouse model	Anti-mir-21 loaded cationic liposomes remarkably enhanced the accumulation in the lung and cellular uptake of anti-mir-21 which improved the anti-fibrotic efficacy in *in- vitro* and *in-vivo*.	90
mRNA	Modified PEI/DSPG/ soy PC, Cholesterol/ DMG-PEG2000	IL-12 mRNA CFTR mRNA CRISPR Cas9 mRNA+sgRNA	IL-12 mRNA-Lewis lung cancer model, CFTR mRNA- CFTR KO mice, CRISPR-Cas9 mRNA- Ai9 mouse strain	This formulation allows the delivery of different size of RNAs and gene editing in lung tissue. Delivery of IL-12 mRNA with this formulation slow down lewis lung cancer progression, CFTR mRNA restores the CFTR protein and CRISPR-Cas9 mRNA delivery can edit the gene in lung tissue.	91
mRNA	PBAEs derivatives/ DOPE/ DMG-PEG2000/ DSPE-PEG2000	Bevacizumab-encoded mRNA	A549 cell lines and Orthotopic NSCLC mouse models	Bevacizumab-encoded mRNA loaded formulation having PBAEs polymer were distributed in lung endothelial cells and can translate the bevacizumab proteins which was more effective than recombinant protein.	92

C12–200: Cationic lipidoid C12–200, DSPC: Distearoylphosphatidylcholine, mPEG-DMG: Methoxypoly(ethylene glycol)-dimyristoyl glycerol, DOTAP: 1,2-dioleoyl-3-trimethylammonium propane, DOPE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, DOTMA: N-[1-(2, 3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA), PEG: Polyethylene glycol, PEI: polyethyleneimine, DSPG: 1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPG), soy PC: Soybean phosphatidylcholine, PBAEs: Poly(beta-amino ester)s, CFTR: Cystic fibrosis transmembrane conductance regulator, CRISPR- Clustered regularly interspaced short palindromic repeats, VEGF: Vascular endothelial growth factor.

Table 2
Various nucleic acid-loaded liposomal formulations for lung diseases.

NCT No.	Title	Disease	Intervention	Phase	Status
NCT01829971	A Multicentre Phase I Study of MRX34, MicroRNA miR-RX34 Liposomal Injection	Primary Liver Cancer SCLC, NSCLC, Renal Cell Carcinoma Lymphoma, Melanoma, Multiple Melanoma,	microRNA (MRX34)	1	Terminated
NCT00004471	Study of Gene Therapy for *CF*	*CF*	pGT-1 gene lipid complex	1	Completed
NCT00059605	Phase I Study of IV DOTAP: CHOL -Fus1 in NSCLC	LC	DOTAP: Chol-fus1	1	Completed
NCT01621867	Gene Therapy in *CF* Patients	*CF*	pGM169/GL67A Placebo	2	Completed

SCLC: Small cell lung cancer, NSCLC: Non-small cell lung cancer, DOTAP: 1,2-dioleoyl-3-trimethylammonium propane, CHOL- Cholesterol, *CF*: Cystic fibrosis, LC: Lung cancer.

the lungs.[94] Researchers are currently fascinated by AMPs owing to their excellent cell selectivity, prompt lethality, and broad spectrum activity.[95] The potential consequences of lung infection include lung fibrosis, which is defined by extreme and prolonged deposition of extracellular matrix caused by chronic tissue injury.[96] IPF is the leading form of both interstitial lung diseases and diffuse parenchymal lung disease, for which therapeutic options remain limited.[93] Peptides with anti-fibrotic activities are progressively being developed. Moreover, peptides and their analogs showed promising anti-cancer activity, and numerous peptide-based drugs have been approved for cancer treatment. Additionally, peptides hold strong promise in the treatment of lung cancer. The therapeutic peptides for the treatment of lung disease with their potential major pathways have been summarized in Fig.2.

Liposomes are promising candidates for delivering peptides into the lung environment. However, extensive research is needed on inhalable liposomal peptide delivery to overcome the limitations of peptides for clinical use, such as rapid degradation and proteolytic digestion. The composition of liposomes greatly affects the delivery of protein, and DPPC was extensively used in the pulmonary protein delivery. Chono et al. formulated the insulin-loaded liposomes using various phospholipids, including dioleoyl, dilauroyl, dimyristoyl, distearoyl, and DPPC. Among them, DPPC-based liposomes demonstrated improved pulmonary insulin delivery in rats.[97] Ponkshe et al. used the Cy5.5-coupled bovine serum albumin (BSA-Cy5.5) as a standard protein for DMPC based inhalable liposomes delivery, using cholesterol and PEG as a surface modifier, and plasma pharmacokinetics were analyzed in mice following intratracheal aerosolization. The systemic bioavailability of inhaled liposomal formulation of BSA-Cy5.5 was higher than inhaled free BSA-Cy5.5, suggesting the inhalable liposomes containing DMPC can achieve systemic delivery of peptides through pulmonary delivery.[98] These studies suggest liposomes can be explored for the delivery of peptides and proteins in lung environment. Studies on other liposomal proteins and peptides that are specific targets for the treatment of lung diseases is limited. Vasoactive intestinal peptide (VIP) is a promising approach for treating severe lung diseases. However, it has a very short duration of activity in the lungs. To address this issue, a liposomal formulation was developed, which prevented enzymatic degradation while preserving and maintaining the biological activity of the peptide. These formulations were tested in BALF, which was used to mimic the proteolytic conditions of the lungs.[99]

Liposomes in the delivery of anti-tubercular agents

Tuberculosis (TB) is the most prevalent contagious disease originating from *Mycobacterium tuberculosis* (*M. tuberculosis*), primarily infecting the pulmonary system. Pulmonary tuberculosis affects nearly one in four people worldwide and remains a major contributor to the top 10 causes of death.[100,101] *M. tuberculosis* is transmitted through

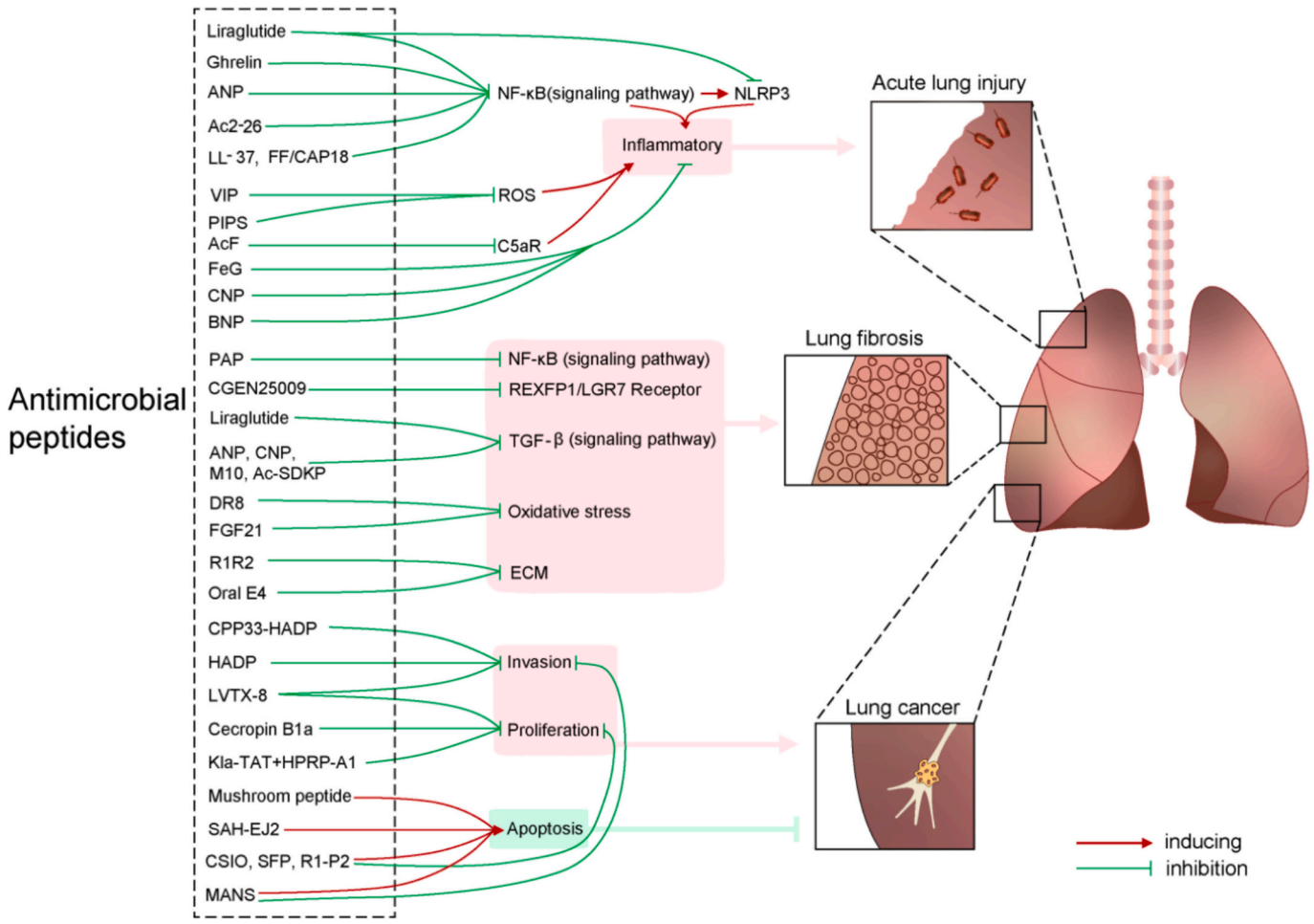

Fig. 2. Therapeutic peptides for lung diseases and their major signaling pathways.[93]

aerosol-based infection, at the initial stage of entering the airways and alveolar regions. Once inside, it is incorporated by alveolar macrophages, which attempt to eliminate the bacilli. This triggers a multicellular immune response, attracting more protective cells to the infection site. As a result, granulomas form, which may develop a solidified caseous region where bacteria can remain viable for years, leading to latent TB. In the final stage, granuloma disintegration and caseous area liquefaction allow bacilli to spread, resulting in pulmonary TB.[102]

M. tuberculosis penetrates and proliferates within macrophages, making them unreachable to antitubercular drugs. Due to limited drug penetration, high doses and prolonged therapy are often required, which can lead to drug accumulation and increased side effects. Initially, patients with pulmonary TB are treated with first-line antitubercular drugs.[102,103] However, non-compliance with these drugs can result in multidrug-resistant tuberculosis (MDR-TB) because of unpredictable chromosomal mutations and genetic alterations in the bacterium.[104] Patient compliance in TB therapy is significantly impacted by drug-induced side effects such as rifampicin can cause hepatotoxicity, renal failure, nausea and flu-like symptoms,[105] isoniazid may lead to convulsive seizures, mental impairment, coma, and vasculitis,[106] pyrazinamide can cause arthralgia, arthritis, and gout, often developing as multiple joint pain within the first two months of treatment.[107,108] MDR-TB strains are resistant to isoniazid and rifampicin, the primary first-line drugs, while extensively drug-resistant tuberculosis (XDR-TB) strains are characterized by resistance to second-line treatment options including amikacin, capreomycin, and fluoroquinolones.[104] To improve TB therapeutic outcomes and lower side effects, various drug delivery strategies and alternative approaches have been explored. Among them, liposomes are the most investigated nanocarriers for delivering antitubercular agents. Liposomal formulations of isoniazid alone or in combination with other antitubercular drugs such as rifampicin, ciprofloxacin, and usnic acid have been developed by various research groups. All these formulations demonstrated enhanced antimycobacterial activity compared to the free drugs.[109–112] Another antitubercular drug rifampicin-loaded liposomal formulation has been studied and with enhanced efficiency.[113,114] Rifampicin-loaded liposomal formulations incorporating polymers such as chitosan and ε-poly-L-lysine have shown improved mucoadhesion and entrapment efficiency. In particular, liposomes containing ε-poly-L-lysine enhanced rifampicin delivery to the lungs.[114] Liposomal formulation of other antitubercular drugs such as amikacin,[115] levofloxacin,[116] clofazimine,[117–119] and rifabutin[120,121] also improves their activity. Obiedallah et al. developed a liposomal formulation containing a novel antitubercular agent, which improved the solubility of the agent and demonstrated antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294).[122] The above-mentioned liposomal formulations for the delivery of antituberculosis agents are further summarized in Table 3.

Liposomal adjuvants for tuberculosis vaccine

The increased mortality associated with TB, MDR-TB, XDR-TB, and TB/HIV co-infection poses a significant public health threat, emphasizing the critical need for a vaccine to prevent the spread of tuberculosis.[123,124] The World Health Organization (WHO) has made

Table 3
Liposomal formulations for the delivery of antituberculosis agent.

Drug	Liposomal component	Bacterial cell/ Model	Outcomes	Ref
Isoniazid	DPPC	Radiometric BACTEC 460 TB	Effectively delivered to the alveolar surface and stabilizes the alveolar structure due to the anti-atelectatic effect of the formulation.	109
Rifampicin	Soya lecithin/ cholesterol	*M. tuberculosis* H_{37} RV (*In-vitro*) Wister rat model (*In-vivo* drug disposition)	Both the liposomal formulation and the free drug showed extended and sustained release, while the liposomal formulation delivered the drug to macrophages more rapidly.	113
Rifampicin	Soybean HSPC/ DPPG	*M. abscessus* (ATCC19977) Human promonocytic THP-1 leukemia cell line	Polymers decorated liposomes improved mucoadhesion without altering liposomal properties or encapsulation efficiency. ε-Poly-L-lysine decorated liposomes exhibited superior antibacterial activity against *M. abscessus* compared to chitosan-decorated liposomes.	114
Amikacin	Egg Phosphatidylcholine	*M. avium* Beige mouse (C57BL/6 J-bg^j/bg^j) acute infection model	The liposomal formulation of amikacin significantly reduced the viable counts of *M. avium* in the spleen and liver compared to free amikacin	115
Levofloxacin	Bovine heart Cl/ PC/Cholesterol	Antituberculosis drug resistant *M. tuberculosis* strain (H37Rv and CN-37 strains)	Effectiveness of the formulation was directly rely on the concentration of CL in the liposomes.	116
Rifabutin	PC/PS	Murine infection model using *M. avium* strain P1581	Intravenous administration of the rifabutin incorporated liposomal formulation reduced the level of infection more than the free drug at both treatment and prophylactic doses.	120
Rifabutin	DPPC/ DPPG	*M. tuberculosis* H37Rv infected BALB/c mice model	Rifabutin loaded liposomal formulation have superior effect than free drug which suggest alternative therapeutic approach for treating extrapulmonary TB in HIV co-infected patients.	121
Clofazimine	DMPC/ DMPG	*M. avium-M. intracellulare* complex (MAC) infection model (beige mouse)	The formulation reduced the *in vivo* toxicity of clofazimine and decreased the CFU count in the spleen, liver, and kidneys. The results suggest that liposomal clofazimine is remarkably efficient in treating MAC infections.	117
Clofazimine	DMPC/ DMPG	Acute and chronic MAC infection model (beige mouse, C57BL/6 J bgj bgj)	The liposomal formulation of clofazimine showed a stronger antibacterial effect against MAC compared to free clofazimine, significantly reducing the live bacterial count in the liver and spleen at higher infection levels, as well as showing a marked decline in the lung involvement in mice with lower levels of infection.	118
Clofazimine	DMPC/ DMPG	*M. tuberculosis*-infection model in BALB/c mice	The liposomal formulation exhibited lower toxicity in all tissues, even at a 10-fold higher dose compared to free clofazimine. This formulation was bactericidal in the liver and spleen, with the growth of *M. tuberculosis* remaining negative for up to 2 months.	119
3-(3,5-dimethylpyrazole-1-yl)-6-(isopropylthio) imidazo [1,2-b]1,2,4,5 tetrazine	Soybean lecithin (PC)/ cholesterol/ Choline palmitic acid ester/ Methyl ester of Palmitic acid	*In-vitro* antitubercular activity against *M. tuberculosis*	The *in vitro* release study highlighted that incorporating the drug into liposomes enhanced its solubility. Antimycobacterial effects against *M. tuberculosis* were observed with both free and liposomal formulations.	122
Rifampicin and Isoniazid combination	Egg PC/ Cholesterol/ CL	*M. tuberculosis*-infected mice model	The results showed an approximately 80 % survival rate after 30 days, with significantly lower CFU values and reduced relative lung weight (RSLW) compared to the free drugs.	110
Isoniazid and ciprofloxacin			pH-sensitive liposomes functionalized with ligands influenced pH dependent drug release, showing slower release at alkaline pH (58.64 %) than at the macrophage pH (82–87 %), due to the destabilization of the pH-sensitive liposomes under acidic environment. Maximum drug accumulation in the lungs was observed with the ligand-anchored liposomes compared to conventional liposomes.	111
Rifampicin/ Isoniazid and Usnic acid	Soya PC, Cholesterol and SA	*M. tuberculosis* H37Rv ATCC 27294 strain and six MDR-TB clinical isolates (MDR-TB 1412, 1619, 0729, 1411, 1409 and 1484)	A synergism between rifampicin and usnic acid was observed, suggesting that this usnic acid-loaded liposome can serve as an effective dosage form to enhance the antimycobacterial efficacy of rifampicin.	112

DPPC: Dipalmitoylphosphatidylcholine, HSPC: Hydrogenated phosphatidyl-choline from soybean, DPPG: 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt, CL: Cardiolipin, DMPC: Dimyristoylphosphatidylcholine, DMPG: 1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol, SA: Stearylamine.

substantial efforts in improving diagnosis and developing new therapeutic vaccines to combat TB, aiming to reduce its prevalence and mortality.125 However, the immune mechanisms responsible for sensitivity to TB disease are not yet fully understood.

Despite significant advancements in pharmacological and diagnostic research, effective immunization remains the only economic solution for the ongoing control of this infectious disease. Among the most effective strategies for TB control is the Bacillus Calmette-Guérin (BCG) vaccine, originally developed by French scientists Albert Calmette and Camille Guérin using an attenuated live strain of *Mycobacterium bovis*.126

However, live attenuated vaccines are not recommended for immunocompromised individuals, such as HIV-positive infants, due to potential safety concerns.127

The development of novel TB vaccines has gained significant interest, with approaches including viral vector vaccines, whole-cell or subunit-based formulations, and adjuvanted recombinant protein components. Various categories of TB vaccines, such as live or attenuated recombinant vaccines, viral vector vaccines, adjuvanted subunit vaccines, and Mycobacterium-derived whole-cell or fragmented vaccines, are being explored as therapeutic vaccines or as adjuncts to chemotherapy to reduce active or latent TB infections. Some of these candidates are undergoing various phases of clinical trials.124,128,129

Subunit vaccines, which contain pathogenic antigens but lack live microorganisms, are considered safer than attenuated live vaccines.130 However, adjuvants are required to modulate the adaptive immune response and provide the crucial innate immunopotentiation.131 Adjuvants are primarily classified into immunostimulants and delivery vehicles. Immunostimulants (*e.g.*, PAMPs, DAMPs, and synthetic agonist drugs) generate danger signals (signal 0) that trigger pattern recognition receptors (PRRs) on antigen-presenting cells (APCs). This enhances antigen presentation on MHC molecules (signal 1) and promotes cytokine release and co-stimulatory molecule expression (signal 2), thereby strengthening the immune response. Lipid nanoparticles (LNPs), poly (lactic-co-glycolic acid) (PLGA), and self-assembled protein nanoparticles improve the efficiency of antigen presentation on MHC molecules, which amplify the immune activation. The precise mechanism of action of adjuvants is illustrated in Fig. 3.132

Among delivery systems, liposomes are widely used as adjuvants and have been applied in TB vaccine development. Since their introduction as an adjuvant in 1974, liposomes have been repeatedly evaluated in clinical trials.133,134 Liposomal adjuvants are extremely flexible, allowing modification through changes in lipid composition, addition of immunostimulatory agents, formulation technique adjustments, and optimization of antigen presentation.135,136 While subunit vaccines are considered safer than live vaccines, they require potent adjuvants to elicit a strong and lasting memory immune response to the vaccine antigen. Currently, no adjuvants approved for clinical use can efficiently induce antigen-specific effector cells and durable memory CD4+ and CD8+ T cells, highlighting the critical need for novel adjuvants capable of eliciting a precise cell-mediated immune response.137 Several adjuvants, AS01E, IC31, GLA-SE, and CAF01 are presently undergoing clinical trials for their potential use in TB subunit vaccines, as summarized in Table 4. AS01E is a liposome-based adjuvants that contain two immune stimulants. One is 3-O-desacyl monophosphoryl lipid A (MPL), which is a detoxified variant of lipopolysaccharide obtained from *Salmonella minnesota*, and it induces activation of innate immunity *via* Toll-like receptor 4 (TLR4). Another is QS21, a saponin molecule from *Quillaja Saponaria*, and it facilitates antigen-specific antibody responses and also stimulates cytotoxic CD8+ T cells.138 The M72/AS01E vaccine is a combination of a recombinant fusion protein and the AS01E adjuvant, which has shown efficacy against pulmonary tuberculosis for three years in the clinical trial NCT0175598.139 IC31 is a synthetic cationic

Table 4
Adjuvants for their potential use in TB subunit vaccines.

Adjuvants	Composition	Developed by	Reference
AS01E	3-O-desacyl monophosphoryl lipid A (MPL), QS21	GlaxoSmithKline	138
IC31	Immunostimulatory oligodeoxynucleotide (ODN1a) and an antimicrobial polypeptide (KLK) in a 25:1 M ratio	Intercell AG (Vienna, Austria)	140
GLA-SE	Stable squalene-in-water emulsion of Glucopyranosyl Lipid A (GLA)	Infectious Disease Research institute	141
CAF01	Dimethyldioctadecyl-ammonium (DDA) and tetrahalose 6,6- dibehenate (TDB)	Statens Serum Institut	144

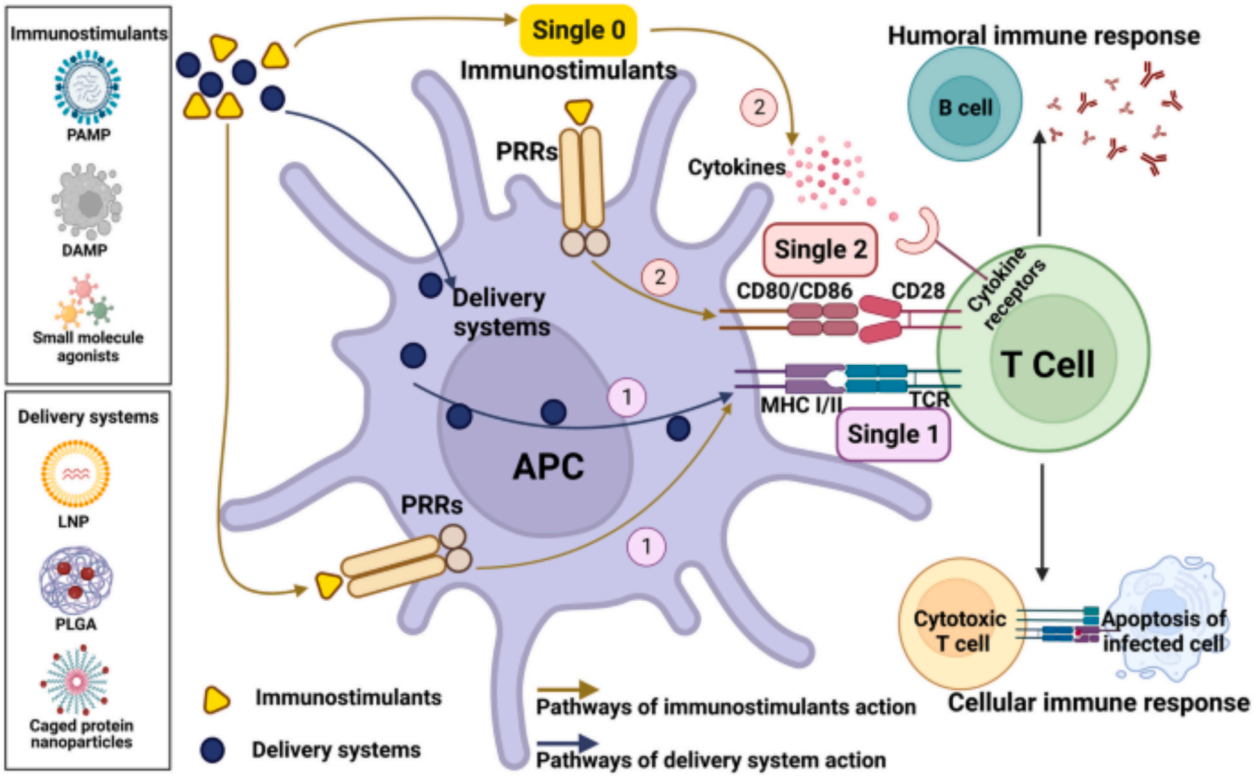

Fig. 3. Mechanism of action of adjuvants. Image was re-produced with permission from Zhao T et al., 2023.132

adjuvants made up of a phosphodiester-backed immunostimulatory oligodeoxynucleotide (ODN1a) and an antimicrobial polypeptide (KLK) in a 25:1 M ratio. It induces a potent and enduring antigen-specific Th1 adaptive immune response through the TLR9 signaling pathway.[140] Glucopyranosyl lipid adjuvant- stable emulsion (GLA-SE) is also TLR4 agonist, and it can trigger myeloid dendritic cells to release high levels of Th1 cell-promoting cytokines.[141] ID93 + GLA-SE is an effective TB vaccine. In a randomized, double-blind, placebo-controlled Phase 2a trial (NCT02465216), the vaccine induced robust and long-term antibody responses. Significantly higher and persistent antigen-specific IgG and CD4+ T-cell responses were observed following two injections of the 5 μg GLA-SE and 2 μg ID93.[142] In the Phase 1 clinical trial (NCT03722472), the single-vial thermostable formulation of ID93 + GLA-SE demonstrated similar safety and improved immunogenicity compared to the non-thermostable double-vial formulation.[143] CAF01 is one of the two component liposomal adjuvant systems consisting of dimethyldioctadecyl-ammonium (DDA) and tetrahalose 6,6 dibehenate (TDB). DDA is a cationic liposome carrier that is stabilized by TDB, a glycolipid-based immunomodulator that serves as a synthetic analogue of the mycobacterial cord factor found in its cell wall.[144]

Liposomes as drug delivery systems for the management of ARDS

Increased pulmonary vascular permeability caused by lung inflammation in acute respiratory distress syndrome (ARDS) results in the release of interstitial fluids in the alveolar cavity, resulting in alveolar collapse and hindering gas exchange.[145] SARS-CoV-2 infection (COVID-19) is also related to ARDS, and patients who suffer from ARDS experience severe hypoxemia and have no curative treatment, while existing options are curative.[146] There are generally three stages of pathological stages: early exudative phase, fibroproliferative phase, and the fibrotic phase.[145] Various drugs have been showing the best results in when they are used to treat the patients with ARDS. Glucocorticoids such as dexamethasone, hydrocortisone, and methylprednisolone, JAK inhibitors such as tofacitinib, baricitinib, fedratinib, ruxolitinib, and others such as ascorbic acid, glycyrrhizinic acid, *N*-acetyl cysteine (NAC), remdesivir, aclatinib, infliximab, and heparin have been used for the curative care of the ARDS patients.[145,147]

Glucocorticoids such as methylprednisolone showed positive treatment outcomes in patients with ARDS and also improved the survival rate, while long-term systemic use is linked to severe adverse effects, including effector inhibition and disruption of the wound healing process.[148,149] JAK inhibitors also improve prognosis and decrease inflammation in ARDS; however the timing of starting treatment affects the effectiveness of JAK inhibitors and they also increase the chance of thrombus development, autoimmunity, and the incidence of anemia and thrombocytopenia.[150] Antioxidants such as ascorbic acid are also used as a supplementary treatment, but didn't improve the clinical symptoms in low or moderate dose.[151,152] Mucolytic agent NAC is also used for the treatment of ARDS, and it has been found that it helps to minimize the duration of stay in the intensive care unit. Due to the substantial first-pass metabolism, the bioavailability of NAC is less than 10 % when oral administration.[145,153,154] Nucleoside analog remdesivir also mitigates cytokine storm damage by inhibiting the dsDNA-associated NF-kB pathway.[155] In order to prevent thromboembolic consequences, especially in patients who are severely ill, heparin, an anticoagulant agent is also utilized in the acute therapy of ARDS. Nebulized heparin shortens ventilator days, lowers inflammatory marker levels, increases coagulation activation, decreases microvascular thrombosis, and enhances functional scores in preclinical trials.[156,157] Liposome-based drug delivery in the lungs has shown a good response in comparison with free drug, and it also shows a good response in the ARDS in an animal model. Scientists have developed variety of ligands including antibodies, peptides, mannosylation, and nucleic acids to enhance the targeted distribution of liposomes. This has provided an opportunity to modify liposomes, thereby improving the therapeutic index of drugs and reducing side effects.[147] Sivan Arbar Raviv et al., formulated DPPC liposomes containing corticosteroid methylprednisolone and the NAC, a mucolytic agent *via* consecutive drug loading. Liposome deposition was greater in an inflammatory lung than in healthy lungs after systemic IV doses, suggesting that liposomes preferentially target the inflammatory lung. In inflammatory lungs, local delivery of liposomes by the ET route results in a higher concentration and longer retention period of liposomes. Another important distinction between the IV and ET routes is that the distribution of IV-administered liposomes covered the entire lung, whereas ET liposomes were primarily identified in the lung parenchyma but absent from some distal regions of the lung. TNF α and NO levels dropped in response to liposomal and *in vitro* free medication treatments of LPS-stimulated macrophages. Since an effect is observed, it can be said that the availability of DPPC liposomal drug is considerable and promising for the management of lung diseases.[145]

Liposomal drug delivery to manage asthma and COPD

Asthma is a chronic respiratory disease that impacts individuals of all ages, being the most prevalent chronic condition in children. It is caused by inflammation and smooth muscle constriction around the airways, causing airway narrowing, making it difficult to breathe. Several factors have been associated with an elevated risk of asthma, including genetics, allergic conditions, urbanization associated with different lifestyle factors, and early life events that affect lung development, such as low birth weight and exposure to smoke and air pollutants.[158,159] Environmental and occupational exposures, as well as being overweight or obese, also contribute to the risk. Asthma is often underdiagnosed and untreated, particularly in middle-income countries. While asthma cannot be cured, it can be controlled with treatments such as inhaled bronchodilators and steroids.[160] The first-line strategy for asthma management involves the combination of steroids and bronchodilators, including leukotriene receptor antagonists and short- or long-acting beta-agonists.[161] A significant problem with asthma management is steroid resistance, which can occur after using high doses of steroids. Other treatment options beyond corticosteroids include cytokine/chemokine antagonists, nebulized glucocorticoids, and monoclonal antibodies. However, these alternatives are often limited by different asthma types and symptoms.[162] Liposomal formulations, especially for the treatment of asthma, offer a novel strategy over current conventional formulations due to their biocompatibility, increased drug retention, reduced extra-pulmonary side effects, and enhanced therapeutic effectiveness.[163,164]

COPD is also a global disease burden and predominant underlying cause of death worldwide, which is marked by chronic bronchitis, emphysema, and mucus hypersecretion, leading to a gradual progression of irreversible airway obstruction.[165,166] Oxidative stress, imbalance of protease/antiprotease, apoptosis, and cellular senescence are the pathogenic events linked with the pathogenesis of COPD.[167–170] Anticholinergics, beta-2 agonists, and inhaled corticosteroids are the recent therapeutic strategies and treatment with antioxidants is an effective way to reduce excessive reactive oxygen species (ROS) in the airways which are one of the main driving factors for the pathogenesis and progression of COPD.[165,171,172]

Honmane et al. developed a liposomal dry powder inhaler (LDPI) for salbutamol sulfate, showing a sustained *in-vitro* release of over 90 % for up to 14 h according to Higuchi's controlled release model. The LDPIs displayed enhanced aerosol performance, featuring a better fine particle fraction, mass median aerodynamic diameter, lower impaction loss, and reduced retention in the capsule and device compared to those prepared using lactose through spray drying. These characteristics help reduce the dosing frequency of salbutamol sulfate and minimize associated adverse drug reactions.[173] Chen et al. developed an aerosolized liposomal formulation of Salbutamol sulfate using the vesicular phospholipid gel (VPG) method. The encapsulation efficiency was 70 %, and the particle size of the liposomal suspension was 57 nm. The liposomal formulation

released salbutamol sulfate for a minimum of 48 h. In contrast, the free salbutamol solution only lasted for less than 8 h, and the liposomal formulation's anti-asthmatic effect was prolonged for 18 h in guinea pigs, as demonstrated by pharmacodynamic study. This study demonstrates that liposomes can enhance the therapeutic efficacy of salbutamol sulfate by increasing its concentration and retention time in the lungs.174 Elhissi et al. prepared liposomal formulations of salbutamol sulfate and beclomethasone dipropionate, both individually and in combination, with or without cryoprotectants. The rehydrated liposomes containing both drugs had particle sizes below 100 nm when sucrose was used as the cryoprotectant and below 136 nm with trehalose. This study suggests that a freeze-dried liposomal formulation combining these two anti-asthmatic drugs can be successfully produced and utilized for pulmonary delivery.175 Konduri et al. compared the effectiveness of weekly therapy using stealth-encapsulated budesonide liposomes to daily therapy with plain budesonide in reducing allergic inflammation in mice. Mice were sensitized with ovalbumin, which led to significant increase in eosinophil peroxidase activity (EPO), peripheral blood (PB) eosinophils and serum IgE levels. Lung inflammation was observed on histologic examination. The therapy was started with weekly budesonide-loaded stealth liposomes, plain budesonide administered daily or weekly and weekly stealth liposomes without budesonide. The results demonstrated that the weekly therapy as effective as daily budesonide in decreasing lung inflammation markers (EPO activity in BAL, PB eosinophils, serum IgE levels, IL-4, IL-5) in experimental asthma.176 Yin Ng et al. evaluated a novel therapeutic approach using curcumin-loaded liposomes, which were able to downregulate pro-inflammatory markers (IL-6, IL-8, IL-1β, and TNF-α) in the BCi-NS1.1 cell line due to the synergistic effect of curcumin and phospholipids. This study suggests that curcumin-loaded liposomes could be a potential pharmacological option for managing asthma, though it primarily provides a foundation for future research.177 The gradual and repeated administration of allergens may lead to a potential cure. However, allergen-specific immunotherapy has drawbacks, such as the failure to modify the immune response, patient non-compliance due to long-term therapy, and the risk of developing anaphylaxis. Alberca-Custodio et al. developed liposomes containing a low allergen concentration (OVA) combined with a synthetic TLR9 agonist (CpG-ON). The cationic liposomes encapsulated the OVA, which significantly reduced anaphylactic reactions. Co-encapsulation of allergens with CpG during the therapy protected against anaphylaxis and provided prolonged effects against different allergens, suggesting this type of immunotherapy as a treatment option for the eosinophilic asthma endotype.178 To explore the immunological and functional responses to a liposome-encapsulated *D. pteronyssinus* vaccine in people with moderate asthma who are sensitive to dust mites, a double-blind, placebo-controlled trial was performed.

The study included 20 asthma patients who received either the vaccine or a placebo for one year. The liposomal *D. pteronyssinus* vaccination decreased the inflammatory and functional alterations brought on by allergen bronchial stimulation and prevent mild asthma patients from their condition getting worse because of extended mite exposure.179 Qingrui li et al. prepared a liposomal sustained-released nebulized aerosol of R-terbutaline hydrochloride. Liposomes were prepared using a thin film hydration method and were loaded with the drug using ammonium sulfate-driven transmembrane electrochemical gradient. This technique successfully resulted in an efficient encapsulation of up to 71.35 % and produced particle sizes of approximately 145 nm. The liposomal formulation of R-terbutaline hydrochloride demonstrated a longer anti-asthmatic effect in guinea pigs compared to non-encapsulated R-terbutaline hydrochloride.180

Studies on the liposomal delivery of antioxidants for the treatment of oxidative stress related COPD have been using hydrophobic (α-tocopherol) and hydrophilic (NAC, glutathione) antioxidants and enzymes (catalase, superoxide dismutase).165 Liposomes can effectively transport the hydrophilic, hydrophobic, and amphiphilic antioxidants to the different tissues and organs. Manconi et al. prepared a curcumin loaded liposomal formulation and surface modified with hyaluronan or chitosan for enhanced protection of the drug and vesicles. Liposomes coated with negatively charged hyaluronan showed improved lung deposition and effectively protected A549 cells from oxidative stress. This study explored liposomal formulations for the delivery of phytodrugs in the treatment of chronic inflammatory conditions. For the delivery of curcumin, hyaluroanan-coated liposomes act as a potential and safe delivery option for the local treatment of lung diseases.181 In another study, liposomal curcumin showed superior biological activity compared to free curcumin powder to inhibit the cigarette smoke-induced inflammation (IL-8, IL-24) and aging in an *in vitro* COPD model using human broncho epithelial cell.182,183

Liposomes in the delivery of anti-cancer agents for the treatment of lung cancers

Lung cancer is major contributor to cancer-related deaths and accounts for more fatalities in women than breast cancer. It originates in the lung parenchyma or within the bronchi.184–186 The rising incidence of lung cancer is primarily linked to smoking habits in both genders, with smoking being one of the most common causes of the disease.187,188 The cellular and molecular basis of lung cancer is not yet fully understood; however, it is hypothesized that frequent inhalation or contact with cigarette smoke or other carcinogens leads to dysplasia of the lung epithelium, resulting in genetic mutations that affect protein synthesis. Mutations in MYC, BCL2, and p53 are significant in small cell lung cancer (SCLC), while EGFR, KRAS, and p16 mutations are associated with non-small cell lung cancer (NSCLC). The treatment and management of both SCLC and NSCLC typically involve adjuvant chemotherapy or surgery followed by chemotherapy, depending on the stage of lung cancer.189–193

Liposomal anticancer formulations have benefited from enhanced efficacy and reduced toxicity, further supported by advancements in liposomal technology for controlled and targeted drug delivery.194 These formulations can be delivered through various routes, including oral, parenteral, and inhalation, with their interaction depending on the chosen administration route. Generally, liposomal anticancer drugs are delivered *via* parenteral routes. Upon entering systemic circulation, the mononuclear phagocyte system (MPS) phagocytizes and breaks down liposomes once they are rapidly absorbed by the reticuloendothelial system (RES) and recognized as foreign particles by opsonin. This process significantly influences their circulation time and overall stability.195,196 Increased vascular permeability and prolonged retention of macromolecules within solid tumors are hallmarks of the enhanced permeability and retention effect.197 Tumor tissues exhibit structurally and anatomically aberrant endothelial cells with increased gaps because of the high oxygen and nutritional demands of fast growth, in contrast to normal tissues where microvascular endothelial gaps are closely packed and intact. The buildup of macromolecules in the tumor microenvironment is caused by the increased obstruction of lymphatic fluid drainage caused by the lack of lymphatic capillaries in tumor tissues. This prolonged retention enables nanoparticles, including liposomes, to persist in tumor tissues for a long period. The enhanced permeability and retention (EPR) effect is recognized as a crucial factor in the accumulation of nanoparticles at tumor sites, and liposomes facilitate drug enrichment in these regions, making it a crucial mechanism for improving tumor treatment outcomes.198,199

Cellular internalization of liposomes depends on their physicochemical properties. Neutral liposomes can readily enter cells *via* passive diffusion, whereas others may undergo active transport mechanisms such as micropinocytosis, caveolae-mediated endocytosis, clathrin-mediated endocytosis, or clathrin/caveolae-independent endocytosis.54,200 Generally, hydrophilic drugs smaller than 5.5 nm are often rapidly eliminated in urine; however, their retention can be enhanced by encapsulating them within a larger liposomal vesicle. The particle size of

liposomes deemed a pivotal factor in determining their half-life, with particle sizes between 20 nm and 200 nm generally avoiding rapid elimination while improving tumor site penetration.201,202

Several liposome types, including PEGylated liposomes, pH-sensitive liposomes, magnetic liposomes, enzyme-responsive liposomes, thermosensitive liposomes, and ligand-targeted liposomes, have been evaluated for their potential in delivering anticancer drugs.196 PEGylation is one of the most widely used strategies for reducing immunogenicity and enhancing circulation time by conferring stealth properties. Hydrophilic polymers such as PEG coatings prevent opsonization, thereby prolonging liposomal circulation. The hydrophilic and flexible nature of PEG chains allows them to extend outward on the liposomal surface. When opsonin bind to the particle surface, the extended PEG chains adopt a more condensed, higher-energy conformation, where repulsive forces counteract attractive interactions between opsonin and the particle surface.203

The first PEG-coated liposomal formulation of doxorubicin, Doxil®, was approved in 1995 to treat AIDS-related Kaposi's sarcoma in 1995, in 2003 to treat metastatic breast cancer, and in 2007 to treat multiple myeloma.196 It was also the first clinically approved pH-sensitive liposomal formulation, designed to release its drug payload in the acidic tumor microenvironment.204 Several drugs have gained regulatory approval for the treatment of various types of lung cancer, including doxorubicin, etoposide, paclitaxel, irinotecan, erlotinib, docetaxel, and cisplatin. These drugs are being formulated into liposomal delivery systems to improve their therapeutic efficacy. The main strategies for lung-targeted drug delivery include tumor microenvironment-specific targeting, overexpressed receptor-mediated targeting, and organelle-specific targeting.205 Numerous clinical trials are evaluating liposomal formulations for the treatment of lung cancer, as summarized in Table 5.

Clinical translation and optimization of liposomal formulations: Prospects and challenges

Nano-based drug-delivery systems show significant potential in combating various diseases. Since the approval of Doxil® for clinical application, numerous types of nano-based drug delivery approaches have been rapidly participating in the pre-clinical and clinical trials. Although considerable studies have been performed in this field, only a limited number of formulations have successfully been translated into clinical applications. Harashima et al. suggests five key areas to consider in nano-based drug delivery research that could support translation from bench to bedside. These include rational design during the research and development phase, deliberate design of clinical trials, development of harmonized and specific protocols, and emphasis on non-classic sponsorships.206 Here, we summarize the some of the efforts that can accelerate the clinical translation of liposomal formulations.

Engineering the liposomes can address the formulation shortcomings and help in increasing the success rate of clinical translation

Among nano-formulations, liposomal formulations are among the most approved nano-formulations for clinical use. Despite the success in clinical translation, there are some basic challenges that remain, such as encapsulation efficiency for both hydrophobic and hydrophilic small molecules, control release of payloads and liposome stability. Approaches such as synthesis of new types of lipids and chemical modifications have been helpful in addressing such shortcomings. The rate-limiting step of passive diffusion of molecules through cellular or engineered membranes (liposomes) is passage through the bilayer, where hydrophobic region is 100–1000 times more viscous than the exterior aqueous phase.207 Kohane and colleagues prepared aromatized liposomes by substituting the phospholipid's hydrophobic acyl chains with aromatic groups at their ends. These aromatized liposomes improve the sustain release of both small and macromolecules and increase the drug loading compared to the commonly used liposomes. A strong correlation between aromatization, molecular weight, and hydrophilicity with the release profile is not observed in small molecules, whereas such a correlation is seen in macromolecules. These aromatized liposomes also prolonged the duration of action of the hydrophilic local anesthetic tetrodotoxin and reduced toxicity in *in vivo* models.207 This study provides a proof of concept that aromatic substitution at the end of hydrophobic tails can solve the problems of low loading of hydrophilic small molecules and prolong the release of drugs and macromolecules such as proteins and peptides.

The clinical translation of liposomal drug delivery is also limited by drug leakage in systemic circulation. Plasma proteins disrupt the bilayer architecture of liposomes and blood shear stress causes the disruption and permeability of liposome membrane leads to premature drug release. Nanobowls can provide mechanical support to liposomes, enhancing their stability during circulation and preventing premature release of the drug due to vascular shear stress and plasma proteins. Fang et al. was first to develop the nanobowl-supported, doxorubicin-loaded liposomes which improved drug loading, reduced drug leakage and enhanced antitumor efficacy. Despite various existing stabilization methods, this approach uniquely embeds a rigid nanobowl within the water cavity of an existing liposome formulation.208 This simple and effective approach may offer valuable benefits for liposomal formulations intended for lung diseases and clinical translation.

Current status and challenges in clinical translation of liposomal formulations

There are limited commercially available liposomal formulations specifically approved for the treatment of lung diseases, although several liposomal formulations are available for the treatment of disease

Table 5
Clinical trials evaluating liposomal formulations for the treatment of lung cancer.

Trial No.	Title	Intervention	Phase	Status
NCT04727853	Study of Irinotecan Liposome Injection as second-line regimen in patients with SCLC	Irinotecan liposome injection	II	Completed
NCT06462105	Liposomal Irinotecan Combination Regimen for First-line Treatment of SCLC	Liposome Injection of Irinotecan Hydrochloride, Carboplatin Injection and Serplulimab Injection	Not applicable	Not yet recruting
NCT06820762	Irinotecan liposome (II) combined with ivonescimab as second-line treatment for SCLC: a prospective, single-arm, multicenter clinical study	Irinotecan liposome (II) plus Ivonescimab *via* intravenously (IV) Q3W for 4–6 cycles, with subsequent Ivonescimab until progression of diseases or intolerable toxicity	II	Not yet recruting
NCT00157209	Phase 2b randomized controlled study of tecemotide (L-BLP25) for immunotherapy of NSCLC	Tecemotide (L-BLP25) And cyclophosphamide given in a Single low dose Best Supportive Care (BSC)	II	Completed
NCT04887298	Study of Liposomal Annamycin for the Treatment of Subjects with Soft-Tissue Sarcomas (STS) with Pulmonary Metastases	Liposomal Annamycin (L-Annamycin)	I/II	Completed
NCT00960115	Combined phase I/II clinical study of EMD531444(L-BLP25 or BLP25 liposome vaccine) in subjects with Stage III unresectable NSCLC following primary chemoradiotherapy	Tecemotide (L-BLP25) and cyclophosphamide given in a Single low dose Saline	I/II	Completed

other than lung diseases. Here, we presented some approved liposomal formulations that are either currently in prescription status or have been discontinued according to the FDA and European Medicines Agency (EMA) databases. ARIKAYCE®, an amikacin liposome inhalation suspension, is the first licensed formulation for the treatment of lung disease caused by a group of bacteria, *Mycobacterium avium* complex (MAC), in a limited number of patients who do not respond to conventional therapy. ONIVYDE®, an Irinotecan liposome injection, is a topoisomerase inhibitor, indicated for the first-line treatment of adult patients with metastatic pancreatic adenocarcinoma in combination with oxaliplatin, fluorouracil, and leucovorin, and adult patients with metastatic pancreatic adenocarcinoma after disease progression following gemcitabine-based therapy in combination with fluorouracil. Myocet®, a liposomal formulation of doxorubicin citrate, is indicated for the first-line treatment of breast cancer in adult women used in combination with cyclophosphamide. AmBisone® is a marketed single bilayer liposomal formulation of amphotericin B used for serious fungal infections. VYXEOS® is a liposomal formulation of daunorubicin and cytarabine indicated for the treatment of newly diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC) in adults and pediatric patients 1 year and older. ZOLSKETIL® is a doxorubicin hydrochloride-loaded liposomes coated with methoxypolyethylene glycol indicated for the treatment of metastatic breast cancer, advanced ovarian cancer, progressive multiple myeloma in combination with bortezomib, and AIDS-related Kaposi's sarcoma. These are some examples of clinically approved and currently used liposomal formulations.

Some marketed liposomal formulations intended for other than lung diseases have been discontinued from the FDA databases. These include DaunoXome® (liposomal formulation of daunorubicin citrate), Depocyt® (liposomal formulation of cytarabine), Marqibo® (liposomal formulation of Vincristine sulfate), and Amphotec® (liposomal formulation of amphotericin B). The reason for the withdrawal varies, such as discontinuation of Marqibo® due to the uncompleted post marketing clinical trial to confirm its clinical benefit and issues with adequate level of patient enrollment.209 Although liposomal formulation has attracted significant interest, their clinical translation faces various challenges, including large scale manufacturing difficulties, batch to batch consistency, availability and affordability, profitability and public acceptance. Moreover, most studies have focused on the formulation optimization and the development of an advanced carrier system. While many *in vitro* studies show the potential efficacy, a large number of studies remain unsuccessful in preclinical studies, approximately 20–25 % of preclinical findings advance to the clinical implementation, and the success rate declines from 94 % in phase I to 14 % by phase III.209,210 The clinically translated liposomal formulation remains limited, not only for lung diseases but across a range of diseases. The translation of liposomal formulations from bench to bedside remains challenging and requires extensive research due to several factors, including difficulties in large-scale manufacturing, complex critical quality attributes, and a lack of harmonization in scientific evaluations. Regulatory authorities such as the FDA, EMA, and Japan's Ministry of Health, Labour, and Welfare (MHLW) have released guidelines to facilitate the registration and market approval process for liposomal products. Key guidelines include: Guidance for Industry: Liposome Drug Products—Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation (FDA), Data Requirements for Intravenous Liposomal Products Developed with Reference to an Innovator Liposomal Product (EMA), and Guidelines for the Development of Liposomal Drug Products (MHLW).211 However, there is still a need for harmonized guidelines to streamline the registration process and ensure the safety and quality of liposomal formulations.

Incorporating all the required pharmaceutical quality system elements into a single formulation is not enough to obtain marketing authorization. Various biological and physicochemical factors significantly affect therapeutic efficacy and must be evaluated on a case-by-case or science-based basis by regulatory authorities, making it difficult to generalize these requirements in a single guideline. However, general guidelines outline common Critical Quality Attributes (CQAs) and define key terms to promote harmonization. There are major challenges in achieving precision in intra-laboratory analysis and analytical techniques for assessing CQAs. Several standardized protocols for CQA analysis have been published, and others are currently under development. Characterization of morphology, size, surface properties, drug loading, release behavior, and stability can be performed using a variety of techniques. Organizations such as the International Organization for Standardization (ISO), the European Nanomedicine Characterization Laboratory (EUNCL), the International Council for Harmonization (ICH), and the American Society for Testing and Materials (ASTM), among others, have published and developed analytical methods for these purposes. Notably, stringent regulatory authorities have made significant progress in the registration of liposome-based non-biological complex drugs. Adherence to good practices in the development of liposomal products has facilitated faster approval of such formulations. However, low- and middle-income countries often lack comprehensive regulatory guidelines for nanomedicine registration, which are crucial to ensure patient safety and treatment efficacy.

Generally, manufacturing methods and physicochemical properties are more prone to change during scale-up or industrial production. The most common challenges in the industrial manufacturing of liposomes include batch inconsistency, lack of reproducibility, scale-up difficulties, limited shelf-life and stability, sterility issues, quality control limitations, and economic pressures—despite advances in various pharmaceutical technologies.212 Developing robust batch manufacturing methods, minimizing multistep processes and the number of batches, applying the principles of Quality by Design (QbD), and strictly adhering to regulatory manufacturing guidelines can help overcome these challenges. Furthermore, continuous efforts toward the development and implementation of new technologies are equally important for improving manufacturing efficiency and product quality. For instance, sterility is one of the major bottlenecks in the development of liposomal formulations, and the advancement of effective sterilization techniques is essential to ensure their successful clinical application. Nevertheless, it is encouraging that researchers are continuing to explore new strategies as well as optimizing liposomal formulation in order to increase their translational success rate from bench to bedside application.

Another major challenge in the development of liposomal formulations is ensuring their safety. Several commercially available liposomal products have been withdrawn from the market due to safety-related concerns. Most of these adverse effects are immune-related and include infusion-related reactions, hand-foot syndrome, hypersensitivity, and both anaphylactic and allergic reactions.213 Unwanted immune responses associated with liposomes include immunogenicity, uptake by the mononuclear phagocyte system (MPS), activation of the complement system, and immunosuppressive effects. Liposomes interact with the immune system through various receptors such as toll-like receptors, scavenger receptors, C-type lectin receptors, apolipoprotein receptors, complement receptors, and folate receptors.213 To reduce these immune responses and improve clinical outcomes, the use of stealth liposomes or modifications aimed at lowering immunogenicity is essential. However, the Accelerated Blood Clearance (ABC) phenomenon should be considered when designing the dosage of PEGylated liposomes. Although this phenomenon has been observed in some preclinical studies, its magnitude appears to be reduced in clinical settings.214 Further research on the biological interactions and underlying mechanisms of liposomes, along with strengthened industry-academic collaboration, is essential to address challenges related to manufacturing and safety.

Harnessing machine learning (ML) to accelerate clinical translation

Optimizing the formulations in a laboratory setting is time-

consuming and expensive, often delaying clinical translation. Various prediction and modeling techniques, such as response surface methodology (RSM), and artificial neural networks (ANNs), are applied for the optimization and prediction of nanoparticle characteristics. They excel through a data-driven approach, proficiency in managing non-linear relationships, and broad use in machine learning applications.215 Alternatively, RSM follows a statistical framework specifically designed for process modeling and optimization, utilizing empirical data and mathematical formulations.216 Machine learning models have been leveraged in predicting and optimizing liposomes. Ensemble learning (EL) modeling is another advanced meta method of machine learning that aims to improve predictive performance by integrating the outputs of multiple learning models. This approach plays an important role in interpreting experimental outcomes and may offer deeper insights into optimal formulation parameters.217,218 The properties of the liposomal formulation can be greatly influenced by the optimization of the independent variables, and EL can act a as tool for supporting decision-making to optimize the best-performing liposomal formulations.219 The optimization of the liposomal manufacturing process can be enhanced through ML and Explainable Artificial Intelligence (XAI), which can also significantly reduce the reliance on empirical methods and enhance the transparency. Luciani and colleagues employed ML to predict critical quality attributes and process parameters for liposome manufacture *via* a microfluidic system. They utilized the concepts of XAI to gain a greater understanding of the complex interaction between different parameters thereby improving the lipid behavior during manufacturing process. This approach also explores similar models for drug encapsulation and supports the broader application of commercial microfluidic chips. In future research, such models can be applied to optimize drug release profiles to ensure the effective and consistent delivery. Emerging ML-based microfluidic production offers considerable promise for enhancing the efficiency of drug formulation development220 as well as their clinical translation, which is often limited by batch-to-batch variation. The integration of design of Experiments (DoE) and AI has enabled the manufacturing of optimized liposomes with high reproducibility. No significant differences were observed between the ANN predicted value and experimented results. Computational tools that support microfluidic-based manufacturing methods offer a promising strategy for accelerating the development of drug delivery systems and clinical translation.221

Future perspectives and conclusions

Over the years, various nanoparticle systems have been studied for the treatment of lung diseases. Among them, liposomal delivery of therapeutic agents to the lungs has shown promising potential for clinical applications. Several liposomal formulations for lung disease treatment have already been approved, demonstrating enhanced clinical efficacy, while others are still in preclinical and clinical trials.

Since the COVID-19 pandemic, the development and formulation of nucleic acid drugs have advanced significantly. Pulmonary delivery of nucleic acids faces several physiological barriers; however, targeted liposomal formulations have shown promising results in preclinical trials, offering precise therapeutic strategies for lung disease treatment in the future.

For the treatment of drug-resistant tuberculosis, novel therapeutic agents have been discovered, and nano-drug delivery systems for existing drugs have been developed to reduce the probability of resistance. Liposomal formulations not only improve patient compliance but also help mitigate drug resistance. Moreover, liposomes are being explored as adjuvants for next-generation vaccines against *Mycobacterium tuberculosis*. Various studies on liposomal formulations for tuberculosis treatment suggest that liposomes are among the most effective nanocarriers for this purpose.

Liposomes have also demonstrated significant potential in delivering chemotherapeutic agents for lung cancer treatment. Inhalable liposomal anticancer drugs help reduce systemic toxicity by localizing drug delivery to the lungs. The development of inhalable liposomal anticancer agents should consider critical factors such as formulation stability, nebulization mechanisms, aerosol targetability to cancer cells, and minimized deposition in the oropharyngeal region.222 In conclusion, although liposomal drug delivery techniques have been used to treat various diseases, including lung diseases, clinical translation remains scarce and necessitates more extensive studies and harmonization of scientific assessments to reduce the translation time, ultimately benefiting patients.

CRediT authorship contribution statement

Saroj Bashyal: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Newton Suwal:** Writing – review & editing. **Rajan Thapa:** Writing – review & editing. **Laxmi Regmi Bagale:** Writing – review & editing. **Vrashabh V Sugandhi:** Writing – review & editing. **Sapana Subedi:** Writing – review & editing. **Sobia Idrees:** Writing – review & editing. **Nisha Panth:** Writing – review & editing. **Bassma H. Elwakil:** Writing – review & editing. **Mostafa El-Khatib:** Writing – review & editing. **Kamal Dua:** Writing – review & editing. **Keshav Raj Paudel:** Visualization, Supervision, Software, Resources, Project administration, Conceptualization.

Consent for publication

All authors have approved to publish this manuscript.

Ethics approval and consent to participate

Not applicable.

Funding

This project was funded by Maridulu Budyari Gumal - The Sydney Partnership for Health Education, Research and Enterprise (SPHERE) -2024, to Keshav Raj Paudel and Kamal Dua.

Declaration of competing interest

All authors declare no conflict of interest.

Acknowledgements

Authors would like to acknowledge the support from University of Technology Sydney, NSW, Australia and Centenary Institute, Camperdown, NSW, Australia.

Data availability

Not applicable.

References

1. Pramanik S, et al. Nanoparticle-based drug delivery system: the magic bullet for the treatment of chronic pulmonary diseases. *Mol Pharm*. 2021;18(10):3671–3718.
2. Yue L, et al. Inhaled drug delivery: past, present, and future. *Nano Today*. 2023;52, 101942.
3. Durham AL, et al. Targeted anti-inflammatory therapeutics in asthma and chronic obstructive lung disease. *Transl Res*. 2016;167(1):192–203.
4. Paudel KR, et al. Recent advances in chronotherapy targeting respiratory diseases. *Pharmaceutics*. 2021;13(12).
5. Burhan E, et al. Isoniazid, rifampin, and pyrazinamide plasma concentrations in relation to treatment response in Indonesian pulmonary tuberculosis patients. *Antimicrob Agents Chemother*. 2013;57(8):3614–3619.
6. Rudrapal M, Paudel KR, Pangeni R. Editorial: drug repurposing and polypharmacology: a synergistic approach in multi-target based drug discovery. *Front Pharmacol*. 2022;13, 1101007.

7. Paudel KR, et al. Computational and biological approaches in repurposing ribavirin for lung cancer treatment: unveiling antitumorigenic strategies. *Life Sci*. 2024;352, 122859.
8. Nagar L, et al. Unravelling the role of nanomedicine in attenuating inflammation, oxidative stress and cellular ageing in chronic obstructive pulmonary disease. *Arch Toxicol*. 2025:1–17.
9.. Kulkarni MP, et al. Interplay of nano-based delivery systems and protein signalling in ameliorating lung diseases. *Journal of Drug Delivery Science and Technology*. 2024;102, 106432.
10.. De Rubis G, et al. Applications and advancements of nanoparticle-based drug delivery in alleviating lung cancer and chronic obstructive pulmonary disease. *Naunyn Schmiedeberg's Arch Pharmacol*. 2024;397(5):2793–2833.
11. Jessamine V, et al. The application of nanoparticles as advanced drug delivery systems in attenuating COPD. *Heliyon*. 2024;10(3), e25393.
12. Wilczewska AZ, et al. Nanoparticles as drug delivery systems. *Pharmacol Rep*. 2012; 64(5):1020–1037.
13. Ibarra-Sánchez L, et al. Nanostructures for drug delivery in respiratory diseases therapeutics: revision of current trends and its comparative analysis. *J Drug Deliv Sci Technol*. 2022;70, 103219.
14.. Carrasco-Esteban E, et al. Current role of nanoparticles in the treatment of lung cancer. *J Clin Transl Res*. 2021;7(2):140–155.
15.. Prasher P, et al. Advances and applications of dextran-based nanomaterials targeting inflammatory respiratory diseases. *Journal of Drug Delivery Science and Technology*. 2022;74, 103598.
16.. Tenchov R, et al. Lipid nanoparticles—from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano*. 2021;15(11): 16982–17015.
17.. Li J, et al. A review on phospholipids and their main applications in drug delivery systems. *Asian journal of pharmaceutical sciences*. 2015;10(2):81–98.
18.. Senjab RM, et al. Advances in liposomal nanotechnology: from concept to clinics. *RSC Pharmaceutics*. 2024;1(5):928–948.
19. Fulton MD, Najahi-Missaoui W. Liposomes in cancer therapy: how did we start and where are we now. *Int J Mol Sci*. 2023;24(7), 6615.
20. Nkanga CI, et al. General perception of liposomes: formation, manufacturing and applications. *Liposomes-advances and perspectives*. 2019:1–24.
21. Mare R, et al. Post-insertion parameters of PEG-derivatives in phosphocholine-liposomes. *Int J Pharm*. 2018;552(1–2):414–421.
22. Working P. Pharmacological-toxicological expert report. CAELYX^< TM>.(Stealth^ (! R) liposomal doxorubicin HCl). *Hum Exp. Toxicol.*. 1996:15.
23.. Laouini A, et al. Preparation, characterization and applications of liposomes: state of the art. *Journal of colloid Science and Biotechnology*. 2012;1(2):147–168.
24. Harashima H, et al. Enhanced hepatic uptake of liposomes through complement activation depending on the size of liposomes. *Pharm Res*. 1994;11(3):402–406.
25. Nagayasu A, Uchiyama K, Kiwada H. The size of liposomes: a factor which affects their targeting efficiency to tumors and therapeutic activity of liposomal antitumor drugs. *Adv Drug Deliv Rev*. 1999;40(1–2):75–87.
26. Allen T, Everest J. Effect of liposome size and drug release properties on pharmacokinetics of encapsulated drug in rats. *J Pharmacol Exp Ther*. 1983;226(2): 539–544.
27. Smith MC, et al. Zeta potential: a case study of cationic, anionic, and neutral liposomes. *Anal Bioanal Chem*. 2017;409:5779–5787.
28. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (part 2). *Trop J Pharm Res*. 2013;12(2):265–273.
29. Wang X, et al. Are PEGylated liposomes better than conventional liposomes? A special case for vincristine. *Drug Deliv*. 2016;23(4):1092–1100.
30. Zhang J-X, et al. Application of liposomes in drug development—focus on gastroenterological targets. *Int J Nanomedicine*. 2013:1325–1334.
31. Senior J, Gregoriadis G. Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol components. *Life Sci*. 1982;30(24):2123–2136.
32.. Vaage J, et al. Therapy of human ovarian carcinoma xenografts using doxorubicin encapsulated in sterically stabilized liposomes. *Cancer*. 1993;72(12):3671–3675.
33. Rangger C, et al. Influence of PEGylation and RGD loading on the targeting properties of radiolabeled liposomal nanoparticles. *Int J Nanomedicine*. 2012: 5889–5900.
34. Ceh B, et al. Stealth® liposomes: from theory to product. *Adv Drug Deliv Rev*. 1997; 24(2–3):165–177.
35. Zhao W, Zhuang S, Qi X-R. Comparative study of the in vitro and in vivo characteristics of cationic and neutral liposomes. *Int J Nanomedicine*. 2011: 3087–3098.
36. Ogiso T, et al. Effect of positively and negatively charged liposomes on skin permeation of drugs. *J Drug Target*. 2001;9(1):49–59.
37. Mahanty A, et al. Bubble liposome: a modern theranostic approach of new drug delivery. *World J Pharm Pharm Sci*. 2017;6:1290–1314.
38.. Sakaguchi, K., et al. Characterization of bubble liposomes by measurements of ultrasound attenuation: effects of shell materials. In 2008 IEEE Ultrasonics symposium. 2008. IEEE.
39. Khan MS, et al. Anti-tumor drug-loaded oxygen nanobubbles for the degradation of HIF-1α and the upregulation of reactive oxygen species in tumor cells. *Cancers (Basel)*. 2019;11(10), 1464.
40. Nsairat H, et al. Liposomes: structure, composition, types, and clinical applications. *Heliyon*. 2022;8(5).
41. Olusanya TOB, et al. Liposomal drug delivery systems and anticancer drugs. *Molecules*. 2018;23(4).
42.. Movahedi F, et al. Stimuli-responsive liposomes for the delivery of nucleic acid therapeutics. *Nanomedicine*. 2015;11(6):1575–1584.
43.. Lee, Y. and D. Thompson, Stimuli-responsive liposomes for drug delivery. Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2017. 9(5): p. e1450.
44.. Abed HF, Abuwatfa WH, Husseini GA. Redox-responsive drug delivery systems: a chemical perspective. *Nanomaterials*. 2022;12(18), 3183.
45. Fleige E, Quadir MA, Haag R. Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: concepts and applications. *Adv Drug Deliv Rev*. 2012;64(9):866–884.
46.. Gai S, et al. Recent advances in functional nanomaterials for light–triggered cancer therapy. *Nano Today*. 2018;19:146–187.
47. Yavlovich A, et al. Light-sensitive lipid-based nanoparticles for drug delivery: design principles and future considerations for biological applications. *Mol Membr Biol*. 2010;27(7):364–381.
48. de Matos MB, et al. Thermosensitive liposomes for triggered release of cytotoxic proteins. *Eur J Pharm Biopharm*. 2018;132:211–221.
49. Tavano L, Muzzalupo R. Multi-functional vesicles for cancer therapy: the ultimate magic bullet. *Colloids Surf B: Biointerfaces*. 2016;147:161–171.
50. Menina S, et al. Bioinspired liposomes for oral delivery of colistin to combat intracellular infections by Salmonella enterica. *Adv Healthc Mater*. 2019;8(17), 1900564.
51. Matharoo N, Mohd H, Michniak-Kohn B. Transferosomes as a transdermal drug delivery system: dermal kinetics and recent developments. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2024;16(1), e1918.
52.. Semyachkina-Glushkovskaya O, et al. Intranasal delivery of liposomes to glioblastoma by photostimulation of the lymphatic system. *Pharmaceutics*. 2022; 15(1), 36.
53. Chalmers JD, et al. Liposomal drug delivery to manage nontuberculous mycobacterial pulmonary disease and other chronic lung infections. *Eur Respir Rev*. 2021;30(161):210010.
54. Sheikholeslami B, et al. Exploring the impact of physicochemical properties of liposomal formulations on their in vivo fate. *Life Sci*. 2022;300, 120574.
55. Chang RYK, et al. Dry powder pharmaceutical biologics for inhalation therapy. *Adv Drug Deliv Rev*. 2021;172:64–79.
56.. Daniels CB, Orgeig S. Pulmonary surfactant: the key to the evolution of air breathing. *Physiology*. 2003;18(4):151–157.
57. Hawco M, Davis P, Keough K. Lipid fluidity in lung surfactant: monolayers of saturated and unsaturated lecithins. *J Appl Physiol*. 1981;51(2):509–515.
58.. Batenburg JJ. Surfactant phospholipids: synthesis and storage. *Am J Phys Lung Cell Mol Phys*. 1992;262(4):L367–L385.
59. Serrano AG, Pérez-Gil J. Protein–lipid interactions and surface activity in the pulmonary surfactant system. *Chem Phys Lipids*. 2006;141(1–2):105–118.
60. Crouch E, Wright JR. Surfactant proteins a and D and pulmonary host defense. *Annu Rev Physiol*. 2001;63(1):521–554.
61.. Winkler C, Hohlfeld JM. Surfactant and allergic airway inflammation. *Swiss Med Wkly*. 2013;143, w13818.
62.. Whitsett JA, Wert SE, Weaver TE. Diseases of pulmonary surfactant homeostasis. *Annual Review of Pathology: Mechanisms of Disease*. 2015;10(1):371–393.
63. Dushianthan A, et al. Acute respiratory distress syndrome and acute lung injury. *Postgrad Med J*. 2011;87(1031):612–622.
64. De Backer L, et al. Bio-inspired materials in drug delivery: exploring the role of pulmonary surfactant in siRNA inhalation therapy. *J Control Release*. 2015;220: 642–650.
65.. Lopez-Rodriguez E, Pérez-Gil J. Structure-function relationships in pulmonary surfactant membranes: from biophysics to therapy. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 2014;1838(6):1568–1585.
66.. El-Sherbiny IM, et al. Overcoming lung clearance mechanisms for controlled release drug delivery. *Controlled pulmonary drug delivery*. 2011:101–126.
67. Ferguson LT, et al. Mechanisms by which liposomes improve inhaled drug delivery for alveolar diseases. *Adv Nanobiomed Res*. 2023;3(3), 2200106.
68. Ibuki R, et al. Effect of lipid composition on the interaction of liposomes with THP-1-derived macrophages. *Biol Pharm Bull*. 2024;47(3):723–731.
69. Zhang J, et al. Amikacin liposome inhalation suspension (ALIS) penetrates non-tuberculous mycobacterial biofilms and enhances amikacin uptake into macrophages. *Front Microbiol*. 2018;9, 915.
70. Zhao J, et al. Exploring the influence of inhaled liposome membrane fluidity on its interaction with pulmonary physiological barriers. *Biomater Sci*. 2020;8(23): 6786–6797.
71.. Ring, N.G., et al., Anti-SIRPα antibody immunotherapy enhances neutrophil and macrophage antitumor activity. Proc Natl Acad Sci, 2017. 114(49): p. E10578-E10585.
72. Liu C, et al. Interactions of inhaled liposome with macrophages and neutrophils determine particle biofate and anti-inflammatory effect in acute lung inflammation. *ACS Appl Mater Interfaces*. 2023;15(1):479–493.
73. Zhao J, et al. Exploring the influence of microstructure and phospholipid type of liposomes on their interaction with lung. *Eur J Pharm Biopharm*. 2024;198, 114271.
74. Wang Q, et al. Recent Progress in nucleic acid pulmonary delivery toward overcoming physiological barriers and improving transfection efficiency. *Adv Sci (Weinh)*. 2024;11(18), e2309748.
75. Zhang X, et al. Role of non-coding RNAs and RNA modifiers in cancer therapy resistance. *Mol Cancer*. 2020;19:1–26.
76. Lu D, Thum T. RNA-based diagnostic and therapeutic strategies for cardiovascular disease. *Nat Rev Cardiol*. 2019;16(11):661–674.
77.. Dilliard SA, Cheng Q, Siegwart DJ. On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc Natl Acad Sci USA*. 2021; 118(52).
78. Fu L, et al. Passive'nanoparticles for organ-selective systemic delivery: design, mechanism and perspective. *Chem Soc Rev*. 2023;52(21):7579–7601.

79. Li Y-X, et al. Targeting pulmonary vascular endothelial cells for the treatment of respiratory diseases. *Front Pharmacol*. 2022;13. p. 983816.
80. Chow MY, Chang RYK, Chan H-K. Inhalation delivery technology for genome-editing of respiratory diseases. *Adv Drug Deliv Rev*. 2021;168:217–228.
81. Kim CS. Physiological factors affecting lung deposition. *J Aerosol Med Pulm Drug Deliv*. 2021;34(3):147–154.
82. Morrison CB, et al. Treatment of cystic fibrosis airway cells with CFTR modulators reverses aberrant mucus properties via hydration. *Eur Respir J*. 2022;59(2).
83. Liu Q, et al. Physicochemical properties affecting the fate of nanoparticles in pulmonary drug delivery. *Drug Discov Today*. 2020;25(1):150–159.
84. Yang J, et al. Pro-peptide-reinforced, mucus-penetrating pulmonary siRNA delivery mitigates cytokine storm in pneumonia. *Adv Funct Mater*. 2021;31(21), 2008960.
85. Zhu J, et al. Surface coating of pulmonary siRNA delivery vectors enabling mucus penetration, cell targeting, and intracellular radical scavenging for enhanced acute lung injury therapy. *ACS Appl Mater Interfaces*. 2022;14(4):5090–5100.
86. Guo Y, et al. Mucus penetration of surface-engineered nanoparticles in various pH microenvironments. *ACS Nano*. 2023;17(3):2813–2828.
87. Watchorn J, et al. Untangling mucosal drug delivery: engineering, designing, and testing nanoparticles to overcome the mucus barrier. *ACS Biomater Sci Eng*. 2022;8(4):1396–1426.
88.. Wang Q, et al. Local administration of liposomal-based Srpx2 gene therapy reverses pulmonary fibrosis by blockading fibroblast-to-myofibroblast transition. *Theranostics*. 2021;11(14):7110.
89.. Taetz S, et al. Hyaluronic acid-modified DOTAP/DOPE liposomes for the targeted delivery of anti-telomerase siRNA to CD44-expressing lung cancer cells. *Oligonucleotides*. 2009;19(2):103–116.
90.. Yan L, et al. Delivery of anti-microRNA-21 by lung-targeted liposomes for pulmonary fibrosis treatment. *Molecular Therapy-Nucleic Acids*. 2023;32:36–47.
91.. Vlasova KY, et al. Synthesis of ionizable lipopolymers using split-Ugi reaction for pulmonary delivery of various size RNAs and gene editing. *Nat Commun*. 2025;16(1), 4021.
92. Le ND, et al. Antiangiogenic therapeutic mRNA delivery using lung-selective polymeric nanomedicine for lung Cancer treatment. *ACS Nano*. 2024;18(11):8392–8410.
93. Li S, et al. Therapeutic peptides for treatment of lung diseases: infection, fibrosis, and cancer. *Int J Mol Sci*. 2023;24(10), 8642.
94. Cookson WO, Cox MJ, Moffatt MF. New opportunities for managing acute and chronic lung infections. *Nat Rev Microbiol*. 2018;16(2):111–120.
95.. Molchanova N, Hansen PR, Franzyk H. Advances in development of antimicrobial peptidomimetics as potential drugs. *Molecules*. 2017;22(9), 1430.
96. Pottier N, et al. FibromiRs: translating molecular discoveries into new anti-fibrotic drugs. *Trends Pharmacol Sci*. 2014;35(3):119–126.
97. Chono S, et al. Aerosolized liposomes with dipalmitoyl phosphatidylcholine enhance pulmonary insulin delivery. *J Control Release*. 2009;137(2):104–109.
98. Ponkshe P, Wang Y, Tan C. Systemic protein delivery via inhalable liposomes: formulation and pharmacokinetics. *Pharmaceutics*. 2023;15(7), 1951.
99. Stark B, et al. Liposomal vasoactive intestinal peptide for lung application: protection from proteolytic degradation. *Eur J Pharm Biopharm*. 2008;70(1):153–164.
100. Zignol M, et al. Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007-2010. *Bull World Health Organ*. 2012;90:111–119.
101.. Migliori GB, Raviglione MC. *Essential tuberculosis*. Springer; 2021.
102.. Nair, A., et al., Advanced drug delivery and therapeutic strategies for tuberculosis treatment. J Nanobiotechnol, 2023. 21(1): p. 414.
103. Costa A, et al. The formulation of nanomedicines for treating tuberculosis. *Adv Drug Deliv Rev*. 2016;102:102–115.
104.. Sung N, et al. Inactivation of multidrug resistant (MDR)-and extensively drug resistant (XDR)-Mycobacterium tuberculosis by photodynamic therapy. *Photodiagn Photodyn Ther*. 2013;10(4):694–702.
105. Zaleskis R. Postgraduate course ERS Copenhagen 2005-the side-effects of TB therapy. *Breathe*. 2005;2(1):69–73.
106.. Bhise S. Isoniazid toxicity. *Journal of Drug Design and Research*. 2017;4(7):1060.
107. Wang YC, et al. Pyrazinamide related prolonged drug-induced liver injury: a case report. *Medicine (Baltimore)*. 2022;101(39), e30955.
108.. Pham AQ, Doan A, Andersen M. Pyrazinamide-induced hyperuricemia. *P t*. 2014;39(10):695–715.
109. Chimote G, Banerjee R. In vitro evaluation of inhalable isoniazid-loaded surfactant liposomes as an adjunct therapy in pulmonary tuberculosis. *J Biomed Mater Res B Appl Biomater*. 2010;94(1):1–10.
110.. Orozco LC, et al. The use of rifampicin and isoniazid entrapped in liposomes for the treatment of murine tuberculosis. *Tubercle*. 1986;67(2):91–97.
111. Bhardwaj A, et al. Pulmonary delivery of anti-tubercular drugs using ligand anchored pH sensitive liposomes for the treatment of pulmonary tuberculosis. *Curr Drug Deliv*. 2016;13(6):909–922.
112. Ferraz-Carvalho RS, et al. Effects of the encapsulation of usnic acid into liposomes and interactions with antituberculous agents against multidrug-resistant tuberculosis clinical isolates. *Mem Inst Oswaldo Cruz*. 2016;111(5):330–334.
113.. Patil JS, et al. A novel approach for lung delivery of rifampicin-loaded liposomes in dry powder form for the treatment of tuberculosis. *Lung India*. 2015;32(4):331–338.
114. Forte J, et al. Mucoadhesive rifampicin-liposomes for the treatment of pulmonary infection by Mycobacterium abscessus: chitosan or ε-poly-L-lysine decoration. *Biomolecules*. 2023;13(6), 924.
115. Cynamon MH, et al. Liposome-encapsulated-amikacin therapy of Mycobacterium avium complex infection in beige mice. *Antimicrob Agents Chemother*. 1989;33(8):1179–1183.
116. Gaidukevich S, et al. Antibacterial effects of liposomes containing phospholipid cardiolipin and fluoroquinolone levofloxacin on Mycobacterium tuberculosis with extensive drug resistance. *Bull Exp Biol Med*. 2016;160:675–678.
117. Mehta RT. Liposome encapsulation of clofazimine reduces toxicity in vitro and in vivo and improves therapeutic efficacy in the beige mouse model of disseminated Mycobacterium avium-M. Intracellulare complex infection. *Antimicrob Agents Chemother*. 1996;40(8):1893–1902.
118. Kansal RG, et al. Therapeutic efficacy of liposomal clofazimine against Mycobacterium avium complex in mice depends on size of initial inoculum and duration of infection. *Antimicrob Agents Chemother*. 1997;41(1):17–23.
119. Adams LB, et al. Effective treatment of acute and chronic murine tuberculosis with liposome-encapsulated Clofazimine. *Antimicrob Agents Chemother*. 1999;43(7):1638–1643.
120. Gaspar MM, et al. Therapeutic efficacy of liposomal rifabutin in a Mycobacterium avium model of infection. *Antimicrob Agents Chemother*. 2000;44(9):2424–2430.
121. Gaspar M, et al. Rifabutin encapsulated in liposomes exhibits increased therapeutic activity in a model of disseminated tuberculosis. *Int J Antimicrob Agents*. 2008;31(1):37–45.
122. Obiedallah MM, et al. Optimization, characterization, and cytotoxicity studies of novel anti-tubercular agent-loaded liposomal vesicles. *Sci Rep*. 2024;14(1):524.
123. Phoswa WN, Eche S, Khaliq OP. The association of tuberculosis mono-infection and tuberculosis-human immunodeficiency virus (TB-HIV) co-infection in the pathogenesis of hypertensive disorders of pregnancy. *Curr Hypertens Rep*. 2020;22:1–13.
124.. Moradi M, et al. Liposomal delivery system/adjuvant for tuberculosis vaccine. *Immunity, Inflammation and Disease*. 2023;11(6), e867.
125. Schrager LK, et al. The status of tuberculosis vaccine development. *Lancet Infect Dis*. 2020;20(3):e28–e37.
126. Hawgood BJ. Albert Calmette (1863–1933) and Camille Guerin (1872–1961): the C and G of BCG vaccine. *J Med Biogr*. 2007;15(3):139–146.
127.. Whitlow E, Mustafa A, Hanif S. An overview of the development of new vaccines for tuberculosis. *Vaccines*. 2020;8(4), 586.
128. da Costa C, Walker B, Bonavia A. Tuberculosis vaccines–state of the art, and novel approaches to vaccine development. *Int J Infect Dis*. 2015;32:5–12.
129.. Saramago S, Magalhães J, Pinheiro M. Tuberculosis vaccines: an update of recent and ongoing clinical trials. *Appl Sci*. 2021;11(19):9250.
130. Kaufmann SH. Vaccination against tuberculosis: revamping BCG by molecular genetics guided by immunology. *Front Immunol*. 2020;11, 316.
131.. Wu Z, Liu K. Overview of vaccine adjuvants. *Medicine in Drug Discovery*. 2021;11, 100103.
132. Zhao T, et al. Vaccine adjuvants: mechanisms and platforms. *Signal Transduct Target Ther*. 2023;8(1):283.
133.. Allison A, Gregoriadis G. Liposomes as immunological adjuvants. *Nature*. 1974;252(5480):252.
134. Di Gioacchino M, et al. Nanoparticle-based immunotherapy: state of the art and future perspectives. *Expert Rev Clin Immunol*. 2020;16(5):513–525.
135. Lovell SC, et al. Structure validation by cα geometry: ϕ, ψ and cβ deviation. *Proteins: Structure, Function, and Bioinformatics*. 2003;50(3):437–450.
136. Schmidt ST, et al. Liposomebased adjuvants for subunit vaccines: formulation strategies for subunit antigens and immunostimulators. *Pharmaceutics*. 2016;8(2016):1–22.
137. Shanmugasundaram U, et al. Pulmonary Mycobacterium tuberculosis control associates with CXCR3-and CCR6-expressing antigen-specific Th1 and Th17 cell recruitment. *JCI Insight*. 2020;5(14), e137858.
138. Didierlaurent AM, et al. Adjuvant system AS01: helping to overcome the challenges of modern vaccines. *Expert Rev Vaccines*. 2017;16(1):55–63.
139. Tait DR, et al. Final analysis of a trial of M72/AS01E vaccine to prevent tuberculosis. *N Engl J Med*. 2019;381(25):2429–2439.
140. Aboutorabian S, et al. A high ratio of IC31(®) adjuvant to antigen is necessary for H4 TB vaccine immunomodulation. *Hum Vaccin Immunother*. 2015;11(6):1449–1455.
141.. Coler RN, et al. The TLR-4 agonist adjuvant, GLA-SE, improves magnitude and quality of immune responses elicited by the ID93 tuberculosis vaccine: first-in-human trial. *npj Vaccines*. 2018;3(1):34.
142.. Day, T.A., et al., Safety and immunogenicity of the adjunct therapeutic vaccine ID93 + GLA-SE in adults who have completed treatment for tuberculosis: a randomised, double-blind, placebo-controlled, phase 2a trial. Lancet Respir Med, 2021. 9(4): p. 373–386.
143. Sagawa ZK, et al. Safety and immunogenicity of a thermostable ID93 + GLA-SE tuberculosis vaccine candidate in healthy adults. *Nat Commun*. 2023;14(1):1138.
144.. van Dissel JT, et al. A novel liposomal adjuvant system, CAF01, promotes long-lived Mycobacterium tuberculosis-specific T-cell responses in human. *Vaccine*. 2014;32(52):7098–7107.
145. Raviv SA, et al. Lung targeted liposomes for treating ARDS. *J Control Release*. 2022;346:421–433.
146.. Lopez L, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in the staff of a public school system in the midwestern United States. *PLoS One*. 2021;16(6), e0243676.
147. Yu Y, Qiu L. Nanotherapy therapy for acute respiratory distress syndrome: a review. *Front Med*. 2024;11.
148.. Stern, A., et al., Corticosteroids for pneumonia. Cochrane Database Syst Rev, 2017 (12).

149. Oray M, et al. Long-term side effects of glucocorticoids. *Expert Opin Drug Saf*. 2016; 15(4):457–465.
150. Gaspari V, et al. Side effects of ruxolitinib in patients with SARS-CoV-2 infection: two case reports. *Int J Antimicrob Agents*. 2020;56(2), 106023.
151.. Sokary S, Ouagueni A, Ganji V. Intravenous ascorbic acid and lung function in severely ill COVID-19 patients. *Metabolites*. 2022;12(9), 865.
152. Truwit JD, et al. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. *JAMA*. 2019;322(13): 1261–1270.
153. Zhang Y, et al. Effects of N-acetylcysteine treatment in acute respiratory distress syndrome: a meta-analysis. *Exp Ther Med*. 2017;14(4):2863–2868.
154.. Tenório MCdS, et al. N-acetylcysteine (NAC): impacts on human health. *Antioxidants*. 2021;10(6), 967.
155. Mechineni A, Kassab H, Manickam R. Remdesivir for the treatment of COVID 19: review of the pharmacological properties, safety and clinical effectiveness. *Expert Opin Drug Saf*. 2021;20(11):1299–1307.
156.. Gupta B, et al. Role of nebulized heparin in clinical outcome of COVID-19 patients with respiratory symptoms: a systematic review. *Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine*. 2023;27(8):572.
157. Dixon B, et al. Nebulised heparin for patients with or at risk of acute respiratory distress syndrome: a multicentre, randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Respir Med*. 2021;9(4):360–372.
158.. Allam V, et al. Nutraceuticals and mitochondrial oxidative stress: bridging the gap in the management of bronchial asthma. *Environ Sci Pollut Res Int*. 2022;29(42): 62733–62754.
159.. Taylor-Blair HC, et al. The impact of airborne particulate matter-based pollution on the cellular and molecular mechanisms in chronic obstructive pulmonary disease (COPD). *Sci Total Environ*. 2024;954, 176413.
160. Bhat AA, et al. The impact of formaldehyde exposure on lung inflammatory disorders: insights into asthma, bronchitis, and pulmonary fibrosis. *Chem Biol Interact*. 2024;394, 111002.
161.. Louie S, et al. The asthma-chronic obstructive pulmonary disease overlap syndrome: pharmacotherapeutic considerations. *Expert Rev Clin Pharmacol*. 2013;6 (2):197–219.
162. Ahmad A. Pharmacological strategies and recent advancement in Nano-drug delivery for targeting asthma. *Life (Basel)*. 2022;12(4).
163.. Ren J, et al. Intranasal delivery of MSC-derived exosomes attenuates allergic asthma via expanding IL-10 producing lung interstitial macrophages in mice. *Int Immunopharmacol*. 2021;91, 107288.
164. Theoharides TC. Ways to address perinatal mast cell activation and focal brain inflammation, including response to SARS-CoV-2, in autism Spectrum disorder. *J Pers Med*. 2021;11(9).
165.. Xu Y, Liu H, Song L. Novel drug delivery systems targeting oxidative stress in chronic obstructive pulmonary disease: a review. *J Nanobiotechnology*. 2020;18(1): 145.
166.. Liu G, et al. Adverse roles of mast cell chymase-1 in COPD. *Eur Respir J*. 2022;60 (6).
167. Montuschi P, et al. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med*. 2000; 162(3 Pt 1):1175–1177.
168.. Maziak W, et al. Exhaled nitric oxide in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1998;157(3 Pt 1):998–1002.
169. Paudel KR, et al. Zerumbone liquid crystalline nanoparticles protect against oxidative stress, inflammation and senescence induced by cigarette smoke extract in vitro. *Naunyn Schmiedeberg's Arch Pharmacol*. 2024;397:2465–2483.
170.. Nucera F, et al. Role of oxidative stress in the pathogenesis of COPD. *Minerva Med*. 2022;113(3):370–404.
171. Paudel KR, et al. Editorial: advanced therapeutic delivery for the management of chronic respiratory diseases. *Front Med (Lausanne)*. 2022;9, 983583.
172.. Mehta M, et al. Cellular signalling pathways mediating the pathogenesis of chronic inflammatory respiratory diseases: an update. *Inflammopharmacology*. 2020;28(4): 795–817.
173. Honmane S, et al. Lung delivery of nanoliposomal salbutamol sulfate dry powder inhalation for facilitated asthma therapy. *J Liposome Res*. 2019;29(4):332–342.
174. Chen X, et al. Liposomes prolong the therapeutic effect of anti-asthmatic medication via pulmonary delivery. *Int J Nanomedicine*. 2012;7:1139–1148.
175. Elhissi A, et al. Development and characterisation of freeze-dried liposomes containing two anti-asthma drugs. *Micro Nano Lett*. 2010;5(3):184–188.
176. Konduri KS, et al. Efficacy of liposomal budesonide in experimental asthma. *J Allergy Clin Immunol*. 2003;111(2):321–327.
177.. Ng ZY, et al. Assessing the potential of liposomes loaded with curcumin as a therapeutic intervention in asthma. *Colloids Surf B: Biointerfaces*. 2018;172:51–59.
178. Alberca-Custodio RW, et al. Allergen-specific immunotherapy with liposome containing CpG-ODN in murine model of asthma relies on MyD88 signaling in dendritic cells. *Front Immunol*. 2020;11, 692.
179. Alvarez MJ, et al. Liposome-entrapped D. Pteronyssinus vaccination in mild asthma patients: effect of 1-year double-blind, placebo-controlled trial on inflammation, bronchial hyperresponsiveness and immediate and late bronchial responses to the allergen. *Clin Exp Allergy*. 2002;32(11):1574–1582.
180. Li Q, et al. Preparation of a sustained-release nebulized aerosol of R-terbutaline hydrochloride liposome and evaluation of its anti-asthmatic effects via pulmonary delivery in Guinea pigs. *AAPS PharmSciTech*. 2018;19(1):232–241.
181. Manconi M, et al. Chitosan and hyaluronan coated liposomes for pulmonary administration of curcumin. *Int J Pharm*. 2017;525(1):203–210.
182.. Kokkinis S, et al. Liposomal curcumin inhibits cigarette smoke induced senescence and inflammation in human bronchial epithelial cells. *Pathol Res Pract*. 2024;260, 155423.
183.. Patel VK, et al. Curcumin liposomes attenuate the expression of cigarette smoke extract-induced inflammatory markers IL-8 and IL-24 in vitro. *EXCLI J*. 2024;23: 904–907.
184.. Malyla V, et al. Recent advances in experimental animal models of lung cancer. *Future Med Chem*. 2020;12(7):567–570.
185.. Sahu P, et al. Pre-clinical lung squamous cell carcinoma mouse models to identify novel biomarkers and therapeutic interventions. *Front Oncol*. 2023;13, 1260411.
186.. Thapa R, et al. Influence of gut and lung dysbiosis on lung cancer progression and their modulation as promising therapeutic targets: a comprehensive review. *MedComm*. 2024;5(12), e70018.
187. Miller KD, et al. Cancer treatment and survivorship statistics, 2016. *CA Cancer J Clin*. 2016;66(4):271–289.
188.. Kocher F, et al. Longitudinal analysis of 2293 NSCLC patients: a comprehensive study from the TYROL registry. *Lung Cancer*. 2015;87(2):193–200.
189. Cagle PT, Allen TC, Olsen RJ. Lung cancer biomarkers: present status and future developments. *Arch Pathol Lab Med*. 2013;137(9):1191–1198.
190. Lindeman NI, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Mol Diagn*. 2013;15(4):415–453.
191. Lindeman NI, et al. Updated molecular testing guideline for the selection of lung Cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Mol Diagn*. 2018;20(2):129–159.
192. Paudel KR, et al. Targeting lung cancer using advanced drug delivery systems. In: *Targeting Chronic Inflammatory Lung Diseases Using Advanced Drug Delivery Systems*. Elsevier; 2020:493–516.
193. Kumari V, et al. An overview of biomedical applications for gold nanoparticles against lung cancer. *Journal of Drug Delivery Science and Technology*. 2023;86:1–15.
194.. Kokkinis S, et al. Liposomal encapsulated curcumin attenuates lung cancer proliferation, migration, and induces apoptosis. *Heliyon*. 2024;10(19).
195. Singh AP, et al. Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. *Signal Transduct Target Ther*. 2019;4(1):33.
196. Chen J, et al. Recent advances and clinical translation of liposomal delivery systems in cancer therapy. *Eur J Pharm Sci*. 2024;193, 106688.
197. van der Meel R, et al. Smart cancer nanomedicine. *Nat Nanotechnol*. 2019;14(11): 1007–1017.
198.. Meel MH, et al. MEK/MELK inhibition and blood–brain barrier deficiencies in atypical teratoid/rhabdoid tumors. *Neuro-oncology*. 2020;22(1):58–69.
199. Zhao Z, et al. Research progress and prospect of nanoplatforms for treatment of oral cancer. *Front Pharmacol*. 2020;11, 616101.
200. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. 2005;4(2):145–160.
201.. Chen CK, et al. Urine biopsy technologies: Cancer and beyond. *Theranostics*. 2020; 10(17):7872.
202. Kong L, et al. RPV-modified epirubicin and dioscin co-delivery liposomes suppress non-small cell lung cancer growth by limiting nutrition supply. *Cancer Sci*. 2020; 111(2):621–636.
203. Storm G, et al. Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. *Adv Drug Deliv Rev*. 1995;17(1):31–48.
204.. Buch, P.J., Y. Chai, and E.D. Goluch, Treating polymicrobial infections in chronic diabetic wounds. Clin Microbiol Rev, 2019. **32**(2): p. https://doi.org/10.1128/cmr. 00091-18.
205. Sharma A, et al. Advances in lung Cancer treatment using nanomedicines. *ACS Omega*. 2023;8(1):10–41.
206. Younis MA, et al. Clinical translation of nanomedicines: challenges, opportunities, and keys. *Adv Drug Deliv Rev*. 2022;181, 114083.
207. Li Y, et al. Aromatized liposomes for sustained drug delivery. *Nat Commun*. 2023; 14(1):6659.
208. Chen Z-J, et al. Nanobowl-supported liposomes improve drug loading and delivery. *Nano Lett*. 2020;20(6):4177–4187.
209. Fernandez-Fernandez A, Manchanda R, Kumari M. Lipid-engineered nanotherapeutics for cancer management. *Front Pharmacol*. 2023;14:1–18.
210.. Đorđević S, et al. Current hurdles to the translation of nanomedicines from bench to the clinic. *Drug Deliv Transl Res*. 2022;12(3):500–525.
211. Liu P, Chen G, Zhang J. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. *Molecules*. 2022;27(4).
212. Basak S, Das TK. Liposome-based drug delivery systems: from laboratory research to industrial production—instruments and challenges. *ChemEngineering*. 2025;9(3), 56.
213. Zahednezhad F, et al. Liposome and immune system interplay: challenges and potentials. *J Control Release*. 2019;305:194–209.
214. Sercombe L, et al. Advances and challenges of liposome assisted drug delivery. *Front Pharmacol*. 2015;6, 286.
215.. León Blanco JM, et al. Artificial neural networks as alternative tool for minimizing error predictions in manufacturing ultradeformable nanoliposome formulations. *Drug Dev Ind Pharm*. 2018;44(1):135–143.
216.. Huang SM, et al. RSM and ANN modeling-based optimization approach for the development of ultrasound-assisted liposome encapsulation of piceid. *Ultrason Sonochem*. 2017;36:112–122.

217.. Džeroski, S., P. Panov, And B. Ženko, Machine learning ensemble methods in. 2009.
218. Neumann D, Merkwirth C, Lamprecht A. Nanoparticle design characterized by in silico preparation parameter prediction using ensemble models. *J Pharm Sci*. 2010; 99(4):1982–1996.
219. Hoseini B, et al. Application of ensemble machine learning approach to assess the factors affecting size and polydispersity index of liposomal nanoparticles. *Sci Rep*. 2023;13(1):18012.
220. Eugster R, et al. Leveraging machine learning to streamline the development of liposomal drug delivery systems. *J Control Release*. 2024;376:1025–1038.
221. Rebollo R, et al. Microfluidic manufacturing of liposomes: development and optimization by Design of Experiment and Machine Learning. *ACS Appl Mater Interfaces*. 2022;14(35):39736–39745.
222.. Rudokas, M., et al., Liposome delivery systems for inhalation: a critical review highlighting formulation issues and anticancer applications. Med Princ Pract, 2016. **25 Suppl 2**(Suppl 2): p. 60–72.