Annu. Rev. Biomed. Eng. 2024. 26:X–X
https://doi.org/10.1146/annurev-bioeng-110122-010848

Recent Developments in Aerosol Pulmonary Drug Delivery: New Technologies, New Cargos, and New Targets

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Keywords

aerosol, inhalation, inhaled biologics, inhaled vaccines, nanoparticle

Abstract

There is nothing like a global pandemic to motivate the need for improved respiratory treatments and mucosal vaccines. Stimulated by the COVID-19 pandemic, pulmonary aerosol drug delivery has seen a flourish of activity, building on the prior decades of innovation in particle engineering, inhaler device technologies, and clinical understanding. As such, the field has expanded into new directions and is working toward the efficient delivery of increasingly complex cargos to address a wider range of respiratory diseases. This review seeks to highlight recent innovations in approaches to personalize inhalation drug delivery, deliver complex cargos, and diversify the targets treated and prevented through pulmonary drug delivery. We aim to inform readers of the emerging efforts within the field and predict where future breakthroughs are expected to impact the treatment of respiratory diseases.

INTRODUCTION

The Burden of Lung Disease and the Need for Inhalation Treatment

When the coronavirus disease 2019 (COVID-19) pandemic brought the world to a standstill, it directed popular attention to the burden of respiratory illnesses and the challenges associated

with treating them. However, the global burden of lung-related illness long predated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and COVID-19. Since at least as early as 1990, lung-related illnesses have led in global cause of death, with chronic obstructive pulmonary disease (COPD) consistently ranking in the top four causes of mortality (1, 2). Moreover, rates of COPD are expected to rise because smoking, pollution, and exposure to biomass fuels all contribute to increased incidence of the disease, making it a concern in countries of all stages of economic development. COPD encompasses chronic bronchitis, obstructive sleep apnea, and emphysema, most commonly observed in individuals over the age of 40. Chronic bronchitis results in restricted airflow through a decrease in airway diameter and an increase in the production of mucus. Emphysema, on the other hand, exhibits a characteristic increase in lung volume by the destruction of the tissue separating gas exchange regions, which limits necessary oxygen transfer (3). The Global Initiative for Obstructive Lung Disease reports the disparity in COPD burden and treatment priority, citing the United Nations' admission that attention to noncommunicable diseases has been inadequate (1). Further complicating the treatment of this disease is the change in airflow distribution in affected patients, with air being skewed away from parts of the lung with the greatest possible ventilation and gas exchange (4). What makes COPD exemplary in pulmonary health is that it progresses over a patient's life, and it exhibits regional effects in the lung. Lung cancer, asthma, idiopathic pulmonary fibrosis, cystic fibrosis, interstitial lung disease, pulmonary hypertension, and infections such as tuberculosis and pneumonia may be acute or chronic conditions but similarly exhibit a spectrum of health effects and regional association. As a result, effective targeting is a critical challenge in addressing lung health. These facts highlight the imperative need for more advanced design and administration of inhalable therapeutics to treat respiratory illnesses as a matter of global importance.

The lung acts as a direct interface between the outside world and the rest of the body, due to its intimate connection with the circulatory system and primary function for gas exchange. As a result, inhalable therapeutics are dually advantageous in the potential for administration at the site of interest, limiting unintended off-target effects, as well as in the potential to deliver therapeutics systemically. Indeed, aerosol administration to the lung can result in as much as 100 times higher local drug concentrations when compared with systemic or oral delivery of the same molecule, dramatically increasing efficacy. Inhaled formulations limit off-target side effects,

avoid first-pass metabolism, and can provide superior patient compliance by avoiding needles and cold chain storage (5–7). Despite these benefits, many persistent technical challenges, regulatory hurdles, and public adoption roadblocks keep aerosol inhalation from being the first-line therapy for many respiratory conditions.

Emerging Paradigms in Inhalation Medicine

Although inhaled medicines have been used in practice since ancient times, the engineered study of their efficacy did not begin until the 1950s (8). Modern advances in inhaler design and more than 230 combinations of devices and therapeutics highlight the field's activity. Devices that are used to deliver inhalable therapeutics in a practical setting for patients generally fall into one of two classes: inhaler or nebulizer. Inhalers typically include dry powder inhalers (DPIs), metered dose inhalers (MDIs), and soft mist inhalers (SMIs). Atomization from a nebulizer usually results from an ultrasonic mechanism, vibrating mesh, or fluid jet. The primary difference between the device classes is the state of aerosol emitted, where DPIs emit solid particles and MDIs, SMIs, and nebulizers emit a cloud of liquid aerosol particles. All devices work with a variety of chemical compositions and can generate respirable aerosols with therapeutic effects. However, depending on the patient ability and dosage requirements, different devices may be more suitable for a given application (9).

Nebulizer: a powered inhalation system that generates a mist from a liquid reservoir for passive administration

Dry powder inhaler (DPI): a class of inhaler that uses breath activation to aerosolize a solid powder formulation into respirable aerosols

In this article, we seek to review the emerging paradigms within pulmonary drug delivery and highlight remaining opportunities in the field (**Figure 1**). First, we address the technologies and advances seeking to customize inhalation medicine, with new approaches aimed at increasing deposition efficiency in diseased lung regions in distinct patient populations. Next, we discuss changes to cargo complexity that have emerged within pulmonary drug delivery, expanding the scope of active pharmaceutical ingredients (APIs) delivered via inhalation. New particle and inhaler technologies have enabled the delivery of not only small-molecule but also macromolecule APIs, including nucleic acid, protein, antibody, and enzyme-based therapies. Accordingly, we discuss the advances in inhaled biologics (i.e., macromolecules such as protein, enzyme, and antibody APIs) and the emerging opportunity for inhaled nucleic acids for gene therapy. Finally, we address the diversified clinical targets being pursued in pulmonary drug

delivery, including inhalable oncolytic, vaccines, and antivirals. While the field of pulmonary drug delivery remains vast, we aim to highlight the most innovative advances over the past decade and predict where the next breakthroughs in pulmonary medicine will emerge.

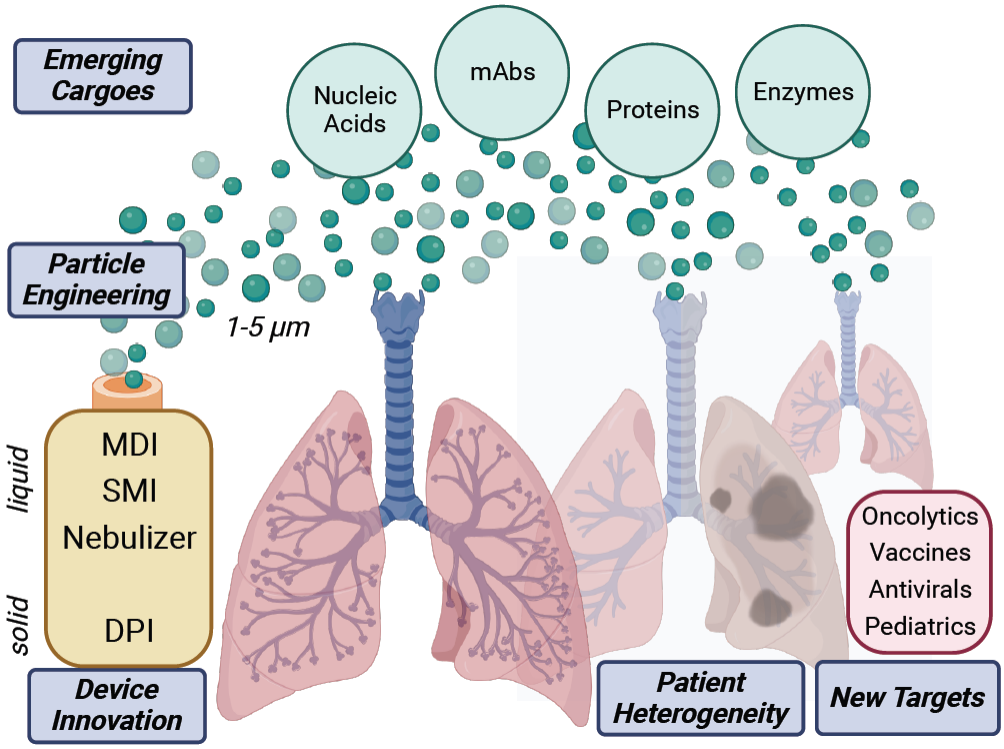

Figure 1 Emerging paradigms of pulmonary drug delivery. Enabling innovations of new cargo modalities, particle engineering technologies, device innovations, and appreciation of patient heterogeneity have led to significant advances in the field of pulmonary drug delivery toward a broader range of therapeutic targets impacting the lung. Abbreviations: DPI, dry powder inhaler; MDI, metered dose inhaler; SMI, soft mist inhaler.

FUNDAMENTALS OF INHALATION DELIVERY

The Lung as an Engineering Feat: Physical Barriers Designed to Keep Aerosols Out

The primary function of the lung is to exchange oxygen and carbon dioxide as required by cellular respiration. It must effectively bring in oxygen from the ambient air and quickly distribute it throughout the body, while simultaneously expelling waste carbon dioxide. Thus, the lungs are intimately connected to the circulatory system as the means of distribution, together forming the cardiopulmonary system. However, because of this close connection between the outside world and the core body, the respiratory system has the additional major functions of

filtering out debris and pathogens, as well as warming and humidifying the incoming air, to prepare it for contact with cells and tissues. These functions are the driving force for the intricate geometry and composition of the lungs.

The lung airways start with the trachea, which bifurcates into the right and left main bronchi at the anatomical feature known as the carina, midway down the thoracic cavity. This is the first point of separation into the macrostructural hierarchy of the right and left lung. Each main bronchus further bifurcates in a symmetric fashion, into the lobar bronchi and again into the segmental bronchi (**Figure 2*a***). These upper airway divisions are used to identify the main airway groups within each lung, referred to as lobes. In the right lung, there are three main lobes—upper, middle, and lower—whereas in the left lung, there are only two—upper and lower. Despite this large-scale regional discretization throughout the lungs, the airway-level structure is similar across common points between the regions, with each passage giving rise to two secondary airways that are smaller in both length and diameter. As a result, the structure and dimensions of the airways are commonly described by tracing sequential bifurcations and naming each subsequent airway branches with an increasing generation number. It is commonly asserted that the adult human lung contains 23 generations beginning with G0 at the trachea and increasing sequentially with each bifurcation until G23 at the alveoli, which are the terminal airway sacs that are roughly spherical in shape and surrounded by a thin epithelial barrier where gas is exchanged with the circulatory system. However, the number of generations may vary depending on the lobe of interest and patient characteristics, and, as such, the true number of generations between an alveolus and the trachea may range from 18 to 30. Regardless, the first ~18 generations function as the conducting airways, responsible for rapidly transporting air in and out of the lung, while the remaining ~5 generations function as the respiratory airways where gas exchange actually occurs. The length scales across these generations span many orders of magnitude throughout the lung; the trachea is roughly 20 mm in diameter and 120 mm in length, while each alveolar sac is roughly 300 μm in diameter. As a result of this complex structure and more than 450 million alveoli (10), the lung has a total surface area and airspace volume on the order of 80 m^2 and 5 L (11). Collectively, these unique structural features dictate fluid dynamics within the airspace, influencing physiological functions and the effectiveness of delivering inhalable therapeutics.

Generation number: a numerical nomenclature to describe the position of airway branches

relative to the trachea; the trachea begins at generation 0 (G0), and each subsequent dichotomous branch increases in number

Alveoli: balloon-shaped structures located at the end of the respiratory tract where gas exchange occurs

Conducting airways: airway segments comprising the trachea, the bronchi, and the bronchioles that function to warm and humidify inspired air and distribute it to the gas-exchanging zone of the lung

Respiratory airways: airway segments comprising the respiratory bronchioles, alveolar ducts, and alveolar sacs that facilitate gas exchange with the vasculature

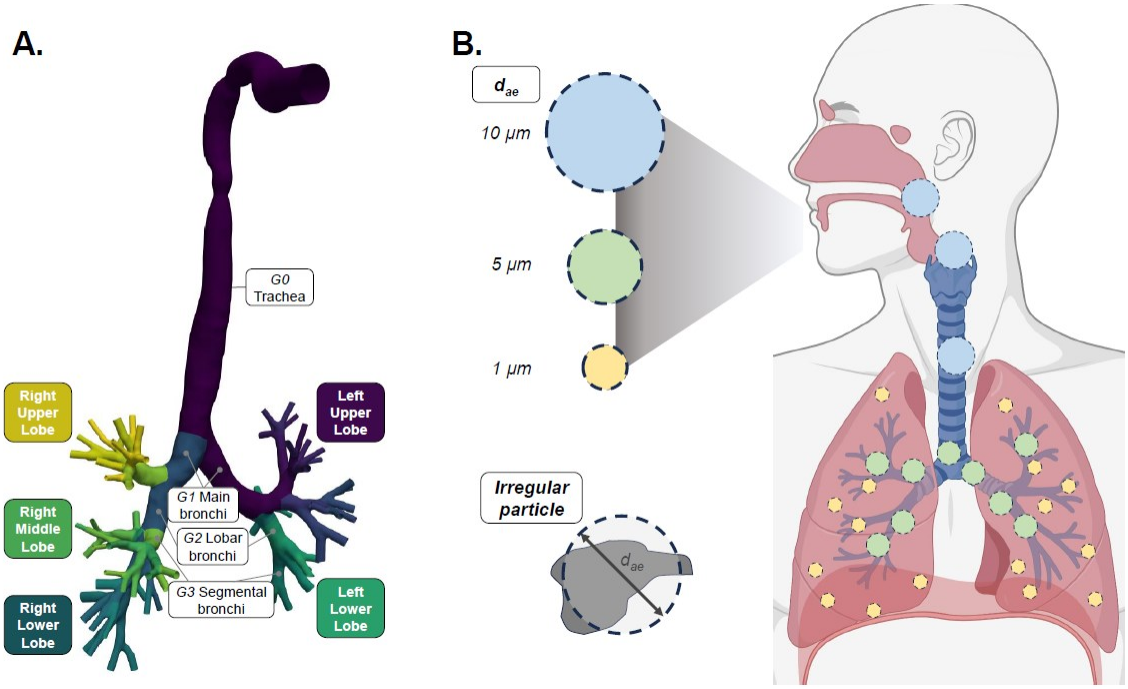

Figure 2 (*a*) Structure of the upper airway of the human lung. Mouth inlet is idealized, while the trachea and bronchi are obtained from a healthy adult male (12). Generations 0–3 (G0–G3) are labeled, and representative generations are indicated for the lobar and segmental bronchi. (*b*) Diagram depicting the role of aerodynamic diameter (d_{ae}) in generational deposition within the lung, where ~10-µm aerosols deposit in the oropharynx and trachea, ~5-µm aerosols deposit in the upper conducting airways, and ~1-µm aerosols reach the respiratory airways (6).

It is important to note that these values should be considered approximate for an adult male, and, in reality, the dimensions of the airways change dynamically depending on patient factors, environmental conditions, the course of breathing, state of health, and age. Certainly, anatomic differences arising during development lead to smaller resting tidal volumes for pediatric patients (~5 mL for newborns) compared with adults (~500 mL for males and 400 mL for females) (13).

Moreover, the overall volume of air exchange distributes unevenly between the five lobes (4, 14). The anatomy of the upper airways, including the trachea and the first few generations, shows an especially impactful degree of interpatient variability (15, 16). This variation goes beyond differences in airway length or diameter and may include presence, absence, or relocation of auxiliary airways, with again significant influences on aerosol transport through these airspaces.

Tidal volume: the total amount of air inhaled or exhaled in a single breathing cycle

Distributing Aerosols to the Lung: Relevant Engineering Phenomena in Modern Applications

These scales and dynamic breathing profiles generate a range of complex, multiphase phenomena that make predicting transport of inhaled therapeutics challenging. Accordingly, inhalable formulations are typically characterized in terms of a particle size distribution based on aerodynamic diameter (d_{ae}), the diameter of a unit density sphere with equivalent settling velocity as the aerosol, as depicted in **Figure 2*b*** (17). Deposition will be influenced by d_{ae} as well as by the local airway flow properties; while turbulent and transitional flows occur in the first few generations, the many bifurcations drastically disperse the local velocities in subsequent generations, leading to low-Reynolds-number developing flows (18). Accordingly, common understanding dictates that aerosols greater than 5 μm tend to deposit primarily by impaction in the bronchi or extrathoracic airways, including the mouth, throat, and larynx. Sedimentation or gravitational settling is the dominant mechanism of deposition for aerosols ~0.5–8 μm and occurs from the influence of gravity; deposition in this size range occurs mainly between the bronchioles and alveoli, with some deposition in the extrathoracic airways. Finally, aerosols < 0.1 μm deposit primarily through diffusion by Brownian motion allowing them to depart from local streamlines and contact the wall, if they are not exhaled before deposition can occur (17, 18). Based on these dominating mechanisms, aerosols between 1–5 μm can deposit at reasonably high efficiencies within the lung and are thought to be the ideal size range for inhaled therapeutics (6, 18).

Breathing profile: the volumetric flow rate over time during a complete cycle of inspiration and expiration, as measured at the mouth; these profiles can be used to establish the tidal volume

Aerodynamic diameter (d_{ae}): the diameter of a unit density sphere with settling velocity equivalent to the aerosol; used to approximate the aerodynamic properties of an aerosol regardless of differences in density, shape, or surface roughness

Thus, characterization of d_{ae} is a mainstay for modern aerosol formulations. Two industry-

standard devices for characterizing formulations by aerosol-generating devices are the Anderson Cascade Impactor and the Next Generation Impactor (NGI) (19). They are designed to separate formulations at physiologically relevant gas flow rates, with the resulting size distribution used to predict the efficiency of pulmonary delivery. The NGI was developed to address the inadequacies of previous impactors, although it is worth noting that both devices fall short in their ability to recreate anatomical geometry, physiological function, and patient-specific metrics, limiting their ability to generate accurate predictions of aerosol deposition (19). Through use of breath simulators and updated protocols, the characterization of the aerosol size distribution through the NGI can yield important predictive properties of the formulation, including emitted dose (the fraction of drug that leaves the device), deposited dose (the fraction of drug that deposits within the lung), and fine particle fraction (FPF) (the fraction of aerosol with a d_{ae} less than 5 μm). Historically, characterization of d_{ae} and FPF have been the main metrics to develop correlations between the aerodynamic particle size distribution and the ultimate fate of a particle in the lungs – termed in vitro–in vivo correlation (IVIVC) (20).

In vitro–in vivo correlation (IVIVC): the ability of in vitro preclinical assessments to predict in vivo clinical responses

Recently, the concept of the FPF has been called into question because it fails to fully describe the characteristics of a formulation, leading to variable results between devices and typically **[**AU: Edit OK? Or change to "general overestimates" or ", in general, overestimates"? Or delete the word "generally"?**]**overestimates total lung deposition (21). In response, a relatively new metric has been proposed based on the method of Efficient Data Analysis (22). In this approach, the small particle mass (SPM) and large particle mass (LPM) are considered along with their ratio (LPM/SPM) to describe the formulation by aerodynamic particle size. This metric has been shown to be insensitive to decision of the cutoff between SPM and LPM for formulations with mass median aerodynamic diameter (MMAD) between 0.3 and 3 μm (22).

Mass median aerodynamic diameter (MMAD): the median aerodynamic diameter of a formulation, by mass; depends on the formulation chemistry and device emission characteristics

Biological Barriers and Targets

Besides the geometry of the lung, environmental composition plays an important role in conditioning the air that enters the airways and reaches the alveoli. While symptoms of disease can manifest in macroscopic changes to the airway that result in airway constrictions,

obstructions, or deterioration (i.e., airway remodeling), disease effects are also observed at the microscopic level in the airway liquid lining fluid. Mucus lines the airways from the trachea to the terminal bronchioles. The conducting airways transition to the respiratory airways over a series of branch points, along which progressively more alveoli populate the airway walls. The alveoli consist of alveolar type I (ATI) and alveolar type II (ATII) epithelial cells; ATI cells are highly elongated epithelial cells that enable gas exchange, while ATII cells secrete pulmonary surfactant. Pulmonary surfactant is distinctly different from airway mucus in that its primary purpose is to maintain equilibrium of the alveoli; the expansion and contraction of the lung leads to volume changes within the alveoli, and the presence of surfactant maintains sufficient surface tension to prevent alveolar collapse (13). Thus, inhaled formulations must penetrate the liquid lining fluid, which is predominantly mucus throughout the conducting airways and is rich in surfactant in the lower respiratory airways.

Airway remodeling: a broad term that describes a change in composition, distribution, thickness, stiffness, and/or number of structural components in the airway due to disease relative to a healthy lung

Not only do inhaled formulations have to navigate the complex airway structure, overcome drastic changes in humidity, and penetrate the liquid lining layer but also they have to contend with cellular barriers of the lung. Throughout the lung tissue, specialized immune cells programmed with the defense of the airspace work to maintain homeostasis, clear inhaled particulates, and mount local immune responses as necessary. As immune cells such as alveolar macrophages, CD11b and CD103 dendritic cells, and interstitial macrophages are tissue-resident cells that are implicated in a range of respiratory diseases (23), they represent burgeoning targets for emerging inhaled therapeutics (24).

PERSONALIZING INHALATION

Delivering aerosols to the necessary site of action within the lung remains one of the largest challenges to pulmonary drug delivery. For many decades, inhalation therapy has used a one-size-fits-all approach, with the main goal being to successfully deliver a critical amount of aerosol to the respiratory tract. However, countless clinical measurements have highlighted both inter- and intrapatient heterogeneity that implies a need for further customization. For instance, the rate of inhalation and exhalation maneuvers, that is, the breathing profile, in a single patient can vary significantly from one breath to the next. Furthermore, breathing functional capacity,

measured by the forced expiratory volume in one second, can change by 60% within the first 20 years of life, vary by 30% between genders of the same age, and decrease by more than 80% for patients with COPD (25, 26). In this section, we address emerging trends within pulmonary drug delivery that have sought to customize inhalation therapeutics, taking into account the heterogeneity in patient anatomy, breathing profiles, and disease presentation.

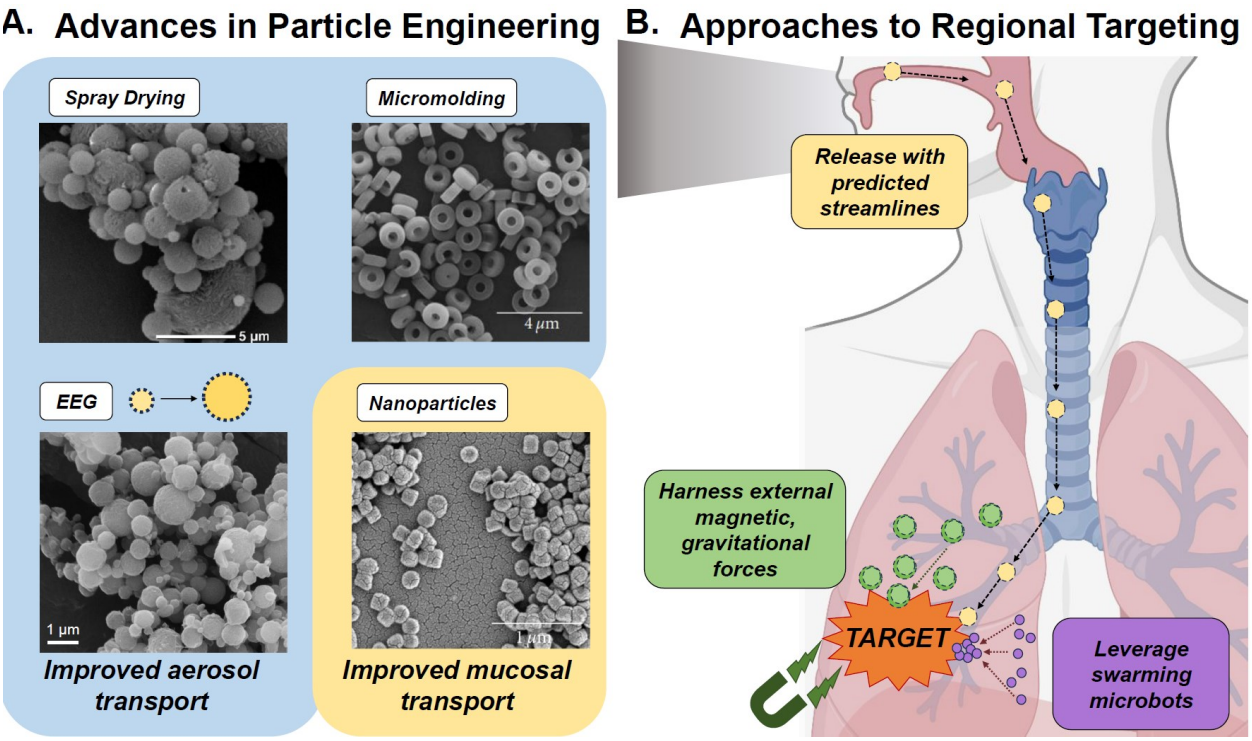

Figure 3 (*a*) Advances in particle engineering include approaches to improve aerosol transport efficiency, including spray drying, micromolding, and excipient enhanced growth (EEG). The use of nanoparticles aims to improve mucosal penetration. Scanning electron micrographs (SEMs) show relative differences of representative formulations from each particle engineering approach. Panel SEM images adapted with permission from References 27–29. (*b*) Regional targeting attempts to deliver aerosols to a target site within the lung that may require lobe- and generation-specific targeting. Approaches for regional targeting are highlighted and include (❶) controlled release positions of aerosols entering the mouth to follow streamlines toward the target location, (❷) use of external forces such as an applied magnet or gravity to direct responsive aerosols to the target, and (❸) design of active microbots that self-assemble after deposition and swarm toward the target location, often with assistance from an external magnetic force.

Enabling Particle Engineering Innovations

The advent of DPIs has offered distinct new opportunities for particle engineering to transform the landscape of inhaled therapeutics, leading to more opportunities to customize delivery

approaches for different applications. Commonly fabricated through spray-drying approaches, dry powder formulations offer patient-actuated control over aerodynamic properties, meaning that patients themselves provide the energy through inspiration that aerosolizes the formulation. Spray drying leads to potentially inexpensive delivery that is compatible with a range of therapeutic modalities delivered at higher efficiencies (30). Recent advances in formulation and packaging approaches enable high therapeutic loadings and long-term stability that avoids cold chain storage. Further particle engineering technologies afford distinct and dynamic control over desirable aerodynamic properties that afford efficient delivery to the lung, leading to enabling particle engineering innovations as discussed in the following. While particle engineering and nanotechnology have been long poised to impact aerosol drug delivery, the past ~5 years have seen tremendous strides in bringing these approaches to clinical success (**Figure 3*a***). Moreover, this heightened control over particle properties lends itself to customizing formulations to better treat heterogeneous patient populations.

The most common DPI particle engineering approaches leverage the scalable, cost-effective, and continuous process of spray drying. Liquid suspensions are atomized into a drying gas, leading to formation of dried particles. Controlling the atomization and drying process leads to control over the resultant particle size and shape, often yielding porous morphologies that are desirable for deep lung deposition. The major advantage of spray drying is the overall versatility, enabling formulation of small molecules, nucleic acids, biologics, and nanoparticle (NP) suspensions (31). However, the drying process can impact stability of thermosensitive cargos, such as biologics; accordingly, spray freeze drying and thin-film freezing have recently emerged as methods to produce high-stability powders for biopharmaceutical delivery (32, 33).

Further control over particle shape, which influences aerodynamics, can be obtained through micromolding techniques, such as PRINT® (particle replication in nonwetting templates), to create desirable inhalation powders (28, 34). Using the PRINT platform, a dry powder treprostinil product for pulmonary arterial hypertension demonstrated a more convenient alternative to nebulized delivery of the same molecule, along with a robust safety profile, in a phase 3 trial (35). These exciting results represent recently achieved clinical milestones that showcase the future opportunities for such precision aerosol powder formulations, leveraging a generic inhaler device.

Other emerging technologies leverage the high humidity of the airspace to induce dynamic

changes in the formulation to ensure deep airway penetration, macrophage avoidance, and high-efficiency deposition. Leveraging swellable hydrogel chemistries, swellable polymeric microparticles have been designed to exhibit volume median diameters on the order of 10 μm that swell to ~70 μm upon hydration, leading to sustained drug release up to 24 days and noticeably decreased macrophage clearance when compared with nonswelling particles (36–38). Excipient enhanced growth (EEG) similarly leverages the high humidity of the lung to induce particle swelling; EEG formulations contain a hygroscopic excipient (e.g., mannitol and sodium chloride) and an initial dry or liquid aerosol size of ~1 μm that rapidly swells within the high humidity of the conducting airways. This allows the initial aerosol to avoid deposition within the extrathoracic space and to deposit more efficiently within the lung (39, 40). EEG formulations have been shown to be especially useful for pediatric delivery (41, 42).

Excipient enhanced growth (EEG): a drug delivery approach that uses formulations containing a hydrophilic excipient additive that promotes aerosol swelling in the high humidity of the respiratory tract to increase aerosol deposition

Finally, in terms of enabling particle engineering technologies, NPs for inhalation remain a high-potential area (43). While these can be delivered as dry powder, advances in nebulizer technologies also afford efficient delivery. Respiratory-specific formulations have emerged in polymeric, liposomal, and lipid nanoparticle (LNP) platforms that show high potential for future translation. The COVID-19 pandemic certainly revitalized this field, with countless NP formulations being tested preclinically following direct airway administration, with a special focus on mRNA delivery. Indeed, within the past 5 years, degradable poly(β-amino ester) (44), poly(β-amino-thio-ester) (45), poly(amine-coester) (PACE) (46), various LNP formulations (47, 48), and even exosomes (49) have shown high lung biocompatibility in small animal models for delivery of inhalable mRNA cargos. This builds off of numerous NP advancements in aerosol drug delivery beginning from liposomes, which have been designed to carry antibiotic, antifibrotic, and antiviral cargos with many recent successes (50–52). Notably, the inhalable NP-based liposomal amikacin formulation Arikace® was approved to treat *Mycobacterium avium* complex lung disease as a part of a combination antibacterial drug regimen (53–55) and serves as a milestone for inhalable NPs.

Lipid nanoparticle (LNP): a nonviral delivery platform capable of delivering nucleic acids with successful transduction

Regional Targeting

Delivering aerosol to specific regions in the lungs is generally accomplished through generational targeting: the process of manipulating the d_{ae} such that aerosols deposit in the generation of interest (56). This is useful for airway diseases that present relatively uniform pathologies, such as asthma (57), and can be accomplished through some of the particle engineering approaches mentioned previously.

An emerging field of study is the delivery of aerosols to directed subregions within the lung, to address disease heterogeneities such as tumors, biofilms, or regions of airway remodeling (**Figure 3*b***, target). In such regional targeting approaches, aerosols are directed via external forces along lobar divisions with the attempt to increase drug concentrations in the lobe of interest (12, 58–60). Gravitational effects on the aerosol through controlled postures have been shown to direct aerosol deposition toward desirable lung regions (56). This involves orienting the patient during deposition to direct sedimentation toward regions of interest. Perhaps the most active area of investigation, the use of magnetically responsive particles and a controlled external magnetic field, has been proposed in numerous scenarios to increase regional targeting within the lung. Preclinical success have been demonstrated in directing aerosol deposition to the right or left lung in mice (59, 61), as well as in human-scale simulations demonstrating increased localization to a tumor in the upper airways (62). Recently, dynamic microbots, that is, synthetic drug carriers that perform programable actions under a controlled stimuli, were designed to self-assemble under magnetic stimuli following deposition, actively swarm together, and migrate to target regions within an in vitro 3D-printed lung model (63). These approaches provide significant proof of concept toward the use of magnetically responsive carriers to dynamically tune airway deposition. Other approaches to drive regional deposition involve controlling the release position at the mouth to direct aerosols to specific lobe locations, as demonstrated by computational models and in vitro 3D-printed lung airway models (12, 60, 64). This approach requires low inspiration flow rates to limit turbulence in the trachea and upper airway (12). A similar realization of this concept has been used to locally deliver chemotherapeutic dosages through a specially designed bronchoscope to only a portion of the airways (65).

Collectively, these emerging approaches afford distinct opportunities to increase drug localization in ways that can overcome disease pathophysiology. However, beyond clinical studies noting the role of posture on regional deposition, these approaches have yet to enter the clinic. Techniques requiring magnetic fields require preclinical testing in larger scale animals

and diverse target sites to ensure adequate penetration of the field throughout the lung tissue. Concerns over inhalation of magnetic carrier particles will also need to be overcome through rigorous biocompatibility studies for both acute and potential long-term effects of treatment. As with many nascent approaches in inhalation, careful consideration of high-impact therapeutic targets and delivered molecules will be needed to pursue clinical translation.

Pediatrics

The development of pediatric-specific therapeutics has been encouraged through legislation such as the Best Pharmaceuticals for Children Act of 2002 and the Pediatric Research Equity Act of 2003 (66). Accordingly, recent innovations to personalize aerosol delivery for children have emerged. Advances in anatomic-based models, both computational and experimental, have allowed for definition of updated aerosol requirements for pediatric patients (16, 67–69). This has brought about growing appreciation for the differences in anatomy between adults and children, especially within the upper respiratory tract during development, that leads to differential aerosol requirements (70). Indeed, while the overall anatomic dimensions of the airway increase in size as children age, additional developmental features further influence shapes, angles, and constrictions throughout the upper airways to impact deposition (16, 67). Certainly, inhalation protocols must also consider challenges surrounding pediatric dosing, including faster respiratory rates, lower tidal volumes, ability for physical coordination, and willingness to interface with this device (71, 72). Accordingly, these changes with age lead to significantly different requirements for aerosol deposition. In **Table 1**, we report impaction parameter, $d_{ae}^2 Q$, estimations to achieve 50% deposition efficiency within different age groups, based on the most established correlations at different ages (16, 69, 73, 74). The impaction parameter is a convenient way to compare deposition studies, as it accounts for both changes in d_{ae} as well as Q, the breathing flow rate. Comparing these across ages, we see orders of magnitude differences in expected $d_{ae}^2 Q$ values throughout childhood, leading to different operational ranges for pediatric patients (70, 75).

Table 1 Representative changes in patient lungs throughout development and the subsequent influence on inhaled drug delivery requirements

	Newborn	3-year-old	10-year-old	Adult	References
Breathing rate (breaths/min)	40–52	25–28	18–22	12–20	76, 77
d_{ae}^2Q corresponding to 50% deposition ($\mu m^2 \cdot cm^3/s$)	100	700	10,000	25,000	16, 69, 73, 74
Corresponding d_{ae} for 50% deposition at ~30 LPM inspiration (μm)	0.5	1.2	4.4	7.0	NA
Corresponding inspiration Q for 50% deposition at d_{ae} ~1 μm (LPM)	0.7	4.7	67.0	167.0	NA

Reference breathing rates shown across average clinical measurements for each age group. The impaction parameter, d_{ae}^2Q, is reported for deposition efficiencies of 50%. The bottom two rows provide examples of how this d_{ae}^2Q can be altered to achieve 50% deposition efficiencies: first by varying the d_{ae} at constant flow rate and second by varying the inspiration flow rate Q under constant particle size.

Abbreviations: LPM, liters per minute; NA, not applicable.

The past decade has seen renewed efforts in pediatric-specific guidelines and considerations that are applicable based on the age of the subject, as well as innovation in pediatric-specific devices, nasal delivery, and aerosol formulations. Notably, use of EEG formulations tested in pediatric in vitro airway models almost eliminates undesired extrathoracic delivery (78), demonstrating the utility of this approach to overcome the challenges in formulating small aerodynamic-sized particles needed for pediatric patients and enable surfactant delivery for pediatrics and neonates (79) that may lower dosage requirements (80). Aerosol delivery via noninvasive ventilation (NIV) to neonates has seen numerous recent efforts and clinical studies for delivery of surfactant, antibiotics, and corticosteroids, although resounding clinical successes have yet to be achieved (81). Advances in device synchronization and improved guidelines on positions of nebulizers within the NIV circuit are expected to lead to future improvements that customize delivery to this distinct population, as more efforts are needed.

Advancements in pediatric-specific formulations and therapeutic modalities remain limited by the difficulty of performing clinical studies in pediatric patients. New formulation and device approaches will benefit from the creation of advanced preclinical testing approaches, including both in silico and in vitro whole lung models, that can take into account pediatric-specific metrics (12, 16, 60, 75, 82).

ADVANCING CARGO COMPLEXITY

Enabled by advanced particle engineering and device designs, pulmonary drug delivery has expanded beyond small-molecule therapeutics to cargos of increasing complexity. These include biologics such as protein, enzyme, and antibody therapies, as well as growing studies using nucleic acids, expanding the scope of inhaled therapeutics to a broader range of respiratory diseases.

Inhaled Monoclonal Antibodies

The global biologics market is expected to grow more than 9% by 2029, with estimates approaching almost $600 billion (83). The inhalation portion of that market is expected to follow similar trends and see substantial growth over the next 10 years, with an estimated 400 molecules currently in the development pipeline. While many of these remain in preclinical testing, notable molecules have advanced to phase 1 and phase 2 clinical trials, as well as approvals for inhaled insulin (Afrezza®) (84) and the dornase alfa enzyme (Pulmozyme®) (85) that support feasibility. Emerging biological molecules include monoclonal antibodies (mAbs), enzymes, peptides, and protein therapeutics, with both nebulized and dry powder formulations in development that have been enabled by innovations in device and formulation technologies as discussed above.

Exciting developments over the past decade have seen the first clinical evaluations of mAbs via inhalation, with a large focus on asthma therapeutics. The anti-interleukin-13 (anti-IL-13) antibody fragment abrezekimab has demonstrated safety and tolerability as a dry powder formulation following single and repeated dosage up to 10 days in a randomized phase 1 clinical study on healthy and asthmatic patients (86). Ecleralimab, an anti–thymic stromal lymphopoietin neutralizing antibody fragment for asthma treatment, similarly showed a strong safety and tolerability in a phase 1 trial when given once daily for 12 weeks, as well as lowered bronchoconstriction in a phase 2a study of mild asthmatics (87). In preclinical studies,

aerosolized IL-4Ra antagonist elarekibep showed positive suppression of acute allergic asthma-like inflammation including eosinophilic contributions in a humanized mouse model to treat type 2 endotype asthma (88). Collectively, these mAb studies further support the safety of inhaled biologics and show strong promise for inhaled mAbs as potential transformative treatments for asthma and beyond.

While these preclinical and early-phase clinical studies signal significant emerging opportunity, such therapeutics continue to face notable challenges. The overall cost associated with mAb production, the relatively high required dosages, and the incomplete deposition efficiency of an inhaled product may limit the overall molecules that are pursued through this route. This also highlights the need for careful selection of targets, disease indications, and mAb molecules that are advanced to ensure successful product approval and patient adoption. The continued and growing interest of inhaled mAbs within both academia and industry signals an optimistic change in the inhalation field; a major blockbuster drug could open the floodgates for additional mAb products, as well as more exploratory cargos. However, the contrast is true as well; a major failure of a leading inhaled mAb product may signal a downturn for the inhalation field.

Inhaled Gene Delivery Vectors

Gene delivery to the lung has persisted as an aspirational goal for the field, as many respiratory diseases may be cured with local elimination of known genetic anomalies. While no inhaled gene therapies have reached clinical approval, work continues in this area to identify appropriate targets, platforms, and formulations suitable for delivery to the lung.

Viral-based vehicles remain at the forefront of investigation, owing to their superior transfection ability. Adeno-associated viruses (AAVs) are an attractive candidate for gene delivery, as this class of virus does not integrate in the host genome. Numerous clinical trials using an AAV2 vector encoding for the human cystic fibrosis transmembrane regulator, the underlying genetic defect that leads to disease in cystic fibrosis, have been performed. Both single- and multiple-dose studies have shown good tolerability but have failed to demonstrate significant improvements in lung function (89). Follow-up preclinical assessments suggest the importance of serotype evaluation for inhalation delivery; while AAV6 is able to penetrate the respiratory mucosa, AAV1 and AAV2 do not, limiting their translational potential (90). Notably, these studies were performed in mucosa collected from human patients, providing potential

insight to the lackluster results seen clinically with AAV2 in lung delivery. AAV6 efficiency was further enhanced through coformulation with extracellular vesicles (EVs), leading to improved transduction in ex vivo human mucus samples as well as in vivo following murine lung delivery (91); this approaches leverages the increased dual-penetration efficiency of both EVs and AAV6 through the mucosa. However, other AAV serotypes have shown preclinical successes; AAV5 delivery of a recombinant IL-4 was able to minimize effects of allergic asthma in a murine model (92). Collectively, these studies suggest that efficacy of AAV vectors is limited by the selection of serotype and its ability to successfully penetrate the mucus, making this a critical evaluation criteria for gene delivery vectors more broadly.

Inhaled delivery of both RNA and DNA cargos with nonviral vectors continues to be desirable to the field, given the opportunities to improve biocompatibility and tolerability in the lung. While naked nucleic acids can be delivered with some efficacy directly to the respiratory tract, delivery is enhanced by mucopenetrating particulate formulations. mRNA delivery especially has advanced over the past decade, with novel LNP (93–95) and polymeric formulations (96, 97) promoting efficient transduction for both nebulized and dry powder treatments of cystic fibrosis, idiopathic pulmonary fibrosis, and cancer. Delivery of plasmid DNA through nonviral platforms has also advanced through clinical testing, including polyplex (98) nebulization and liposome formulations (99), where the latter has advanced to phase 2b trials for cystic fibrosis treatment. Interestingly, inhalation of CRISPR/Cas9 plasmid DNA has also been evaluated through in vitro studies following delivery of a chitosan nanocomplex, which showed good mucus penetration and particle stability following nebulization (100). Despite the interest and growing body of preclinical approaches for nonviral gene therapy approaches, most of these studies remain in preclinical evaluation. Such platforms have yet to achieve transduction efficiencies in vivo reaching those of their viral counterparts, which remains a limiting challenge for this area. Moreover, development of new materials delivered to the lung coincides with general concerns for overall lung biocompatibility; thus, novel gene delivery vectors, as well as other drug delivery vehicles discussed in this review, will face significant regulatory evaluations for future translation. Such clinical evaluations will likely not be pursued without significant gains in preclinical mucosal penetration and transduction efficiency.

SHIFTING FOCUS TO NEW THERAPEUTIC TARGETS

Further enabled by new cargo modalities, pulmonary drug delivery has expanded to address a wide range of new therapeutic targets and to address complex local pathologies within the lung. The recent few years have seen a resurgence in innovative approaches to anticancer oncolytics, mucosal vaccines, antiviral treatments, and immune engineering (24) that are likely to reshape the pulmonary drug delivery landscape for years to come.

Inhaled Oncolytics

In 2020, lung cancer was the second-leading diagnosed cancer and, despite advances in screening and treatment options, remains the leading cause of deaths from cancer, with a low 5-year survival rate that has increased minimally in the past decades (101). The current standard method of treatment for lung cancer is a combination of surgery, radiation, and intravenous chemotherapeutics (102); however, it has been shown that inhalable therapeutics could improve on-target delivery, increase retention, and reduce off-target delivery (103, 104).

Systemically administered chemotherapy is limited by off-target dose-limiting toxicities, leading to treatment interruption and lowered therapeutic efficacy, which, importantly, can be overcome through direct administration via inhalation. However, for inhalation, the choice of drug to limit local adverse effects in the lung is critical for avoiding adverse pulmonary events. Clinical trials for inhaled chemotherapies have advanced through phases 1 and 2 that assess molecules such as doxorubicin (105), cisplatin (106, 107), and 9-nitrocamptothecin (108), among others, generally finding overall acceptable safety profiles in the limited patient population tested. Notably, cisplatin delivery using a lipid formulation has shown (106, 107) potential in early clinical studies; other particle engineering approaches have evaluated formulations for nebulized and dry powder delivery of the same molecule (109). In preclinical models, inhaled cisplatin has been shown to enhance the effects of traditionally administered immune therapy (110), while combination formulations of cisplatin and antitumor small interfering RNA (111) or alternative chemotherapy drugs (112) showed improved therapeutic benefit; these approaches may afford distinct combinatorial treatment modalities moving forward. With continued clinical investigations of these molecules ongoing, efficacy and approval of such approaches may represent a significant breakthrough for lung cancer treatments.

Immunotherapy and cancer vaccine treatments have also demonstrated benefits from local administration, largely in the preclinical space. Inhaled mAbs such as cetuximab, an anti–epidermal growth factor receptor, or G6–31, an anti–vascular endothelial growth factor, have

been well tolerated in macaques and show reduced tumor volumes and improved anticancer profiles (113, 114). Locally delivered checkpoint inhibitor antiprogrammed cell death protein ligand 1 (aPD-L1) formulations have shown selected tumor killing and strong cytotoxic T cell responses, leading to increased survival rates (115, 116). Modification of the local tumor immune environment has been demonstrated through delivery of stimulator of interferon genes (STING) agonists, which activate the immune system to an antitumor response, with effects observed both locally in the lung as well as in distal metastatic sites (117). Inhaled cytokines, such as IL-2 and interferon-γ, can further modify the local tumor immune microenvironment and have been shown to be feasible in early clinical studies (118). Locally administered NP cancer vaccines delivering a tumor-specific antigen and appropriate adjuvant generated a local population of effector memory T cells capable of tumor reduction in the lung (119), and similar cancer vaccines have also been demonstrated following local mRNA delivery (120). Collectively, this active research area points to numerous opportunities for future clinical translation.

For both small-molecule and biologic delivery for lung cancer, a major limiting factor to advancing these treatment modalities involves the late stage in which lung cancer is typically diagnosed, when the disease has already metastasized. Metastatic disease impacting multiple organ systems beyond the lung lowers the potential benefit of direct and restricted lung delivery, decreasing enthusiasm for the effort involved in developing inhaled lung cancer treatments. However, ongoing advances in lung cancer screening, early detection, artificial intelligence, biomarkers, and public health initiatives are poised to mitigate this current challenge (121). Thus, continued efforts in inhaled therapeutics now may be well timed for significant impact as diagnoses of earlier-stage lung cancer becomes more prevalent.

Vaccines

The successful mRNA-based LNP vaccines developed for SARS-CoV-2 serve as a major milestone for vaccine development. Despite their overall success, these vaccines yield limited mucosal protection at the site of infection in the lung and nasal passages, by virtue of their intramuscular administration (122). Lacking this mucosal protection, even vaccinated individuals are susceptible to airway infection and are more likely to transmit disease to others (123). Local delivery of vaccines through inhalation can overcome this challenge, providing organ-specific responses and universal protection across alternative mucosa for improved barrier protection

(124, 125). Indeed, experts have called for an Operation Nasal Vaccine to support the existing efforts to bring aerosol vaccination forward with the same rapid commitment as the first-generation vaccines to overcome this critical gap in protection (126).

Numerous aerosol vaccines have emerged throughout the development pipeline in response to the COVID-19 pandemic. Within the clinical sphere, between 20–30 aerosol candidates have reached human testing worldwide, with platforms ranging from live attenuated, viral vector, and protein subunit, among others (127). Notably, CanSino Biologics' Convidecia Air™ (an adenovirus type-5 vector) was approved in 2022 as an inhaled booster dose administered via nebulizer for oral inhalation (128). While data supporting the mucosal immune response in humans have yet to be reported, this concept of boosting with an inhalation vehicle has been demonstrated in small animal models using the stabilized SARS-CoV-2 spike protein and spike-encoding mRNA delivered via polymeric PACE NPs and has been shown to produce robust mucosal cellular and humoral responses (129). Hundreds of early-stage COVID-19 vaccines across a range of platforms remain in development for aerosol administration. One notable innovative example includes an intranasal vaccine that employs albumin hitchhiking for increased lymph node accumulation. These protein-lipid conjugates were designed to include the receptor binding domain (RBD) of the SARS-CoV-2 spike protein and show enhanced production of neutralizing antibody production in the lung when compared with the RBD alone in both mice and nonhuman primates (130). Other examples include local administration of lung cell-derived small EVs that outperformed protection of liposome vaccines when delivering recombinant RBD (131), a chitosan NP vaccine that delivers the spike protein (132), adenovirus type-5 vaccination encoding the spike protein (133), and LNP formulations developed specifically for respiratory tract delivery (134).

Certainly, these efforts accelerated aerosol vaccine development for many other respiratory pathogens. Innovative NP, viral, and virus-like platforms in both nebulizer and dry powder formulations have emerged for inhaled vaccination of tuberculosis (135–137), anthrax (138), and influenza (139), among many others. Recent work has shown that the deposition site in the lung may influence different vaccine platforms by altering overall immune response, pointing to a continued need to understand and tune airway deposition (140). As a further roadblock to more widespread adoption of inhaled vaccines, development of response-enhancing adjuvants approved specifically for the respiratory tract remains a major limitation; however, recent efforts

to develop novel inhaled adjuvants have started to emerge (141, 142).

Antiviral Approaches

COVID-19 also brought a resurgence in research surrounding local delivery of antiviral treatments. These included a wide range of approaches, from nebulized delivery of known antivirals such as remdesivir and NA-831 (National Clinical Trial number NCT02408874) to biologics and innovative decoy-based approaches. Clinical phase 1 results demonstrated utility of AMP5A, an anti-inflammatory biologic based on a low-molecular-weight fraction of human serum albumin (<5 kDa) that was administered via nebulizer for 5 days to COVID-19 patients along with the standard treatment regime. Coadministration improved clinical outcomes, leading to fewer deaths, shorter hospital stays, and fewer intensive care unit admissions in the small patient population studied (143). Local delivery of dexamethasone via NP has been shown in preclinical murine and nonhuman primate studies to mitigate airway inflammation and protect against lung injury (144).

A few interesting decoy-based approaches have demonstrated promising preclinical successes that may point to utility of this concept. Local inhalation delivery of exosomes engineered to express angiotensin-converting enzyme 2 (ACE2) showed robust protection against SARS-CoV-2 infection in both mice and macaques, binding to the viral particles that minimized host infection (145). Similar innovative approaches include molecular masks, which are ACE2-mimicking peptoids that, when delivered locally, block viral entry into cells (146); decoy nanoparticles, which are membrane-wrapped NPs that express ACE2 and sequester both virus and extracellular inflammatory cytokines (147); and camouflaged microspheres, which can perform the same dual-sequestering functionality (148). The Swedish company Masker MedTech AB is currently pursuing development of a recombinant protein-based ACE2 mimic on the basis of this concept for inhalation via both nebulized and dry powder for the treatment and prevention of COVID-19.

Respiratory infections encompass much more than SARS-CoV-2, and efforts to improve treatment strategies through local delivery must continue. Inhaled zanamivir, the small-molecule prophylactic influenza drug marketed as Relenza®, has been approved since 1999. Building on Pulmozyme's success, an emerging enzyme to treat influenza infection is DAS181 (Fludase®), a dry powder recombinant sialidase that works to remove sialic acid from respiratory epithelial cells and prevent viral binding. Results from phase 1 and 2 trials show reasonable tolerability for

1-day and 3-day dosing regimens, but adverse effects begin at 7 days, along with an undesirable host antibody response (149). Broad-spectrum antivirals have also emerged; a spray-dried formulation of tamibarotene, a retinoid derivative, has shown broad protection against SARS-CoV-2, influenza A, and Middle East respiratory syndrome–related coronavirus (MERS-CoV) in relevant small animal infection models (150). These collective examples point to emerging trends to diversify the standard small molecules historically used in inhalation antiviral approaches, with the expectation of improved long-term benefits to the patient.

CONCLUSIONS

Since the onset of the COVID-19 pandemic, inhaled therapeutics have come into the limelight and experienced a resurgence of interest from the broader drug delivery community. Such reinvigorated efforts have seen rapid development of new cargos and new targets for inhalation delivery, capitalizing on decades of advances in particle engineering and device development. Continued commitment to this field is needed to overcome the many unique challenges faced by this delivery route to translate these efforts into successful products.

One of the major roadblocks to development of inhaled therapeutics has been the lack of tools available to predict local therapeutic response in the lung, compounded by the challenge of directly sampling drugs in the airspace. In parallel to the formulation advances discussed in this article, the exciting advancements in the development of new preclinical tools specifically to address aerosol effects in the lung are expected to support acceleration of pulmonary drug delivery. Biological assays including air–liquid interface cultures, lung-on-a-chip microfluidic systems, and pulmonary organoids all offer advanced assessment of cellular responses distinct to the lung, especially as these incorporate a growing number of respiratory-specific cell types (151, 152). Further advances in in silico, in vitro, and in vivo models of the airways that can predict aerosol deposition under varied interpatient anatomies and diseased breathing profiles will also accelerate pharmacokinetic understanding of therapeutics in the lung (153). Ultimately, improved IVIVC from these advanced preclinical tools will assist in translation of emerging aerosol modalities, allowing for increases in efficiency and customization to drive down development costs.

Beyond this practical limitation, adoption of innovative inhalation treatments faces steep economical and societal challenges that remain inescapable for the field. Through the selection

of impactful biological targets, formulation modalities, and patient-friendly device designs, a major breakthrough drug in the inhaled biologic or vaccine space would go a long way toward continuing the revitalization of this field and making inhalation therapeutics the first-line approach. Given the recent growth of and remaining growth potential for the field, we remain optimistic that pulmonary drug delivery will offer innovative solutions to solve the global burden of respiratory diseases.

FUTURE ISSUES

1. Can the development, validation, and adoption of advanced preclinical testing paradigms that evaluate regional deposition, patient heterogeneity, immune targets, and mucosal clearance improve in vitro–in vivo correlation and decrease development timelines?
2. Of the emerging formulations that show strong preclinical potential (including biologics, gene therapy, antivirals, and immune engineering formulations), which area will see investments in clinical development?
3. Which formulation platforms will prove compatible, efficient, and safe for repeated respiratory dosing?
4. Can existing inhaled devices support the emerging formulation pipeline?
5. How can formulations and devices be improved to reduce the interference effects of heterogeneity in patient anatomy, pathology, and compliance?
6. What best practices should be established for characterization in formulations and trials, to facilitate longitudinal studies and cross comparison across studies of delivery and pharmacokinetics?
7. How will inhalable medicine evolve to meet the needs of an increasingly aging and connected world?

DISCLOSURE STATEMENT

At the time of publication, C.A.F. and I.R.W. have a pending US patent application to develop an in vitro model of airway deposition; C.A.F. has received funding from the National Institutes of Health and the National Science Foundation to develop inhalable formulations and advance airway models. The authors are not aware of any other affiliations, memberships, funding, or

financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award R35GM142866A and the National Science Foundation under award 2237430. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation.

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