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Nanomedicines via the pulmonary route: a promising strategy to reach the target?

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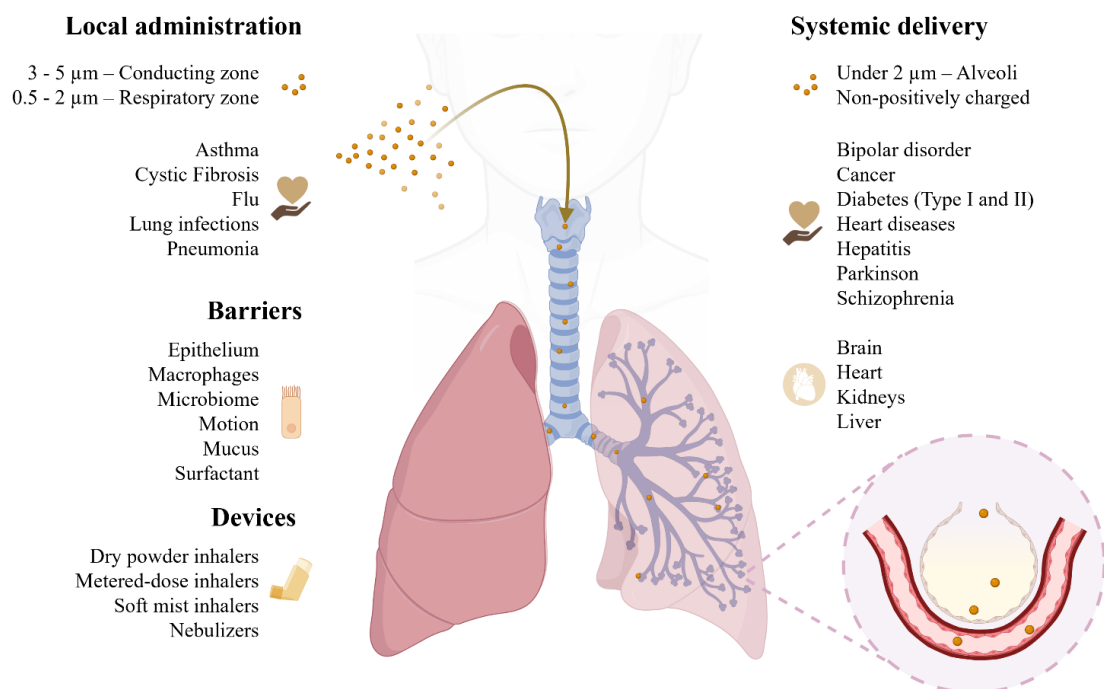
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Abstract

Over the past decades, research on nanomedicines as innovative tools in combating complex pathologies has increased tenfold, spanning fields from infectiology and ophthalmology to oncology. This process has further accelerated since the introduction of SARS-CoV-2 vaccines. When it comes to human health, nano-objects are designed to protect, transport, and improve the solubility of compounds to allow the delivery of active ingredients on their targets. Nanomedicines can be administered by different routes, such as intravenous, oral, intramuscular, or pulmonary routes. In the latter route, nanomedicines can be aerosolized or nebulized to reach the deep lung. This review summarizes existing nanomedicines proposed for inhalation administration, from their synthesis to their potential clinical use. It also outlines the respiratory organs, their structure, and particularities, with a specific emphasis on how these factors impact the administration of nanomedicines. Furthermore, the review addresses the organs accessible through pulmonary administration, along with various pathologies such as infections, genetic diseases, or cancer that can be addressed through inhaled nanotherapeutics. Finally, it examines the existing devices suitable for the aerosolization of nanomedicines and the range of nanomedicines in clinical development.

Graphical abstract



I – Introduction

While technologies and technics related to human health are constantly evolving, some pathologies remain difficult to cure or to treat, including cancers, neurodegenerative diseases, and mobility issues. There are various approaches to diagnose or to cure those different health issues, one of them being to reduce the size of the compounds to reach more effectively and specifically the target of interest. One format in this regard, which is explored during the last decades, is the use of nanometric objects: nanoparticles. The definition of the latest term is a necessary preliminary endeavor. According to the International Organization of Standardization (ISO), it is a “nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly” [1]. The scientific community calls “nanoparticle” every object whose external dimensions are below 1 μm . Their tiny size confers them unique properties due to their huge interaction surface [2]. Other than their size, their shape and their supramolecular structure vary as well. Indeed, adjusting these three last parameters allows to obtain specific physico-chemical properties leading to the existence of many kinds of nanoparticles which can be categorized depending on their properties, shape, and size [2]. Nanoparticles have been employed by humans since early history for crafting everyday objects. For example, nanofibers were used to solidify ceramic more than 4500 years ago or for hair dying by Egyptians 4000 years ago [3]. Historical references are plentiful, but the first scientific description of nanoparticles has been done in 1857, by Michael Faraday who synthesized colloidal gold nanoparticles in solution [4,5]. The researcher characterized the presence of very fine particles in his reaction media while studying the properties of light and matter. Under these conditions, he produced transparent gold films by chemical treatment involving reducing agents. During the process, he washed the gold films, leading to the formation of a reddish fluid that he used for optical

experiments, passing a light beam through it. He observed a cone effect and attributed it to the presence of suspended gold particles that were too small to be detected by the instruments then in use: nanoparticles. This optical effect is now called the Faraday-Tyndall effect [5–7]. As shown in **Figure 1**, nanoparticles can be produced by two main methods. The first method involves obtaining nanometric objects from a macrometric object, gradually shaping it down: it is the top-down approach. It is possible to decrease the size of the objects by using ultrasonication, laser ablation, etching, sputtering or thermal decomposition. A necessary but impractical condition to this end is to work under high pressure and the processes cost a lot of energy. Moreover, those practices do not easily allow to reach size under 100 nm [8,9]. The second main method consists of building-up the objects by assembling atoms or molecules together, to then have clusters that may form nanoparticles: it is the bottom-up approach [8,9]. Nanoparticles offer a myriad of applications, and our focus will be on their utilization in the therapeutic field: nanomedicines, a relatively

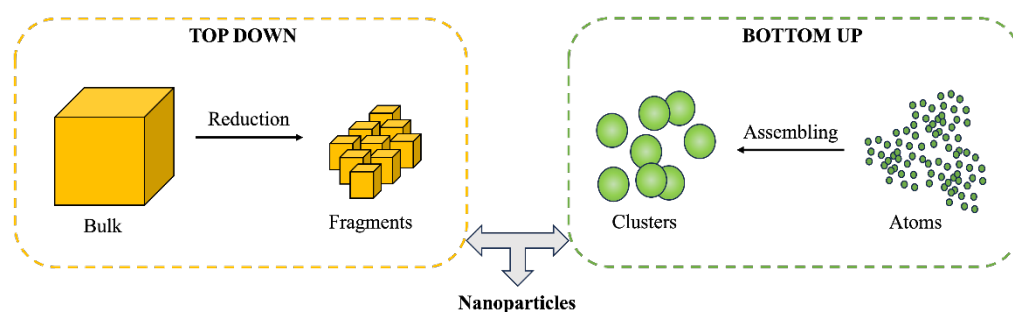


Figure 1. Schematical representation of the top-down and the bottom-up production of nanoparticles, adapted from [14].

newly described field [3]. The earliest references to what could be described as nanomedicine date back to the 17th and 18th centuries, while the start of the nanomedicine research field strongly began in the 1990s [10,11]. This research field provides the opportunity to overcome numerous challenges in drug delivery within the body, such as transportation, protection, enhancement of solubility, and improvement of the drug bioavailability. Its main objective being to enable the delivery of active ingredients to specific targets while enhancing their biological activity. Some other goals pursued in nanomedicine are the use of nanovectors for imaging, specialized medicine, and even theragnostic when a single vector enables both diagnosis and treatment concurrently [12]. Nonetheless, nanomedicine encounters various challenges, including the complexity of characterizing nanoobjects, the hurdles associated with large-scale production, and the need for reproducibility in manufacturing protocols, already challenging in small-scale production [13]. Despite this, the multitude of possibilities within the field of nanomedicine not only holds great promise but is also intricately connected to the diverse nature of existing nanoparticles, suggesting an abundance of potential applications. Indeed, nanoparticle for therapeutic applications may encompass organic entities like liposomes and polymeric micelles [14], inorganic materials [15] such as carbon-based materials [16], metal nanoparticles [17], or metal-organic framework nanoparticles (MOF) [18]. All these variants find application in the realm of nanotherapeutics research [19–23]. The diversity in nanomedicine extends beyond the nature of the nanoparticles to include a variety of administration routes, such as intravenous, oral or intramuscular. In literature, the systemic administration by intravenous injection is probably one of the most studied routes, mainly in the cancer field, where the well-known and controversial Enhanced Permeability and Retention (EPR) effect allows an accumulation of nanomedicines in some solid tumors [24–26]. This effect, explained by the over-vascularization of tumors and

abnormal permeability of recruited vessels, as well as an insufficient lymphatic drainage in tumors compared with healthy tissues, seems to be different in human and xenograft rodent model metabolism. Indeed, despite an excellent efficacy of nanomedicines in preclinical mice xenograft cancer models, once administered to human cancer patients, the nanomedicines do not exhibit the same level of effectiveness [27]. The difference in the effectiveness of anticancer nanomedicine delivery using EPR effect in xenograft mouse models compared to humans can be attributed to an oversimplified and overestimated representation of the EPR effect in xenograft models [28]. These models, obtained through subcutaneous injection of tumor cells, fail to accurately mimic the complexity and heterogeneity of human tumor cells and new models are in development to limit the problem: patient-derived xenografted (PDX) models grafted with surgically removed tumor tissues of patients and offering the opportunity to develop personalized medicine [28]. Administering nanomedicine *via* intravenous injection may encounter challenges, such as the immune system response. Once in the bloodstream, nanoparticles can be opsonized and immune system sentinels, such as monocytes, dendritic cells or macrophages, intensely interact with the nanoobjects, leading to their phagocytosis and subsequent clearance from the bloodstream [29]. The oral route is probably the most difficult administration route: drugs and nanomedicines must be protected from the acidic conditions of the stomach, from biliary salts, from enzymatic pancreas degradation and must be able, at the same time, to be released in the digestive tube and pass through the gut membrane while avoiding the mucus entrapment [30]. Another way of administration, known by the great public, is the intramuscular injection and became famous since the SARS-CoV-2 mRNA and DNA vaccines [31]. However, as shown in **Figure 2**, administration of nanomedicines to human is not so new with the first FDA-approved nanomedicine called Doxil®, commercialized in 1995 [32,33]: these doxorubicin loaded liposomes, surface-decorated with polyethylene glycol (PEG) were first indicated for the treatment of AIDS-related Kaposi's sarcoma. Another route that is used by humans since antiquity but had been all but forgotten before being reexplored few decades ago is the pulmonary route. It is nevertheless a very interesting one as it is non-invasive, it circumvents the first pass metabolism and system toxicity, decreases the number of necessary doses, and allows to directly target the lung epithelium,

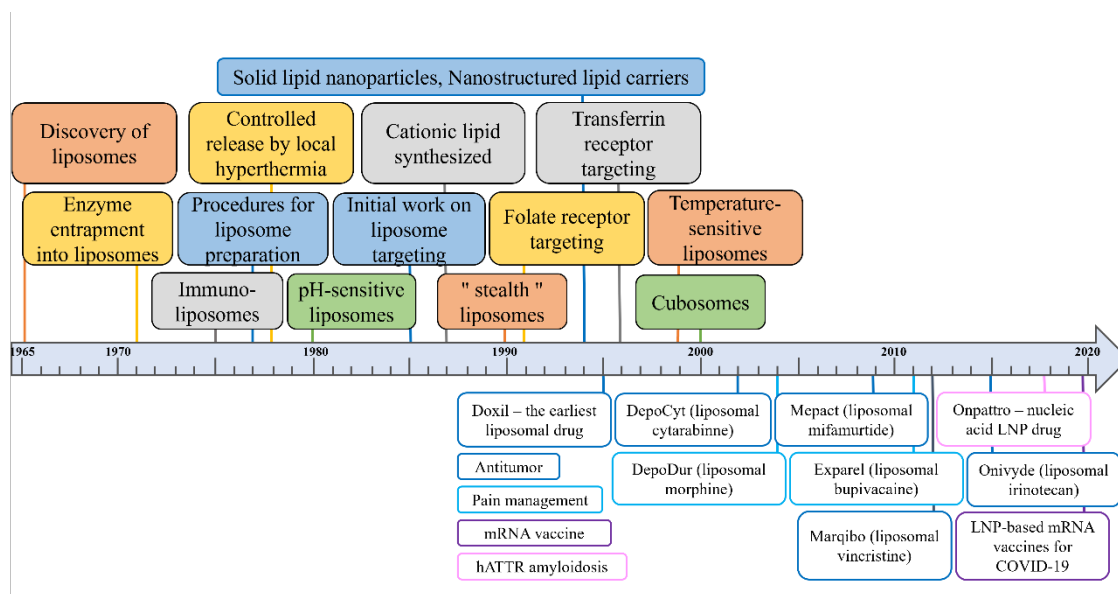


Figure 2. Timeline of lipid-based nanomedicine history, from their discovery to their market launch. Adapted from [24].

enhancing local drug concentration. Most importantly, this route is perfectly compatible with the administration of nanomedicines. Indeed, nanoobjects demonstrate aerosol transfer capability, resilience against forces generated during aerosolization, biocompatibility, the capacity to target specific sites or cells in the lung, potential for controlled drug release, and for biodegradation within an acceptable timeframe [34,35]. Another advantage provided by the pulmonary route is the direct connection of the lungs to the systemic circulation, allowing in certain conditions the nanoparticles to reach directly the bloodstream and organs as the heart, the spleen, the liver, the bone marrow or the placenta, as represented in **Figure 3**, by crossing the air-blood barrier [36]. Despite its numerous advantages, the pulmonary route of administration can raise concerns when non-biodegradable or non-biocompatible materials, such as silica and metal-based nanoparticles or carbon nanotubes, are utilized, potentially leading to toxicity issues [34]. To gain a deeper understanding of the benefits and drawbacks of delivering nanomedicines through the pulmonary route, we will first delve into the interaction between respiratory organs and nanoparticles. We will then describe the essential characteristics that nanoobjects should possess to effectively reach their target through this administration route. Following that, we will introduce devices specifically designed for inhalation delivery, with a focus on delivering nanomedicines. This will include highlighting their strengths and weaknesses in nanoparticle delivery. Finally, we will explore diseases that can be treated through nanomedicine inhalation, offering insights into promising research endeavors.

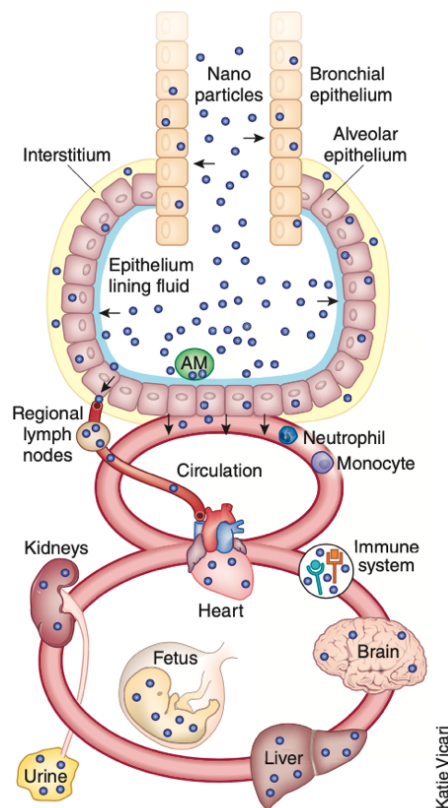


Figure 3. Translocation of nanoparticles from the alveolar epithelium to the regional lymph nodes and the systemic circulation. AM: Alveolar Macrophages. Adapted from [28].

II – The respiratory system

The historical practice of administering medicine through the pulmonary route dates to ancient Egypt, as evidenced by the Ebers' Papyrus from 1554 BC. This ancient document describes the delivery of henbane plants through the inhalation of their smoke to enhance patients' respiratory conditions [37]. In contemporary times, the understanding of therapeutic inhalation has expanded, and our focus will now be shifted to the delivery of inhaled nanomedicine: the characteristics of respiratory organs and the interactions between nanoparticles and lung barriers will be explored. The respiratory system begins with the nose and mouth, passes through the trachea, and ends with the lungs. The first challenge posed by the inhalation of nanomedicine is to prevent deposition in the upper airways. To address this, we will later delve into the significance of propelling the nanovectors into the lungs in droplets or aggregates ranging from 0.5 to 5 μm , facilitating deposition in the lungs without being exhaled [38]. The left lung is composed of two lobes leaving some place to the heart whereas the right one is composed of three lobes. Each lobe is made up of primary bronchi, branching out into secondary bronchi, which in turn divide into smaller air ducts, the bronchioles, before ending in more than 300 million alveoli, grouped together in alveolar sacs in the deep lung. Furthermore, each alveolus contains many pulmonary capillaries, in total, there are 280 billion of them in both lungs. This entire network provides a surface area of 70 m^2 of blood-gas barrier, enabling the body to internalize air by admitting it to the circulatory system with direct access to the heart [36,39]. The alveolar region of the lung, illustrated on **Figure 4**, constitutes 90% of the

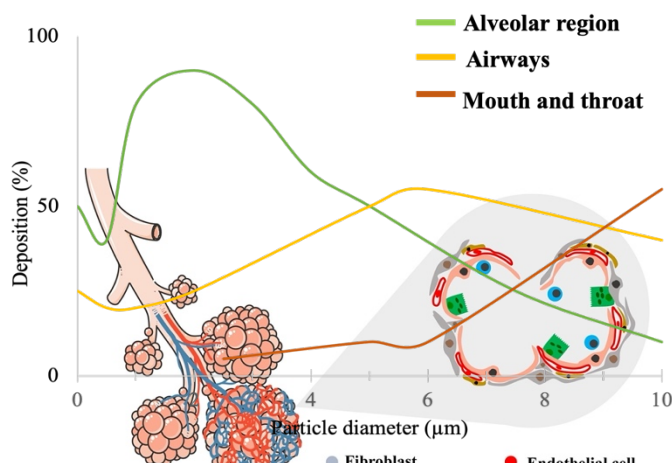


Figure 5. The deposition percentage of particles in the different airway zones, depending on their diameter. Adapted from [32].

Figure 4. Schematical representation of the alveolar region vascularization and its 3-layers structure (on the right). Adapted from [30].

lung volume [40]. The figure also presents the “3-layers structure” between the air and the alveoli, containing alveolar macrophages, alveolar type I and type II cells, endothelial cells, fibroblasts and pericytes. It is the third layer which contains venous blood, allowing the exchanges of a O_2 and CO_2 [40].

The vital respiratory function allows the body to uptake oxygen, ensuring its normal functioning, and despite its self-cleaning mechanism, it also represents an open door for particles in the air, and this could be used for the delivery of nanomedicines. In other terms, if the systemic circulation is desired, the alveolar sacs are the place to target to

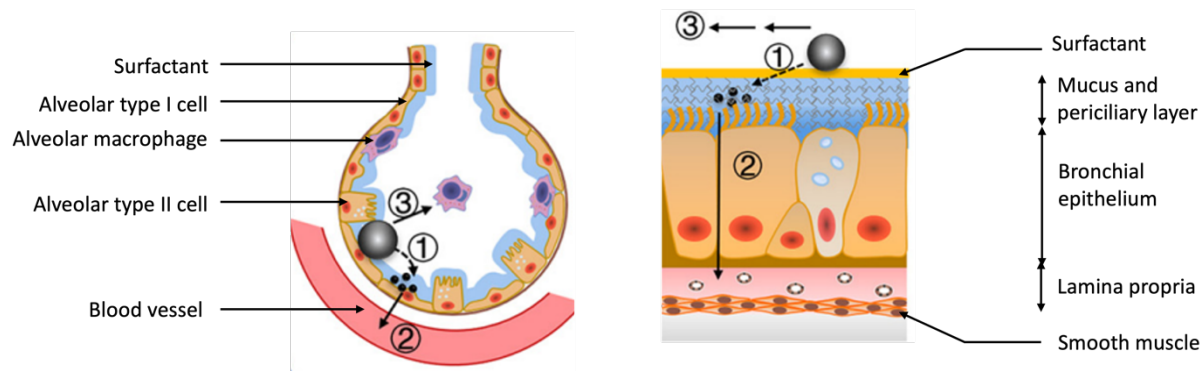


Figure 6. Structure of healthy alveoli sacs, of their epithelium and effect of the barrier on clearance and absorption of nanoparticles. (1) Erosion and dissolution of a nanoparticle after contact with the lung fluids; (2) Transport of the nanoparticle or dissolved drugs across the mucus/surfactant and epithelial layers; (3) Mucociliary and macrophage clearance of the nanoparticles. Modified from [34].

administrate nanomedicines through the pulmonary route as it is the place where the lung epithelium is the thinnest. However, their access is not easy as the lungs continuously sweep out air and molecules. Indeed, along this way, different forces come into play. First, inertia affects the way for the largest particles, which then collide with the walls and settle there until being cleaned out by the ciliated cells. Further on, gravity filtrates the particles. The smallest ones continue their path as their movement, controlled by diffusion based on Brownian motion [41], depose them down in the alveolar region. This description reveals that the deposition zone of spherical particles in the respiratory tract is largely dependent on their diameter, as shown in **Figure 5**. Furthermore, particles with a diameter larger than 1 micrometer tend to remain on the epithelial surface before being exhaled through coughing or eliminated via mucociliary transport or macrophages phagocytosis [42]. This exposes one advantage of delivering nanomedicine. Because they are smaller than 1 micrometer, once the droplet or aggregate deposits on the epithelium, it may release individual nanosized objects that may not be exhaled. More precisely, the particle deposition in the lung depends on the aerodynamic diameter, a parameter involving geometric diameter, shape and density of the particle [43,44]. This is why the pulmonary route can be used for local or systemic administration, depending on the diameter of the droplet, in the case of liquid state formulations and of the dry powder in the case of solid-state formulations.

The lung airflow is not the only biological barrier to overcome before arriving in the alveolar bags as these organs have a unique composition made of surfactant, mucus, immune cells (as shown on **Figure 6**) but also pathogens and molecules brought by the respiration [45]. In other words, the constant flow of air in the respiratory tract is a vehicle for foreign bodies capable of infecting the system, explaining the unique composition of the lungs. The immune system protects the airways first of all thanks to macrophages that are constantly patrolling in the lungs [46]. In the healthy lungs, those immune cells are the first line of defense against pathogens and exogenic molecules as they possess a great ability to phagocyte. This enables them not only to protect the respiratory tract against external aggression, but also to clean it from dead or senescent cells as they regulate the amount of surfactant produced by lung epithelium [47]. Macrophages are very effective, but their phagocytosis capacity depends on the size of the particles. They can capture particles with a diameter from 0.5 to 3 μm , but they allow particles with a diameter of less than 0.26 μm to pass through [48]. Another inconvenient of macrophage function is that their clearance is slow as it can take from few

hours to few weeks [49,50]. The clearance kinetic depends on nanoparticle size, as it may allow them to interact with immune cells, resulting in either immunosuppression or immunostimulation [51]. This interaction may enhance or decrease the therapeutic effect of nanomedicines and two main mechanisms are observed for nanoparticles uptake by macrophages. Firstly, the non-selective mechanism encompasses micropinocytosis, where tiny soluble substances are taken up, and phagocytosis, where larger and solid particles are involved. For nanoparticles with a diameter under 50 nm, clathrin-mediated endocytosis uptake is mostly observed [51]. The second type of mechanism is a selective uptake, which depends on an interaction between macrophage receptors and the decorated surface of nanoparticles. Not only the size of particles impacts their interactions with macrophages, but also their properties. Indeed, hydrophilicity of the surface reduces the possibility of interactions with proteins, decreasing the nanoparticle opsonization and macrophage capture, while a hydrophobic surface enhances engulfment by immune cells, with a macrophage clearance of 1-2 hours. Surface charge also plays a key role in macrophage uptake, as positively and negatively charged particles present a higher risk of being phagocytosed than neutral ones [51]. Finally, the aspect ratio must also be considered for immune system interaction with nanomedicines, as a smaller aspect ratio facilitates phagocytosis. This effect could be avoided by adding stealth properties to nanoparticles, which is possible by coating them with hydrophilic polymers such as polyethylene glycol or polysarcosine, for example [51,52]. Once again, the dimensions of nanomedicines can be compatible with a systemic delivery *via* pulmonary administration given their nanometric size could enable them to avoid the capture and the clearance by pulmonary macrophages. However, it's important to remember that not only the size of the particle is important, but also its physicochemical properties.

Further barriers that need to be crossed before reaching the alveolar region is the surfactant (Surface Active Agent) located at the air-liquid interface and the mucus composing the liquid layer of the alveolar walls [53]. The surfactant is produced by alveolar type II cells, as described in **Figure 7**. Mostly composed of phospholipids (around 90% of its molecular mass) and of proteins (around 10% - SP: Surfactant Proteins – SP-A, SP-B, SP-C and SP-D), this surface-active material is crucial to maintain the integrity of the lung as it avoids the atelectasis at end-expiration. In addition to this essential function, lung surfactant is also involved in the immunity of the organ. Two surfactant constituent proteins, SP-A and SP-D are part of the immune system. They belong to the collectin protein family, possessing a lectin domain, interacting with pathogens. Thanks to this interaction, SP-A and SP-D help complement activation and phagocytosis, killing pathogens [54]. The surfactant also decreases nanoparticles uptake in the deep lung [55,56]. Indeed, their internalization by immune cells and translocation through epithelial cell layer is directly linked to their interaction with the surfactant. For example, positively charged silica nanoparticle uptake by A549 and NHI-H441 cells is inhibited by surfactant, as they strongly interact together notably *via* electrostatic forces [56]. The mucus is a viscoelastic gel principally composed of mucin fibers and continuously produced and cleared at a rhythm of around

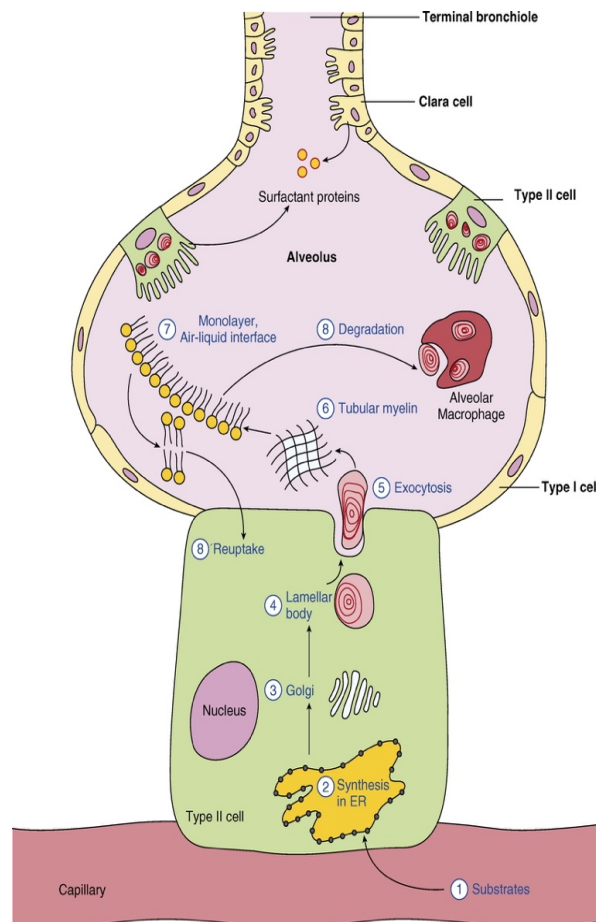


Figure 7. Production and recycling of surfactant. (1) Dietary substrates arrive from the systemic circulation to the type II cells cytoplasm; (2) Surfactant synthesis in the type II cells endoplasmic reticulum (ER); (3) Transport of the surfactant in the Golgi for more modification; (4) Stockage of the surfactant in the lamellar bodies; (5) Secretion into liquid hypophase during the alveoli exocytosis; (6) Formation of tubular myelin by the surfactant; (7) Transport at the air-liquid interface and formation of a monolayer surfactant film; (8) Internalization or degradation by the alveolar macrophages; (8') Reuptake by type II cells for reuse; Some surfactant proteins are also synthesized at the terminal bronchiole region. Adapted from [42].

2.4 mm/min in the bronchial and around 1 mm/min in the small airways [57]. This material acts like a semi-permeable barrier, able to trap molecules and nanoparticles that will then be quickly cleared by the ciliated cells, after a few hours [58]. The nanoscale proves advantageous in dealing with the mucus, where large molecules are easily entrapped, and nanovectors carrying the drug are more likely to diffuse within this network. Moreover, modifying the nanovector surface charge enables interactions with the mucus when positively charged, or avoids such interactions when negatively charged. To evade mucus clearance and enhance the diffusion of nanomedicines within the mucus, various approaches can be employed. For that, simple methods such as PEGylation, surface decoration using mucolytic agents, muco-inert polymers, or the use of osmotic agents are possible [59,60].

Another barrier inherent to lung administration that should be discussed is the epithelium when the systemic circulation is targeted. Indeed, reaching the blood circulation is impossible without crossing it. This pseudostratified epithelium' structure changes as we go deeper into the lung, shown in **Figure 8**: in the bronchia, it is composed of ciliated cells (responsible of the brushing movement which moves the mucus), goblet cells (secreting the mucus), basal cells (the epithelium progenitor cells) and brush cells, set on the basement membrane [61]. This cell layer is 58 μm thick and it is covered with 8 μm of lung fluid. In the terminal bronchia area, the same structure is observed but thinner with a cell

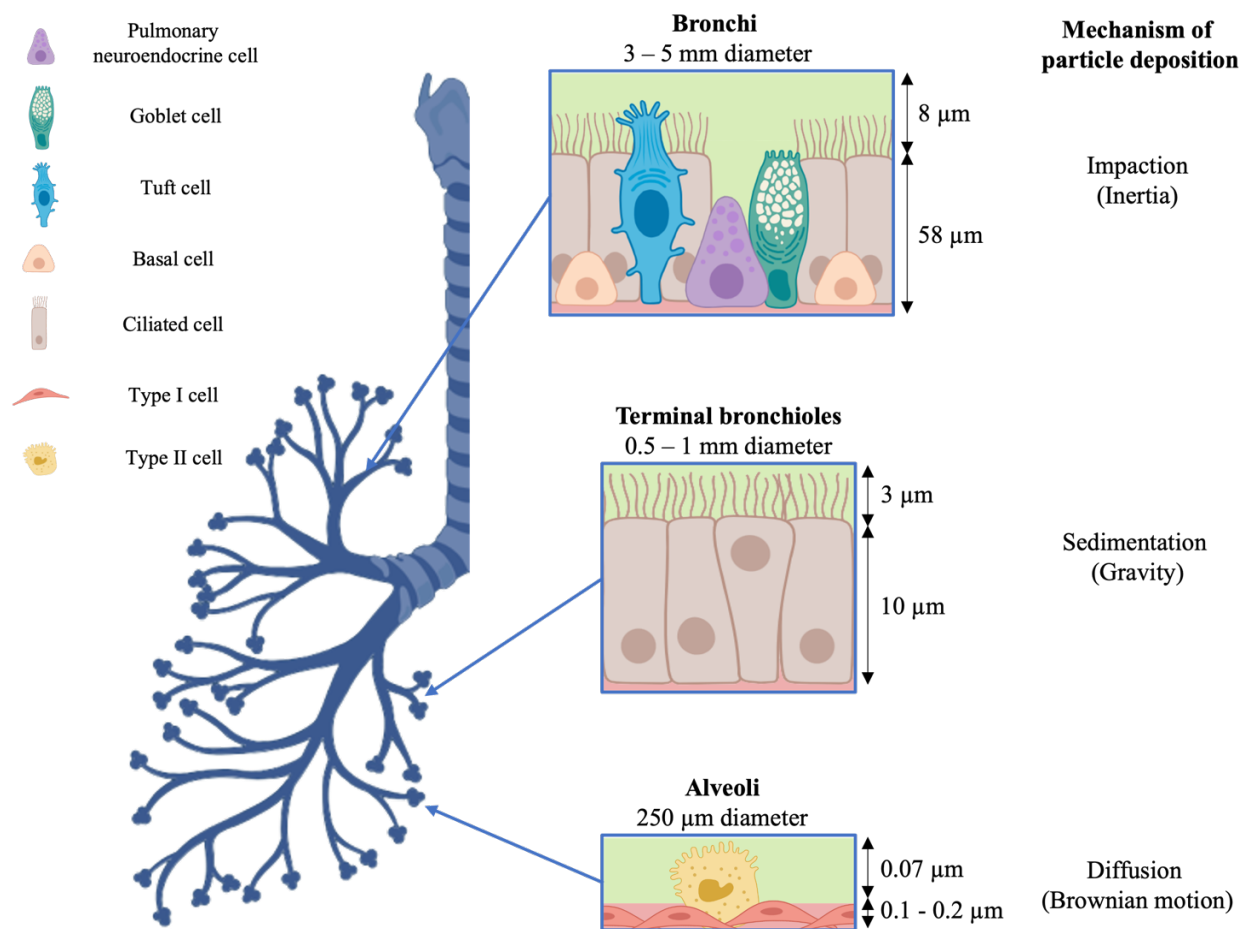


Figure 8. Structure and thickness of lung epithelium at different zones with the main forces controlling the particles movements. Adapted from [46].

layer of 10 μm and a fluid layer of 3 μm . In the alveoli, more than 95% of the cells are replaced by type I cells forming a thin cell layer of 0.1 – 0.2 μm , covered by only 0.07 μm of lung fluid. It is through this thinner barrier in the deep lung region that particles may enter in the system [43,61]. The transport of molecules across the epithelium depends on their physico-chemical properties. Thus, hydrophilic compounds will be transported by extracellular pathways such as through tight junctions or by active transport via endocytosis and exocytosis. Lipophilic molecules, for their part, will be transported by passive diffusion [62]. The second transport being slower, opting for a lipophile drug may allow it to stay longer in the deep lung as it slows its clearance, leading to a sustained action [63]. The charge is also important to pass through the epithelium as positively charged particles cannot cross this barrier [64]. This could be attributed to the limited presence or absence of anionic sites within the air-blood barrier contrary to what is generally observed for epitheliums [65].

The last barrier discussed was brought to light thanks to advanced genetic material sequencing methods called “culture-independent analysis”. These methods allowed to identify microorganisms composing the healthy human flora or microbiota [66]. Contrary to common belief, the deep lung is not sterile but has its own local microbiota [67,68]. The comprehensive characterization of lung microbiota revealed variations in its composition and diversity from birth to adulthood. Depending on the mode of delivery, species like *Staphylococcus* or *Ureaplasma* will be dominant the first weeks of life, then the population will become richer in *Streptococcus*, *Prevotella* or *Veillonella* [69]. Furthermore, the density, the composition of respiratory system microbiota and its diversity decrease while reaching the deep airways as shown in **Figure 9** [70]. However, these conclusions must be nuanced as the collect of samples *via* bronchoalveolar

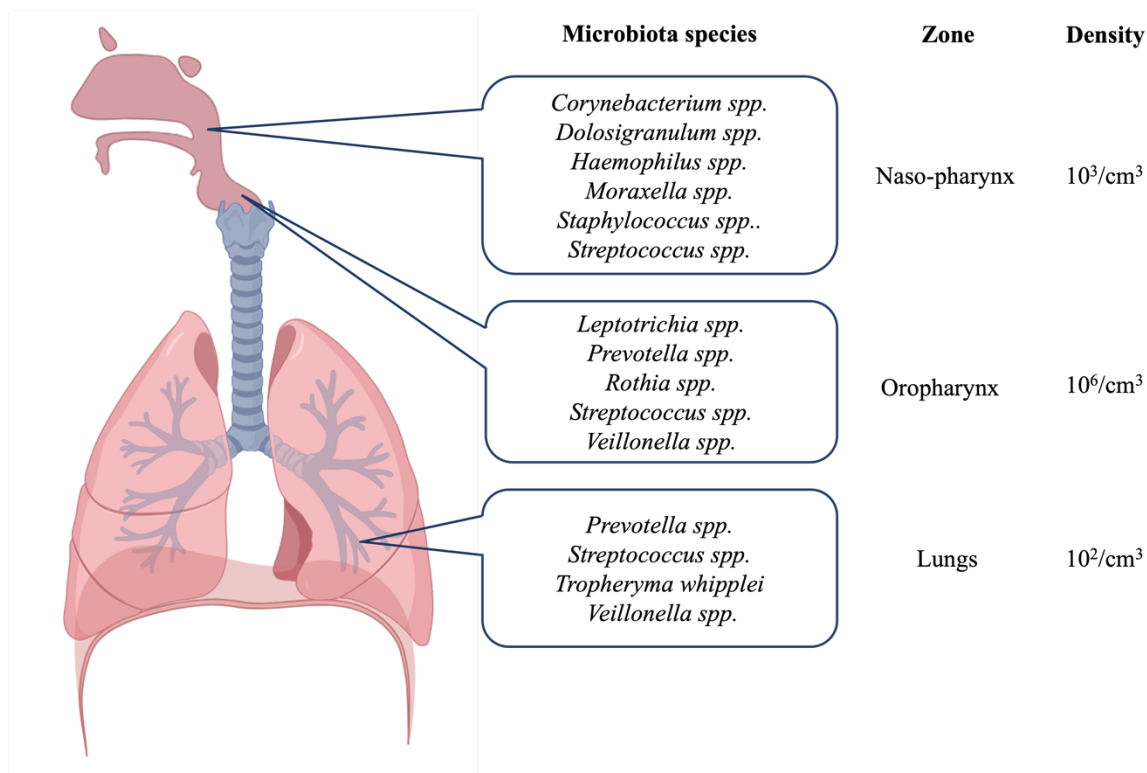


Figure 9. Composition and density of respiratory system microbiome.

lavage fluids can be contaminated and not reflected the clinic reality of microorganisms and their respective density according to their localization in the respiratory tract.

Additionally, according to literature, it is becoming increasingly difficult to ignore the role of the microbiota in different physiological processes, including tumor development [71,72] and biological response to cancer treatments [73–76]. The local microbiota must be considered as a real biological barrier and have to be studied to develop treatments of tomorrow, notably for lung cancers. Finally, many studies show variations in the respiratory microbiota composition in patients suffering from pathologies such as asthma, cystic fibrosis or lung cancer and it is important to take those differences in account to develop tomorrow treatments [77–79].

To conclude, the pulmonary route offers two possibilities: the lung local administration or the systemic administration. For the first one, the diameter of the droplet or of the solid particle containing the nanoparticles must be micrometric. Thus, it will be deposited in the conducting zone (3-5 μm) or in the respiratory zone (0.5 to 2 μm), while individualized particles with a diameter lower than 0.5 μm will be easily exhaled [38]. As will be explained later, not only the formulation has to be taken in account but also the device used to pulmonary delivering drugs. Indeed, depending on the device, droplets or powder aerosols are produced, forming larger or smaller particles. For the systemic administration, drugs must be transported in the deep lung *via* a 0.5 to 2 μm of aerodynamic diameter aerosol in order to reach alveolar region without being exhaled, and to sediment in the alveoli [38]. They must not be positively charged to avoid the mucus and surfactant trap, to cross the air-blood barrier [56,64,65]. They also have to be nanometric to avoid the macrophage clearance (less than 0.26 μm) once deposited in the alveoli. Then they may pass in the systemic circulation to reach desired organs.

III – Inhalation drug-delivery technologies and nanomedicines

After elucidating the respiratory system and its inherent barriers for, the focus of our analysis will now shift to examining various strategies for administering nanomedicines through this route. Opting for nanomedicine over traditional formulations offers a myriad of advantages. One notable benefit is the potential to reduce the administered dose while concurrently enhancing therapeutic effects. Moreover, nanomedicine facilitates the delivery of hydrophobic molecules as shielding them from degradation. However, the first step allowing to deliver nano-compounds in the lung is to choose the adapted inhalation device. Pulmonary administration can occur from a liquid or solid sample to generate an aerosol and four main inhalation technologies are important to distinguish: the pressurized metered-dose inhalers (MDIs), the dry powder inhalers (DPIs), the nebulizers and the soft mist inhalers (SMIs) [44,80]. First, it is important to realize that all the existing technologies have their own advantages and inconvenients and each of them correspond to a certain type of formulation. It is then necessary to choose the device which is adapted to the formulation as the aerosol characteristics will determine the therapeutic efficacy and the deposition zone. We will see that some devices are more conducive for nanodrugs delivery than others [81]. However, in general, the ideal device should generate aerosol droplets in the range of 0.5 to 5 μm of diameter enabling them to reach the deep lung: it should provide a reproducible drug dosing without damaging the physico-chemical properties of the formulation. It should also be easy to use by patients, inexpensive and, depending on the administrated drug, it should be portable [44]. The use of

inhalation devices for medical applications began in the late 19th century and **Figure 10** shows the evolution of those technologies for the past hundred years [82–84].

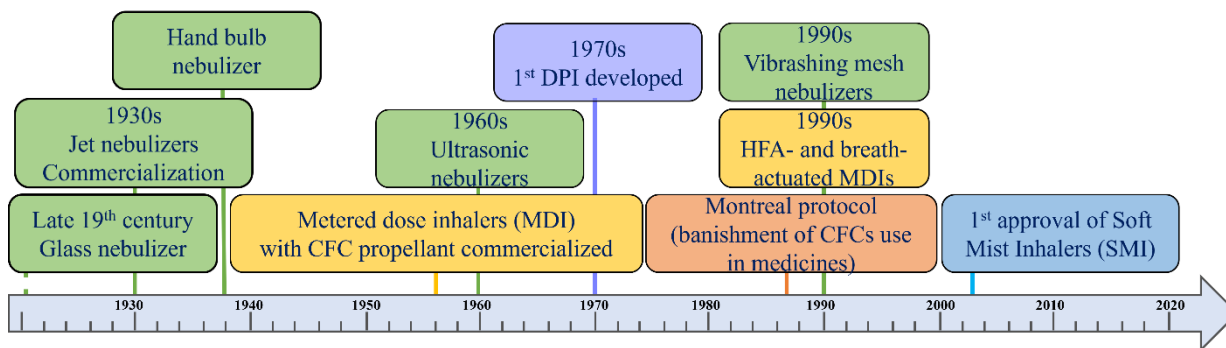


Figure 10. Timeline of inhalation devices development. CFC: Chlorofluorocarbons; HFA: Hydrofluoroalkane.

a) The nebulizers

The nebulizer is the oldest form of inhalation devices. It appeared in the late 19th century and can also be defined as modified atomizer. Indeed, atomizers are devices able to generate a mist of fine droplets from a liquid sample thanks to a high velocity air-jet that propels the liquid forward as a thin layer that will be fragmented into droplets by shear-induced instability. The nebulizer works in the same way but has also an impactor in front of the jet, allowing to remove large particles from the air stream [3]. This was the first device authorized on the market for inhalation of therapeutics, it is easy to use, and its main advantage is that it does not require any breathing technique when inhaled. However, it's also described as uncomfortable to use by patients [80,85]. **Figure 11** shows the three main types of nebulizers: the jet, the ultrasonic and the mesh nebulizers. These devices allow the formation of 1 to 10 μm droplets permitting a deposition in all lung zones. Nebulizers provide the opportunity to deliver larger amounts of drugs compared to DPIs and MDIs [86]. They can be employed for delivering nanomedicines to the lungs, particularly in the treatment of lung cancer using a suspension, where they exhibit greater efficiency compared to DPIs [87]. These devices could also be used to deliver mRNA in the lung when encapsulated in lipid nanoparticles containing surface stabilizers [88]. However, for the delivery of nanomedicines via nebulization it is crucial that the particles exhibit the ability to withstand high shear forces to prevent aggregation and premature release of the cargo [89]. Significantly, lipid-based nanoparticle formulations are susceptible to shear degradation, leading to a potential loss of the active compound cargo, especially when using hydrophilic materials [90]. To overcome this issue, the design of lipid nanoparticles must include shear-stress resistant compounds [90].

First, the jet nebulizer works thanks to a gas flow which passing through the reservoir to atomize the liquid sample. This process generates large and small droplets that are driven to the baffle. At this point, the baffle allows the small droplets to leave the nebulizer for being inhaled by the patient while the bigger droplets go back to the reservoir and redo the process. It is estimated that 50 % of the drug loaded mass is delivered, generated by a compressor or by a central air supply [85]. This low deposition efficacy is one of the jet nebulizer weaknesses. Another weakness being that it can partly destroy the active compound or modify its structure (antibodies, liposomes) or even increase the drug release from more flexible structure such as liposomes [85]. Indeed, the gas compression used to form the jet of formulation may cause a shear-stress concern and break lipid-based vesicles [91].

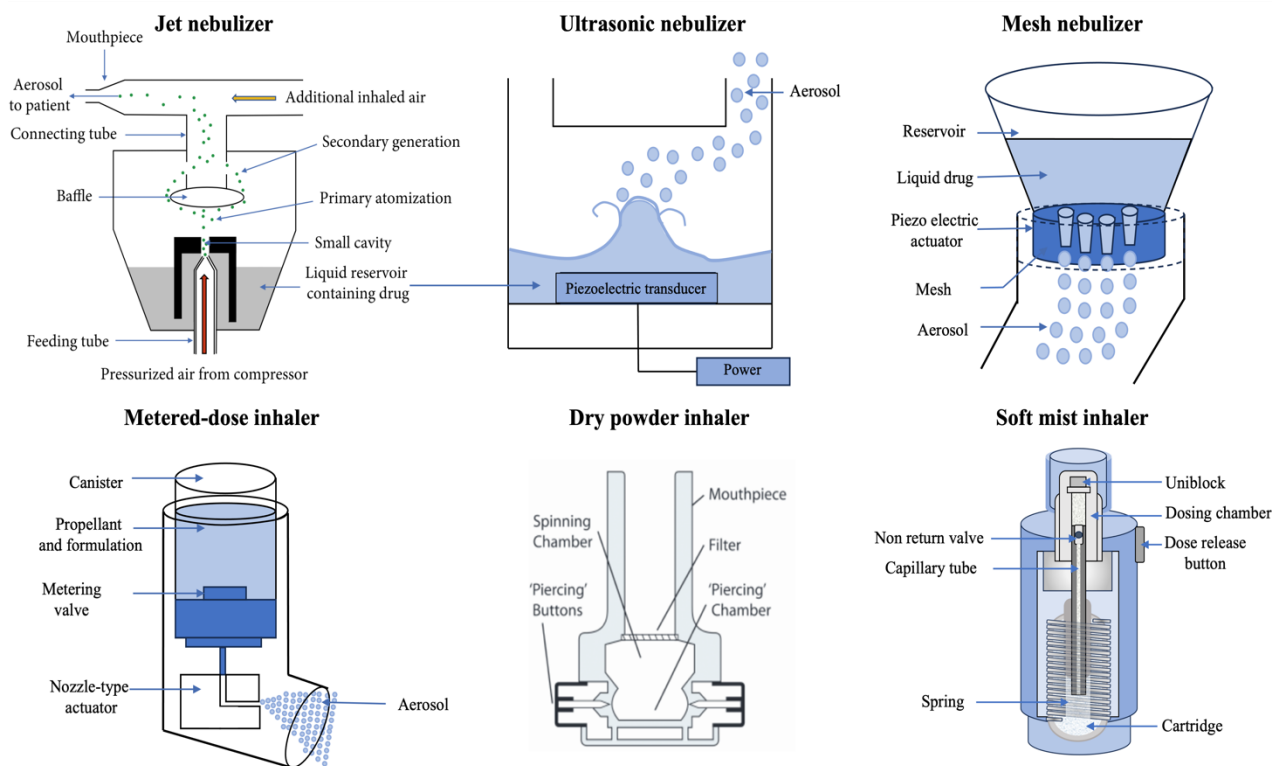


Figure 11. The three main types of nebulizers, general structure of metered-dose inhaler, dry powder inhaler and soft mist inhaler. Jet nebulizer adapted from [76]; Structure of a dry powder inhaler adapted from [66].

Ultrasonic nebulizers work with vibration of a piezo-electric crystal between 1.2 and 2.4 MHz, forming a fountain-shape jet of small and bigger particles. As for the jet nebulizer, the bigger droplets are returned to the reservoir to restart the process. The small droplets go to the nebulization chamber to be inhaled by the patient. This device also traps 50% of the sample but the main difference with the jet nebulizer is the absence of gas source, leading to a small leakage during exhalation. Ultrasonic nebulizer differs from the jet nebulizer thanks to its ability to nebulize more viscous liquid. This advantage also reduces the range of drug formulations that can be administrated with the device. Moreover, the solutions are faster nebulized, but it cannot be used for suspensions and the piezoelectric crystal provoke an increase of the temperature that can degrade the drug or cause aggregation of thermosensitive molecules and damage thermolabile compounds [85,91].

The third type, mesh nebulizers, can be divided in two subtypes: vibrating and static. The first one has an ultrasonic transducer which is in contact with the liquid and vibrates at 180 kHz. The vibrations are pushing the liquid through the static mesh's 1000 to 6000 holes of 3 to 6 μm of diameter. This generates particles that can directly be inhaled by patients. The vibrating mesh nebulizers has a piezo element instead of the transducer, causing the deformation or the vibration of the mesh. Here, the holes are conic, the widest part being in contact with the liquid. As for the static subtype, the system produces droplets that can be directly inhaled by the patient. The particularity of this device is that the produced aerosol is comparable to the drop-by-drop technic [85]. The vibrating mesh nebulizer is the most commonly used device in the literature to study RNA delivery [86]. Nevertheless, in the context of nanotherapeutics, mesh nebulizers exhibit a non-negligible drug leakage effect from lipid nanoparticles, primarily attributed to significant shear stress and heat generated by the vibrating mesh [90].

b) The metered-dose inhalers (MDIs)

The MDIs, developed in the 50's mostly to treat asthma, are likely the most widely recognized devices among the public. **Figure 11** shows the structure of a typical MDI [92]. A solution in liquid propellant or a suspension of the drug is contained in the metal canister which keeps it under pressure. A predefined amount of propellant and a dose of drug are expelled from the canister after the actuation, leading the propellant to vaporize and delivering an aerosol spray containing the drug [93]. It is the actuation by the patient on the canister that propels the formulation and forms an aerosol spray that goes in the throat where it can then be inhaled. This device is known to have bad effects on environment because the propellant gas used contain hydrofluorocarbon (HFC) that are powerful global greenhouse gas [94]. Furthermore, they have a low efficacy of drug delivery due to the fast escaping of drug from the device and a desynchronization between actuation and inhalation. Therefore, it is estimated that 70 to 80% of the formulation is lost before its deposition [44]. This low delivery efficacy could be explained by patients' utilization. Indeed, to use this kind of system, patients are bound to respect some rules like a breath-holding time, synchronization with the breathing, or even shaking the inhaler before the use but a lot of people do not respect those rules, impacting the efficacy of the technologies [95]. Furthermore, the MDIs generally cause discomfort during intake due to the fast released associated with the expansion of the compressed gas leading to a "cold Freon effect" [96]. Significant research efforts have been directed towards the development of nanomedicines through MDIs administration: liquid propellant make the use of most of classic nanomedicines (lipid or water insoluble polymer based) not possible or at least very challenging. Despite these difficulties, literature describes MDI devices as highly promising as it could be used to deliver various nanoobjects, including liposomes, solid lipid nanoparticles, inorganic nanoparticles, and polymer hybrid nanoparticles [97]. This device is also highly valued for its robustness, compactness, and portability [97].

c) The dry powder inhalers (DPIs)

DPIs devices, shown on **Figure 11** are smaller devices than nebulizers, such as MDI and SMIs, making them more cost-effective [98]. They do not possess propellants and so they are environmentally friendly. They are also easier to use as they don't require the patient to coordinate his inhalation with the device actuation unlike for MDIs, even if a breath-holding time is necessary. They combine two technologies, the powder and the dispersion of dry particles in one aerosol that the patient aspirate [32,64]. They are generally preferred to the other devices because of their stability due to the solid phase of the particles administrated [44,99]. One of the important factors to take in account for the formulation delivered with DPIs is the size of dry powder particles. Indeed, the aerodynamic diameter of the drug particle size must be between 1 and 5 μm , otherwise, particles could be deposited in the oropharynx instead of the deep lung. Especially, the powder administrated by spray-drying have the tendency to be cohesive, reducing the dispersion quality during aerosolization [99]. Recent advancements in the technology of spray drying for nanocompounds enable their administration using DPIs. Nano Spray-Dryers have the capability to generate particles as small as 300 nm by converting a solution, a nano-emulsion, or a nanosuspension into a powder. This drying system can be complemented by freeze-drying to produce particles with even lower density and smaller aerodynamic diameters [100–104]. However, it is typically necessary to encapsulate nanoparticles within microparticles for delivery using a DPI or engineer reversible nanoparticle aggregates capable of being inhaled. The nanoparticles will then be individualized upon contact with lung fluid once released from the microparticles [105]. This represents an additional production step that requires

the addition of extra excipients in the formulation. This can lead to the dilution of the final drug content and may potentially cause tolerance issues [105].

d) Soft mist inhalers (SMIs)

Soft mist inhalers (SMIs), as well as DPIs and MDIs, are portable inhalation devices. The drug formulation is inserted into a unique nozzle called ‘uniblock’, which propels the solution into two fine and converging jets at a predefined angle. The collision of these two jets enables the production of a light mist highly composed of fine particles (between 65 and 80 %). Easier for the patient to use, its relatively long aerosol generation time (1.5 seconds) allows for better coordination of inhalation and actuation. Finally, as its operation is purely mechanical, it does not require a propellant gas, unlike MDIs [106]. The development of this device was guided by the principles of an ideal inhaler, considering patient feedback, pharmaceutical standards, and ecological considerations as well as the objectives of drug delivery efficacy with a high deposition of particles in the lung and a low one in the oropharyngeal region [107].

To conclude with the different inhalation devices on the market, the choice should be made on the basis of the formulation used and of the targeted organ since, as shown in **Table 1**, the local or the systemic administration depends mainly on the particle diameter [63,64,108]. Depending on the device, the aerosol generation process may potentially damage nanomedicines. Therefore, the formulation should be engineered to ensure the vector resistance. Addressing this issue often requires the use of excipients or stabilizing compounds, adding more complexity to the nanoparticle fabrication process, which already exhibits some lack of reproducibility. Furthermore, MDIs and DPIs are both good for therapeutic use as they are generally well accepted by patients, but they often produce a lot of large particles, increasing the inertial impaction in the upper airways [109]. The recent nebulizers on the other hand, generate smaller particles, increasing the amount that can reach the deep [109]. However, they also introduce shear stress, which may potentially break sensitive nanoobjects. Finally, the more recent device seems to combine the appreciation of great public with the delivery efficacy, maximizing the amount of drug that reach the lungs [107].

Table 1. Overview table of the particles size effects on their deposition zone and clearance.

Particle diameter	Zone		Generation	Force	Clearance	
					Hydrophobic surface	Hydrophilic surface
> 6 μm	<u>Conducting zone</u> Tracheobronchial region	Tracheal region	0	Inertial impaction	Mucociliary escalator	Diffusion Pinocytosis
		Bronchi aera	1		Endocytosis (epithelial macrophages)	Cough Mucociliary escalation
2 - 6 μm		Bronchioles aera	2 - 16	Sedimentation	Cough	Chemical binding (epithelial,

		Terminal of bronchial aera				sub-epithelial, secretion)
0.5 - 2 μm	<u>Respiratory zone</u> alveolar region	Respiratory aera	16 - 23	Diffusion	Phagocytosis (alveolar macrophages)	Diffusion Pinocytosis
		Alveolar ducts			Endocytosis (type I cells)	Endocytosis (alveolar macrophages, type I cells)
		Alveolar sacs			Fluid flux	
	<u>Systemic circulation</u>		-	-	To avoid clearance by macrophages, particle diameter should be less than 0.5 μm	To be translocated from the lung to the systemic circulation or lymph nodes, particles have to be negatively charged

IV – Pulmonary administration: for which disease?

As previously exposed, the oldest documented use of pulmonary administrated treatment was the delivery of henbane plants on Ebers' papyrus [37]. Since then, many other devices and applications had been described in history until the development of modern inhalation devices. Indeed, the first clinical practice of such an apparatus began in 1956, using MDIs only six years after its invention [110]. **Table 2** lists some drugs whose administration by inhalation had been approved on the market by U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) for local administration while **Table 3** shows approved inhaled drugs for systemic administration. As expected, most of the inhaled medicine currently available are design to target the lungs for local applications. One of the lung diseases commonly treated by inhalation is asthma. This world spread inflammatory disease is characterized by many symptoms like wheeze, difficulty to breath, caught and chest tightness due to narrowing and swelling of the airways [43,111,112]. The disease is usually treated using salbutamol. This active compounds specifically targets the β_2 -adrenergic receptors, relaxing the airways smooth muscles [113]. This treatment is commonly administered using affordable portable metered-dose inhalers. However, it is known that the use of these devices can be uncomfortable for patients due to coordination difficulties and nebulizers would be a good alternative [114]. Another pathology that can be treated by pulmonary route is cystic fibrosis. The most recognized symptom associated with this genetic disease is an excessive mucus production in the lungs. The thick mucus produced becomes difficult to clear due to a malfunction of cystic fibrosis transmembrane receptor (CFTR), increasing the susceptibility to recurrent bacterial and viral infections in the

respiratory tract. This pathology not only affects lungs but also other organs such as pancreas, sweat glands, digestive system, reproductive system and so on [115,116]. Cystic fibrosis treatment delivered by inhalation consists generally of antibiotics as tobramycin, aztreonam or amikacin which help to fight against bacterial infections but don't cure the disease [112,117,118]. More common medical conditions are also candidates for inhalable medications, such as the widely recognized seasonal flu, which can be treated with inhaled zanamivir [112]. Pulmonary arterial hypertension, bronchospasms, pneumonia, non-tuberculous mycobacterial lung infection and chronic obstructive pulmonary disease (COPD) can also be cured by inhalation, for a local action [112,118–120].

As discussed previously, respiratory route of administration also gives the possibility to delivery drugs into the systemic circulation. One of those organs and probably the easiest to be reached by pulmonary administration, is the heart. Indeed, some recent publications show that nanomedicines can be administrated to the heart from the respiration thanks to the thinness of lung epithelium (0.1 – 0.2 μm), its large surface of absorption ($\pm 100 \text{ m}^2$), its proximity with the blood vessels and the high local blood flow (5 L/min) [44,121,122]. The heart is not the only organ that could be targeted *via* pulmonary administration. Almost every organ could as soon as the drugs are able to reach the systemic circulation as described in **Figure 3**. Even the brain could be targeted by inhalation, but this would mostly concern nasal route due to the direct contact of the afferent olfactive nerves with the nose inner chamber instead of pulmonary route [36,123]. Therefore, as indicated in **Table 3**, lung-to-brain delivery is also a possibility, as Adasuve® and Inbrija® are delivered through the lungs into the bloodstream [124,125]. This method to accessing the bloodstream was recently explored and there are still not so many drugs available on the market. As visible on **Table 3**, these drugs arrived on European and US market in the 2010's and there are not so numerous yet. They are all delivered to the patient through dry powder inhalators.

Insulin was the first commercialized active compound concerned, with a first commercialization in 2006 both in Europe and USA, followed by another formulation FDA-approved in 2014 [126,127]. Surprisingly, the two others inhaled drugs on the market are treating mental illness as schizophrenia and Parkinson disease while the heart appeared to be the easiest organ to target [112,124]. A substantial amount of research is presently underway to harness the pulmonary route for systemic delivery. Some inhaled medicines could treat hepatitis, heart diseases or HIV, pain, anxiety, cardiovascular crises, insomnia, cancer and so on [97,127–130]. Some other are even already patented, delivering immunoglobulin, mRNA, liposomes containing gene silencing therapeutics and may appear on the market soon [131]. Finally, despite the limited availability of inhaled medicines targeting the systemic circulation on the market, this method of administration holds great promise. However, there are currently no nanomedicines on the market for systemic delivery through the lungs, despite the potential opportunity it presents. Nanomedicines could be delivered through the lungs to the systemic circulation, as their characteristics enable them to pass through the air-blood barrier [36]. Furthermore, when focusing on the inhaled nanomedicines approved on the market, it is challenging to find examples, as demonstrated by **Table 4**, which only contains three instances. One of those three example, Amikacin, can be delivered daily in the form of a liposomal formulation called Amikacin Liposome Inhalation Suspension (ALIS) commercialized as Arikayce®. This formulation was specifically developed for nebulized administration of the broad-spectrum aminoglycoside, polycationic, semi-synthetic antibacterial agent amikacin. This antibiotic binds to the 30S ribosomal subunit, inhibiting bacterial protein synthesis and causing the death of micro-organisms [132]. Amikacin as

well as the two other approved inhaled nanomedicine mentioned in **Table 4**, Curosurf® and TOBI Podhaler® are for local administration in the lungs [133,134]. The scarcity of aerosolized nanomedicine for lung administration, despite the numerous advantages highlighted throughout this review, may find its roots in certain technical explanations. Firstly, the issue of nanomedicine breaking during aerosol generation, caused by the devices, could be one such factor. Further enhancements of these devices are essential to be adapted for the delivery of nanoobjects. Furthermore, inhaled nanomedicines still have to be improved to be used in therapeutics as for the moment, they can show some toxicity. Generally, their development process stop in clinical phases 1 or 2 because of reported side effects including nausea, cough or tiredness [135]. However, we haven't been able to identify any ongoing 2 nor 3 clinical trials. This could be attributed to a lack of understanding regarding the interaction of nanoparticles with cells or their targets and to a gap between the animal models used to characterize the biological efficacy and the clinic reality [136]. Indeed, to progress to clinical phase 1, preclinical research should include safety data along with a comprehensive study of the pharmacodynamics and pharmacokinetics of the drug [137]. Nevertheless, the scientific community is actively engaged in the development of inhaled nanotherapeutics, and numerous promising studies are currently underway.

Table 2. Non-exhaustive list of inhaled medicines approved on the market by U.S Food and Drug Administration (FDA) and European Medicines Agency (EMA) for lung local administration and DPIs, MDIs or nebulizers' delivery.

Local administration				
FDA approval year	EMA approval year	Active Pharmaceutical Ingredient	Brand name	Disease
Dry powder inhalers				
2000	2006	Zanamivir	Relenza®	Flu
2015	-	Salbutamol sulfate	ProAir RespiClick®	Asthma
Metered-dose inhalers				
2001	-	Salbutamol sulfate	Ventolin-HFA®	Asthma
1996 1 st Hydrofluoro-alkaline MDI	-	Salbutamol	ProVentil HFA®	Asthma
Nebulizers				
1996	-	DNAse	Pulmozyme®	Cystic fibrosis
-	2000	Hydrocortisone	DuoResp Spiromax®	Asthma
2001	-	Salbutamol sulfate	AccuNeb®	Asthma
2005	-	Levalbuterol hydrochloride	Xopenex®	Bronchospasms
2010	2009	Aztreonam	Cayston®	Cystic fibrosis

2016	-	Ribavirin	Virazole®	Infection pulmonary arterial hypertension
2017	-	Glycopyrrolate bromide	LONHALA Magnair®	COPD
1996	-	DNase	Pulmozyme®	Cystic fibrosis
-	2000	Hydrocortisone	DuoResp Spiromax®	Asthma

Table 3. Inhaled medicines approved on the market by U.S Food and Drug Administration (FDA) and European Medicines Agency (EMA) for systemic administration.

Systemic circulation				
FDA approval year	EMA approval year	Active Pharmaceutical Ingredient	Brand name	Disease
Dry powder inhalers				
2006 (removed)	2006 (removed)	Insulin	Exubera®	Type I and II diabetes
2012	2013	Loxapine	Adasuve®	Schizophrenia / Bipolar disorder
2014	-	Insulin	Afrezza®	Diabetes
2018	2019	Levodopa	Inbrija®	Parkinson

Table 4. Inhaled nanomedicines and nanomedicine-based approved on the market by U.S Food and Drug Administration (FDA) and European Medicines Agency (EMA) for lung local administration. DPPC: Dipalmitoylphosphatidylcholine – DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine – MAC: Mycobacterium Avium Complex – CF: Cystic Fibrosis – API: Active Pharmaceutical Ingredient

Approval year		Formulation	Diameter of the nanoparticle	API	Excipients	Brand name	Disease
FDA	EDA						
2018	2020	Liposome	294 nm	Amikacin	Cholesterol, DPPC, NaCl, NaOH, Water	Arikayce®	MAC lung Disease Cystic fibrosis

							Pneumonia
1999	1992	Liposome	80 and 800 nm	SP-B and SP-C	NaCl, NaHCO ₃ , Water	Curosurf®	<i>Pseudomonas aeruginosa</i> infection in CF patients
1997	2011	Powder / Lipid Nanoparticle	1-5 µm	Tobramycin	DSPC, CaCl ₂ .H ₂ O, H ₂ SO ₄	TOBI Podhaler®	Cystic fibrosis

V- Promising nanomedicines for lung administration: few concrete examples

a) local administration as a target

Cell penetrating peptide (CPP) for gene therapy

The local administration of nanoparticles could theoretically be used to treat every common lung disease, and it also can be used to perform gene therapy. That's the objective Osman *et al.* pursued using nanoparticles interacting electrostatically with DNA to treat cystic fibrosis [138]. The incurable inherited disease is caused by a CFTR gene mutation, as explained before [115,116] and as many as 1990 different mutations have been reported, not all of them leading to a pathologic condition [139]. Nevertheless, most of them alter the production of CFTR proteins, preventing the correct regulation of the cyclic adenosine monophosphate (cAMP)-dependent chloride channel and bicarbonate secretion as well as epithelial sodium channel causing improper transport of ions, mucus stasis, chronic lungs infections and pulmonary failure [138]. The strategy of Osman *et al.* is to develop a nanoparticle composed of a cell penetrating peptide (CPP) as a gene vector to target alveolar epithelium stem cells. For that, they modified a CPP into a glycosaminoglycan-binding enhanced transduction (GET) peptide containing heparan sulfate (HS) cell targeting sequence. Indeed, the GET peptides are known for enhancing transduction for difficult-to-transduce cell lines. After that, they covalently coupled the GET-peptide to a PEG through a maleimide-thiol coupling reaction, to obtain *via* self-association DNA-loaded nanoparticles enabling lung gene therapy. After *in vivo* assays on healthy mouse lung model by pulmonary administration, the team showed promising results for gene therapy by inhalation: the formulation still must be tested on CF-mice models.

Polymeric nanovectors

One of the recent proofs that nanomedicines by inhalation delivery could be a great medical strategy has been brought by Patel *et al.* with their paper about silibinin loaded poly(ϵ -caprolactone)/pluronic F68 inhalable nanoparticles against lung cancer. In this study, silibinin, a natural polyphenolic compound derived from milk thistle seeds and one of the two diastereoisomers composing the silymarin complex, was employed. It is renowned for its hepatoprotective

properties and utilized in the treatment of liver diseases [140,141]. In addition to its hepatoprotective effects, silibinin has shown significant antiproliferative effects, limiting tumor growth and inducing strong apoptotic death in endothelial cells [141]. More specifically, silibinin may exert a potent effect on both small and non-small human lung carcinoma cells by inhibiting their growth, moderating cell cycle arrest, and inducing robust apoptotic death [141,142]. Despite promising effects on cancer cells, silibinin presents poor aqueous solubility and oral bioavailability [143,144]. To overcome the drug poor bioavailability and solubility, Patel *et al.* loaded the molecule into poly(ϵ -caprolactone) / pluronic F68 nanoparticles. Poly(ϵ -caprolactone) (PCL) is a non-toxic, biodegradable, and biocompatible polymer known for its hydrophobic nature [145,146]. This polymer is extensively utilized for drug delivery, and various formulations, including micelles, hydrogels, scaffolds, fibers, films, and microspheres loaded with antitumor agents, have been developed. Its gradual degradation profile enables the prolonged release of drugs [144]. PCL is frequently co-polymerized with pluronic F68 surfactant to obtain an amphiphilic co-polymer, in order to produce nanocarriers. Additionally, as a poloxamer, pluronic F68 is able to mitigate the initial drug burst release by slowing down drug diffusion through the polymeric matrix [147]. In Patel *et al.* study, it is utilized both as a pore-forming agent to enhance drug release in PCL and for its interference with multidrug-resistant (MDR) tumors, making them more sensitive to anticancer treatments. They produced PCL/pluronic F68 polymers inhalable nanoparticles loaded with silibinin by solvent displacement process. The nanoparticles were then freeze dried to permit *in vivo* delivery through a modified DPI, before being used for a complete study including: characterization of nanoparticles and optimization of their production methods, *in vitro* drug release, *in vitro* cytotoxicity and clonogenic assays on A549 cells, ROS production measurement, *in vitro* pulmonary deposition tests, completed by *in vivo* antitumor activity, biodistribution and pharmacokinetic study and colloidal stability studies on Sprague-Dawley rats NNK lung cancer models. The outcome of this study revealed the successful generation of uniform spherical nanoparticles with a good drug loading, exhibiting favorable aerosolization behavior and sustained release characteristics. When compared with the efficacy of the drug administered alone and compared with other silibinin-loaded PCL/Pluronic F68 nanoparticles, Patel *et al.* results surpassed those reported in literature. They asserted having conceived an inhaled nanoparticle formulation that holds promises for future preclinical and clinical studies, representing a significant advancement in the treatment of lung cancer through inhalation delivery.

Inhaled polyplexes

Well known by the great public since the COVID-19 crisis, the use of mRNA-based therapy is gaining more and more importance. Already on the market, notably with mRNA-based vaccines targeting the SARS-CoV-2 virus, mRNA can also be used for gene editing, protein replacement therapy and treatment of genetic diseases, hypercholesterolemia, muscular dystrophy and even acute hepatic porphyria [86,148]. However, exogenous mRNA is easily recognized by host immune system and must be modified to allow their transcription into cytoplasm by host cells. The production of modified and optimized mRNA occurs *in vitro* and the resulting biomolecule is called *In vitro* Transcribed (IVT) mRNA [149–151].

Inhaled mRNA delivery to the lungs could be significantly enhanced using IVT-mRNA, and this is precisely the focus of Patel *et al.* who engineered mRNA polyplexes. Polyplexes are formed by complexing mRNA with cationic

polymers, enhancing mRNA transfection efficiency, which is significantly low when using mRNA alone due to its anionic nature and its large molecular weight [152]. Hence, Patel *et al.* synthesized hyperbranched poly(beta amino esters) (hPBAEs), cationic biodegradable polymers, to create stable and concentrated inhalable polyplexes nanoformulations. The luciferase coding mRNA- hPBAEs polyplexes were then administered to mice through the use of a vibrating mesh nebulizer [151]. The particles demonstrated stability even after nebulization, as evidenced by electron microscopy. Furthermore, the mRNA within lipoplexes, encoding luciferase, exhibited lung biodistribution 24 hours post-nebulization, with luminescence levels surpassing the control lacking hPBAEs. These results demonstrated the protective impact of lipoplexes on mRNA. Additionally, a uniform distribution of polyplexes in the lungs was observed 24 hours after nebulization across all five lobes. In conclusion, this study presents a promising tool for delivering mRNA to the lungs using a vibrating mesh nebulizer, potentially paving the way for clinical applications in the treatment of lung diseases [151].

Inhaled mRNA loaded lipid nanoparticles

One of the most studied mRNA delivery systems is lipid nanoparticles (LNPs), composed notably of ionizable lipids bringing: protection of the mRNA from enzymatic degradation, improvement of cell internalization and endosomal escape [153]. Sarode *et al.* team used this LNP system in their research to develop a mRNA-lipid nanoparticle dry powder product (DPP) using spray drying technology to deliver in the lung *via* inhalation [154]. As discussed in their article, delivering mRNA through inhalation could represent an excellent strategy to treat acute respiratory distress syndrome (ARDS), cystic fibrosis (CF), lung cancer, pulmonary alveolar proteinosis (PAP), pulmonary arterial hypertension (PAH), and primary ciliary dyskinesia (PCD). DPIs deliver dry powder products (DPP) that show higher stability than suspension or solution. This could represent an advantage for mRNA delivery, but the solid state for biological molecules requires the use of stabilizing agents or excipients because of their natural instability in solid form [155]. Nonetheless, there are very few reports in literature on excipient screening, formulation property optimization, or studies on the aerosolization of spray-dried mRNA LNPs. This lack of information prompted Sarode *et al.* to focus on the optimization of the spray drying process for LNP formulations in hydroalcoholic solutions, employing novel ionizable lipids along with mannitol and leucine as excipients [154]. The two novel ionizable lipids were evaluated in this study, the first one based on a phenolic acid core and the second one on a citric acid core, exhibiting potential biodegradability due to the presence of ester linkers in their molecular structure. This innovative family of ionizable lipids, rooted in phenolic acid and citric acid cores, demonstrated as a valuable candidate for the delivery of mRNA through inhalation, showcasing their promise in advancing respiratory drug delivery strategies. Following the optimization of their formulation and intratracheal administration to CD-1 male mice, Sarode *et al.* convincingly demonstrated that their optimized dry powder formulation of mRNA-LNPs provided an effective and functional delivery system for administering mRNA therapeutics to the lungs through inhalation [148,154].

Another example of inhaled mRNA loaded LNPs is the study of Mukherjee *et al.* on cystic fibrosis treatment [156]. As explained previously, cystic fibrosis is a genetic disease due to a CFTR receptor gene mutation leading to the cyclic adenosine monophosphate (cAMP)-dependent chloride channel dysregulation and a bicarbonate secretion and epithelial sodium channel malfunction causing improper transport of ions, mucus stasis, chronic lungs infections and

pulmonary failure [115,116,138]. Furthermore, the excessive activity of epithelial sodium channel (ENaC) could explain two cystic fibrosis consequences: airway dehydration and impaired mucociliary clearance [156]. ENaC, a tetrameric channel (composed of α , β , γ and δ subunits), is highly selective for Na^+ and K^+ ions and it is expressed in several organs, including lungs where it regulates fluids balance and viscosity [157]. In the airways, electrogenic absorption of Na^+ from mucosal surface, resulting in water absorption, is operated by ENaC [156]. The objective of this research is so to inhibit ENaC, reducing sodium absorption, to restore an effective airway liquid surface hydration. The research team formulated lipid nanoparticles loaded with inactivating mutant ENaC α mRNA (α_{mut} ENaC). They studied the transfection of their nanoparticles both *in vitro* and *in vivo*. For this animal study, they administered the mRNA-LNP *via* nasal instillation on BALB/c and *CTFRKO* mice. *In vitro*, they obtained an efficient inhibition of ENaC activity, reducing amiloride-sensitive current by 85 % with a transfection of ENaC α mRNA altering selectively ion transport in ENaC expressing cells, minimizing off-target effects. They also measured an increase in airway surface liquid of 34 %. *In vivo*, they observed after a two-days delivery of the ENaC α mRNA-LNP the amiloride response decreased suggesting a potential therapeutic impact. In conclusion, Mukherjee *et al.* engineered mRNA-LNP which could constitute a universal treatment for cystic fibrosis, regardless of CTFR mutations. Their future studies will now occur on “ α -ENaC-overexpressing mouse model that replicates inspissated mucus phenotype observed in cystic fibrosis patients” which will allow inhalation lung delivery [156].

b) The systemic circulation as a target

Silica nanoparticles coated with PLGA

Considerable research is being conducted to deliver inhaled nanomedicines into the systemic circulation, and some of these approaches have even reached the *in vivo* stage. It is the case for Santin *et al.* whose article was published recently, their objective being to restore normal heart function after doxorubicin treatment [158]. Doxorubicin is an anthracycline anticancer drug, firstly isolated in the 70's from *Streptomyces peucetius var. caesioides*, used to treat breast, lung, ovarian, gastric cancers and so on. Its mechanism of action can be explained in two manners: intercalation into DNA, disrupting topoisomerase-II-mediated DNA repair or free radicals generating, damaging cellular membranes, DNA and proteins [159]. In addition to its therapeutic effects, doxorubicin exhibits cardiac side effects leading to potentially lethal cardiomyopathy after only few days of administration [160]. One recently formulated explanation of this doxorubicin cardiotoxicity is the blocking of autophagic flux in cardiomyocytes by the antimalignant drug. This blockade disrupts the functioning of lysosomes, making them less acidic [161]. The idea proposed by Santin *et al.* is to formulate PLGA-grafted silica nanoparticles (NPs-PLGA) to target the lysosomes and reestablish their acidic pH to restore their normal function. The poly(lactic-co-glycolic acid) (PLGA) being a biodegradable polymer of lactic acid (LA) and glycolic acid (GA), it has the capacity of acidifying lysosomes [162]. Thus, in parallel of a doxorubicin treatment, a suspension of the NPs-PLGA was pulmonary co-administered intratracheally to mice to pass the air-blood barrier and directly reach the heart. The team obtained promising results with prevention of lysosomes alkalization, restoring their activity as well as the autophagic flux and preserving the mitochondrial function. Authors demonstrated

as well that the nebulization of NPs-PLGA was cardioprotective against doxorubicin: cardiac fibrosis was decreased, cardiomyocyte atrophy was prevented, and a limited cardiac dysfunction was observed.

Peptide loaded calcium phosphate nanoparticles

Myocardial diseases are typically addressed through oral or injection-based treatments. However, the oral route can exhibit unreliable enteral resorption, while injections, whether intravenous, subcutaneous, or intramuscular, often lead to patient discomfort [121]. The objective of Miragoli *et al.* is to deliver heart failure treatment by inhalation to avoid those discomfort and absorption issues. They previously conceived biocompatible and biodegradable negatively charged calcium phosphate nanoparticles (CaPs). They used a biomineralization-based strategy to obtain 20 to 50 nm of diameter particles as a micro RNA nanovector that successfully crossed cardiomyocyte cellular membrane and released the biomolecular cargo into the cells [163]. Recognizing the capability of CaPs to cross the cardiomyocyte membrane, they opted to design peptide-loaded CaPs for delivering R7W-MP peptide into the myocardium through pulmonary administration. This peptide is a cell penetrating peptide mimicking melusin natural protein, a chaperon protein highly expressed in cardiac muscle. Melusin plays a protective role for the heart after pathological injuries by phosphorylating Akt, Gsk3beta, and Erk1/2, activating the Akt myocardial signaling pathway [164]. R7W-MP also targets Cav β 2 cytosolic subunit of the L-type calcium channel (LTCC), improving cardiac contractility in pathologies with LTCC alterations by restoring the channel density at the plasma membrane [121]. After *in vitro* characterization of the inhalable CaPs, they proved the stability of their ζ -potential at -32 ± 3 mV and a diameter of 80 nm stable for 8 days at 5°C. They determined an interaction of CaPs with proteins by incubating in fetal bovine serum. An increase of the diameter reaching 280 nm after 7 days and an increase of the ζ -potential from -30 to -10 mV were observed, confirming the interaction between CaP surface and proteins. Then, they prepared fluorophore-loaded CaPs to evaluate their *in vivo* distribution through oral, intraperitoneal, intravenous and inhalation routes. The oral route failed to specifically target heart, but the three other administration routes showed a rapid delivery of the CaPs after 40 min, especially via the inhalation route which demonstrated the best results. For peptide-loaded CaPs, the surface charge steadily stayed at -31 ± 3 mV and they presented a round shape with a diameter of 20 to 50 nm. After peptide loading evaluation, they found that almost the entire amount of peptide was successfully loaded on CaPs. The loading was stable for 10 hours storage at room temperature then a small decrease of loaded-peptide amount was observed after 24 hours. The formulation has been tested *in vivo* on a mouse model of streptozotocin (STZ)-induced diabetic cardiomyopathy *via* inhalation of CaPs loaded with R7W-free therapeutic peptide. They observed a total recovery of cardiac function and an improvement of molecular and cellular defects due to the diabetic cardiomyopathy, explained by a direct targeting of the peptide to LTCC, restoring the channel protein levels and recovering its current as well as LTCC-related contractile properties. In conclusion, Miragoli *et al.* showed that peptide-loaded CaPs represent a promising tool to administrate therapeutics peptides to the heart through inhalation administration.

Conclusion

Pulmonary route is a non-invasive way of administration allowing to avoid the first pass metabolism and systemic toxicity. Despite some inherent barriers, nanomedicines could be an opportunity to deliver non-soluble or sensitive compounds to the lung or through the lung to the bloodstream. Indeed, their characteristics enable them to counteract

these biological barriers. Their nanometric size as their surface properties are assets for avoiding macrophage entrapment and penetrating lung epithelia, ensuring they are transported in droplet or aggregate form to prevent exhalation. The diversity of nanoparticles and the ability to decorate their surface with stealth molecules such as PEG, mucolytic agents, muco-inert polymers, and more, enable them to evade mucus and surfactant clearance or target them. The ability to design positively or negatively charged nanoparticles is also a strength for targeting or avoiding the mucus. It also allows for the design of nanovectors capable of crossing the air-blood barrier or, conversely, remaining in the lungs. The translocation of nanomedicines across the air-blood barrier to the bloodstream opens up a new pathway to target various organs, including the heart, liver, and even the brain avoiding the issues brought by intravenous or oral route. Delivering nanomedicines through the lung appears to be a promising and innovative route of administration. However, there is still a need for advancements in this field, ranging from the inhalation devices available on the market to the enhancement of characterization techniques, the animal models used and pharmacokinetic/pharmacodynamic studies.

ETHICAL STATEMENT:

Ethics approval and consent to participate

Not applicable to this review.

Consent for publication

Not applicable to this review.

Availability of data and materials

Not applicable to this review as no data were generated or analyzed during the current study.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Authors' Contributions

The first draft of the manuscript was written by Méline Guérin and Elise Lepeltier commented and corrected the different versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

Data sharing not applicable to this article as no dataset was generated or analyzed during the current study.

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