# Nano-delivery to the lung - by inhalation or other routes and why nano when micro is largely sufficient?

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**Abstract**

Respiratory diseases gather a wide range of disorders which are generally difficult to treat, partly due to a poor delivery of drugs to the lung with adequate dose and minimum side effects. With the recent developments of nanotechnology, nano-delivery systems have raised interest. In this review, we detail the main types of nanocarriers that have been developed presenting their respective advantages and limitations. We also discuss the route of administration (systemic versus by inhalation), also considering technical aspects (different types of aerosol devices) with concrete examples of applications. Finally, we propose some perspectives of development in the field such as the nano-in-micro approaches, the emergence of drug vaping to generate airborne carriers in the submicron size range, the development of innovative respiratory models to assess regional aerosol deposition of nanoparticles or the application of nano-delivery to the lung in the treatment of other diseases.

**Key-words**

Nano-delivery; Lung diseases; Inhalation; Nano-carriers; Aerosol; Nanomedicine

**Table of content**

## 1. Introduction

Respiratory diseases gather a wide range of disorders including acute and chronic respiratory infections, lung cancer, asthma, chronic obstructive pulmonary disease (COPD), pneumonia, cystic fibrosis and tuberculosis [1,2]. Unfortunately, respiratory diseases are frequent, with a high incidence worldwide, and are responsible for almost 4 million deaths every year representing a major public health issue [1]. In particular, lung cancers are among the most frequent cancers with 2.21 million cases in 2020, and exhibit the highest rate of mortality with 1.8 million deaths in 2020 worldwide [2–4]. Lung cancer prognosis is poor often due to an advanced stage at time of diagnosis [5]. In addition, the lung is one of the most frequent sites of metastasis from primary cancers [3]. COPD is also a concerning disease, it has become the third leading cause of death worldwide, with 3.23 million deaths in 2019, and the number of deaths is growing annually [2,6].

Although progress has been made in the diagnosis and treatment, an effective therapy for severe and chronic diseases is still missing. Moreover, certain lung diseases such as COPD and pneumonia are becoming increasingly difficult to treat. Several factors can be involved such as the difficulty for drugs to reach the lower respiratory tract with adequate dose and minimum side effects or the development of multidrug-resistance [1,2,5]. In the treatment of COPD, antibiotics, bronchodilators, and glucocorticoids can be used. However, they are not specific drugs and their long-term use can be associated with adverse effects [2]. Furthermore, most obstructive pulmonary disorders are associated with chronic inflammation, leading to cough and secretion of mucus in the airway, which forms a "barrier" to an efficient drug delivery [2,7–9]. In addition, bacteria growing in mucus form a dense bacterial biofilm resistant to drugs [2]. As poor delivery of drugs to the lung is a factor responsible for the lack of success of lung disease treatment, new ways to reach this objective have been considered to improve it. In particular, with the recent developments of nanotechnology, the development of nano-delivery systems has raised interest.

## 2. Nano-delivery systems

### *2.1. Definition*

Nanomedicine can be defined as the application of nanotechnologies to health and its related research. According to the European Science Foundation, the aim of nanomedicine includes the comprehensive monitoring, control, construction, repair, defense and improvement of all human biological systems, working from the molecular level using engineered devices and nanostructures, ultimately to achieve medical benefit [10]. Thus, nanotechnology consists of

the design, characterization, intentional production and application of nano-objects of controlled physicochemistry. In this context, nano- and submicron particlescan be used to deliver drugs, genes or vaccines to specific tissues or cells by targeted delivery or systemic route [7,11]. It is here important to mention that strictly speaking, the nanoscale ranges from 1 to 1000 nm but nanoparticles are defined as particles which size is lower than 100 nm [1]. Bigger particles (100 nm - 1 µm) are consequently referred to as submicron particles.

Nanoparticles (NPs) can thus be used as carriers, allowing the delivery of therapeutic and/or diagnostic agents. These agents are either encapsulated, adsorbed or covalently bound to the NP surface [2,12]. Due to its nanoscale size, nanomedicine brings new functionalities in comparison with classical formulations [3]. For instance, nanocarriers allow improving the stability of the transported active agent, preventing its degradation by protecting it from extracellular enzymes or avoiding clearance systems, fostering its cellular uptake, and allowing a controlled and targeted delivery to a specific site with a homogenous distribution, also prolonging its retention time in the target tissue, while reducing the side effects by shielding. All these factors contribute to improve the pharmacokinetics and pharmacodynamics of the active agent [3,7,13].

Different types of nanocarriers have been developed as detailed below. Ideally, they should be biocompatible, biodegradable, non-immunogenic, and with high stability and scalability [13]. When administered by intravenous route, nanocarriers can accumulate preferentially in tumors due to the enhanced permeability and retention (EPR) effect [3,14–16]. Indeed, they are able to cross tumor vascular endothelium but not healthy tissue vascular endothelium because tumor vascularization is altered and more permeable. This passive accumulation of nanocarriers resulting from a mechanical and spontaneous effect, allows tumor targeting.

To increase the delivery of active agents to specific target cells or tissues, active targeting is an option consisting of the addition of a targeting ligand to the surface of the nanocarrier. These ligands can be proteins, peptides, polysaccharides, glycolipids, glycoproteins, or antibodies with receptors that are overexpressed at the surface of target cells or tissues [3,13,14,16].

### *2.2. The different types of nano-delivery systems*

Several materials have already been approved by the Food and Drug Administration (FDA) as drug delivery systems. They include liposomes, polymeric nanocarriers, dendrimers, inorganic nanoparticles and proteins [1]. Not all of them are used in clinical practice, the most complex materials still need further research and are rather being developed in the context of pre-clinical

studies. Figure 1 illustrates some examples of the main nanomaterials used or in development for medical purposes (not exhaustive); a brief description is also given for each below.

Liposomes consist of vesicles made of phospholipids and cholesterol bilayer. Due to their amphiphilic nature, they can carry both hydrophilic and lipophilic formulations: the inner core can encapsulate hydrophilic drugs while hydrophobic drugs can be entrapped in the lipid bilayer [1,5,17]. In addition, because of their composition similar to that of cell membranes or components of lung surfactant, liposomes appear biocompatible, with a low toxicity. Furthermore, their lipidic nature can allow them to cross biological barriers and hence promote their absorption [1]. Based on these advantages, liposomes are one of the most extensively studied systems for controlled drug delivery to the lungs [17,18]. In 1995, they became the first FDA-approved nanocarrier (Doxil®: a doxorubicin PEGylated liposome, which has been clinically used in the treatment of lung cancer) [1,2,19]. Since then, many different drugs have been encapsulated in liposomes [17] such as antibiotics [20,21], bronchodilators [22,23], immunosuppressants [24,25], anticancer drugs [26], sex hormones [27,28], peptides [29], proteins [30] and oligonucleotides [31].

Solid lipid nanocarriers (SLNs) are also based on a lipid structure but they are slightly different from liposomes in the sense that they are made of solid lipids (*i.e.* lipids solid at room temperature), surfactant(s) and water [1,18].

Polymeric nanocarriers have also been designed for drug delivery. Polymers can be natural (such as albumin, gelatin, alginate, collagen, cyclodextrin and chitosan) or synthetic (*e.g.* poly lactic acid (PLA), poly-lactic-co-glycolic acid (PLGA), polycaprolactone (PCL), polyacrylates, polyethyleneimine (PEI), polyethylene glycol PEG, polyanhydrides and poly-l-lysine) [1,18,32]. These materials are characterized by their biocompatibility and biodegradability [18,32]. For the treatment of respiratory diseases, polymeric nanoparticles have been intensively studied to carry pulmonary drugs such as anti-asthmatic drugs [33,34], anti-tuberculosis drugs [35,36], pulmonary hypertension drugs [37], and anticancer drugs [38]. PLGA, that has been approved by the FDA, has also been considered for gene transfer applications as an efficient non-viral vector [1,39]. Similarly, PEI and chitosan are commonly used in gene therapy [1].

Dendrimers are three-dimensional structures consisting of multi-branched polymers, their surface can be functionalized with various groups. These later enhance dendrimers versatility and biocompatibility. Furthermore, they can be modified by other charged compounds through electrostatic interactions. Dendrimers can carry different drugs with variable solubility [1]. An

example of the use of dendrimers lies in the treatment of inflammatory respiratory diseases related to asthma [1].

Inorganic nanocarriers including gold, silica, iron oxide, alumina or titanium dioxide nanoparticles, exhibit several advantages. For instance, they are highly biocompatible, stable, resistant to microbial degradation and show a high delivery efficiency as well as magnetic properties [1]. Iron oxide nanoparticles can be used for the diagnosis of respiratory diseases, using positron emission tomography, computed tomography, magnetic resonance imaging and gamma scintigraphy [40,41]. In addition, their heating using an external magnetic field can result in the induction of apoptosis in the neighbor cells, potentially leading to the elimination of cancer cells [1]. Still regarding the treatment of respiratory diseases, gold nanoparticles have been used as nanocarriers in drug delivery systems [1].

### *2.3. Advantages of the nano-delivery systems*

Nano-delivery systems provide several advantages compared to classical formulations or micron-sized delivery systems.

#### *2.3.1. Targeted drug delivery*

As mentioned before, a poor drug delivery to the lung is a reason for the failure of the treatment of respiratory diseases. Nano-delivery systems have been considered to increase the local drug concentration in areas of interest such as tumors, rising therapeutic effects, also decreasing the global dose to administer, and consequently inducing less side effects. Nano-delivery systems can help control the release of the drug, overcome the lung barrier, especially mucus secretions that can be abundant in some diseases, and increase the permeability of the drug in the lung. This finally results in enhanced cell uptake and consequently, therapeutic effects may be reached with lower drug doses [1,2,13]. In addition, as mentioned before, the EPR effect can enhance passively the targeting of the drug in tumors [3]. Active targeting is also possible to further target the nanocarrier towards a specific cell type or tissue, expressing specific receptors. Two recent reviews illustrating the application of targeted drug delivery can be quoted: Rommasi and Esfandiari [42] discussed the application of liposomes in drug delivery in cancer therapy while Habib and Singh [43] reviewed the merit of the lipid-mediated gemcitabine delivery, especially with regards to overcoming the obstacles associated with conventional chemotherapy.

#### *2.3.2. Increased drug bioavailability*

Nano-delivery systems can increase the bioavailability of the drug they carry by allowing a better water solubility (in particular for insoluble hydrophobic drugs) and stability [44,45]. Indeed, by suspending the drugs as nanoparticles, one can achieve a dose that is higher than that of a solution, which is thermodynamically limited by the aqueous solubility of the drug [2,46,47]. Another way for a nanocarrier to improve drug bioavailability is by protecting it from degradation [2]. Indeed, when encapsulated in the nanocarrier core, drugs are protected from enzymatic attacks [13]. In addition, to further enhance the drug bioavailability various stabilizers, absorption enhancers, and mucoadhesive adjuvants, such as fatty acids, surfactants, and protease inhibitors, can be used [13,48,49]. A typical example is PEGylation, the addition of safe, non-biodegradable and FDA-approved polyethylene glycol (PEG) chains at the surface of the nanocarrier. It is a common process to improve the therapeutic value of a medicine by prolonging its body residence time [2,5,17]. The presence of PEG prevents opsonins from binding to the nanocarrier (by steric hindrance) and thus prevents the elimination of the nanocarrier by phagocytosis. Therefore, PEG provides a "stealth" shield to the drug to bypass the body's defense mechanisms, allowing to prolong its half-life [5].

### *2.4. Limitations of the nano-delivery systems*

Despite their numerous advantages previously detailed, nanocarriers also exhibit some limitations.

#### *2.4.1. Access to target tissue, bypassing the physiological barriers that are respiratory secretions*

The EPR effect is acknowledged to contribute to the passive accumulation of intravenously injected nanocarriers in a tumor. In practice, it appears to be quite limited in clinical trials as it is highly dependent on the patients, the tumor type and can vary over time [3,14,15]. Furthermore, the penetration and diffusion of a nanocarrier within a tissue are closely related to its physicochemical features, especially size, shape and surface chemistry [3,14,15]. In addition, when nanocarriers are introduced in the blood stream, the many present proteins bind at the surface, leading to the formation of the protein corona [50–52]. This new interface screens molecules grafted on purpose at the surface of the nanocarrier for active targeting, resulting in ineffective targeting of the drug. Another strategy to overcome this issue is to deliver nanocarriers directly into the tumor, *i.e.* through inhaled route for lung diseases [3]. Although this administration route has advantages, as will be discussed with more details later, it has also to face some challenges. Indeed, after local administration of the nano-delivery systems, they have to overcome some physiological barriers, particularly present in obstructive diseases and

consisting of respiratory secretions, such as mucus and alveolar fluids (*e.g.* pulmonary surfactants), in order to gain access to the target cells or tissue. Nanocarriers can thus be entrapped in mucus through steric, electrostatic, or hydrophobic interactions [11,53]. Nanocarrier properties play a key role in these interactions. As an example, it has been reported that small nanocarriers (~ 100 nm) can better cross mucus layers than larger ones (> 250 nm) [11,54]. Another way to ease the transport through the mucus secretions is by PEGylating nanocarriers, this coating will reduce the nanocarrier adhesion to the mucus, allowing them to diffuse more freely [7,11,55].

#### *2.4.2. Clearance*

Nanocarriers have to overcome the natural defense systems of the organism such as mucociliary clearance and alveolar phagocytosis [7]. Indeed, nanosystems can be trapped in the airway mucus that forms an extracellular gel mainly composed of water and mucins (heavily glycosylated proteins). They are then transported out of the lungs by means of ciliary beating and cough. After mucus ascends the trachea, it is propelled by ciliary epithelium in the larynx and is swallowed [56]. Nanocarriers can also be recognized as foreign bodies by cells from the immune system (reticuloendothelial system), such as hepatic and splenic macrophages in systemic delivery and alveolar macrophages in topical delivery [11]. Here again, the physicochemical features of the nanocarriers will have an impact on their tendency to be phagocytized by alveolar macrophages, especially size, as it has been reported that optimal size for phagocytosis is 500-3000 nm [13]. PEGylation by preventing opsonins to bind to the nanocarrier surface and induce their phagocytosis is a way to cope with this early clearance [51,53].

#### *2.4.3. Toxicity*

The potential toxicity of nanocarriers has also to be carefully taken into account. The smallest nanocarriers are not easily phagocytized by macrophages and, consequently remain in the alveoli, they can then translocate to the interstitium potentially causing side effects [18]. Moreover, by their nature, some nanocarriers may be more toxic than others. For instance, while liposomes seem relatively safe because of their similarity with biological compounds, polymeric and inorganic nanocarriers could be more prone to induce adverse effects [17]. For instance, although PLGA is biocompatible, its low degradation may result in excessive accumulation in the respiratory tract, generating a pH drop and an acidic environment that will lead to damages [1,17,57,58]. In addition to the possible inherent toxicity of the nanocarrier,

some excipients used in the nanoformulation can exhibit adverse effects [3,46,53]. As an example, polyvinyl alcohol (PVA) is the most commonly used emulsifier in the formulation of PLGA nanoparticles. Despite repeated washings, PVA is not completely eliminated and the residual PVA remaining associated with the nanoparticles can be responsible for adverse effects [59]. Inorganic nanocarriers are made of metals to which exposure of significant quantities in the lungs could lead to acute pulmonary inflammation [60–62] and oxidative stress [63].

#### *2.4.4. Technical challenges*

A major issue of nanoformulation is achieving a suitable drug payload in the nanocarrier, which is often in the range of 1-10% w/w [3]. In particular, liposomes exhibit a low encapsulation capacity (2 to 5%) [64,65] while polymeric particles can exhibit up to 10% drug loading [17]. In addition, some nano-delivery systems such as liposomes may show a low stability, a poor retention of drugs of intermediate solubility and a challenging large-scale production [5,66]. PEGylation has been proposed to prevent the aggregation of nanocarriers and thus improve their stability during storage [1].

#### *2.4.5. Nano vs micro-drug delivery systems*

Compared to formulations containing larger particles, nano-delivery systems offer several advantages. As nanoparticles are characterized by a large surface area to volume ratio, a great number of molecules can be added at their surface [17,46,47]. This large surface can be functionalized (with targeting agents) or loaded with the active agent to transport. On the contrary, more drug can be encapsulated inside sub-micron and microparticles (size typically ranging between 0.1 and 500 µm) which are also physically and chemically more stable than some nanosystems such as liposomes [5]. The use of submicron-sized particles is also safer when the formulation is administered via systemic route. Indeed, particles larger than 5 µm can cause pulmonary embolism, potentially resulting in fatal outcomes [18,67].

In addition, the optimal particle size for phagocytosis ranges from 0.5 to 3 µm, and it has been reported that particles smaller than 0.26 µm can escape phagocytosis by macrophages [17,18,68].

Nanoparticles can also show an enhanced cell internalization compared to larger particles and therefore potentially an enhanced drug delivery [46]. For instance, pulmonary epithelial cells internalize particles of 0.5 µm or smaller 10 times more than 1 µm particles and 100 times more than 2 or 3 µm particles [69]. Nanoparticles are also more internalized by vascular endothelial cells than their micro-sized counterparts (typically >1 µm in diameter) [70].

## 3. Administration route

The release rate of a drug carried by a nano-delivery system and its deposition in the lung parenchyma depends on the nanocarrier characteristics but also depends on the method of delivery [5]. Different administration routes are possible: intravenous injection, oral administration, or inhalation. Ideally, it should enable as much drug as possible to reach the target area. When choosing the route of administration one should take into consideration several parameters such as the physicochemical properties, pharmacology, and toxicology of the drug and delivery vehicle, as well as the anatomical features related to the disease [2].

### *3.1. Systemic administration*

Nano-delivery systems offer the possibility to carry active agents while altering their pharmacokinetics and biodistribution profile toward a preferential accumulation in affected tissues while sparing healthy tissues. They are mainly administered through systemic route. For instance, in the treatment of cancer, chemotherapy, consisting of intravenous injection or sometimes oral administration of non-specific and non-selective drugs is distributed not only to the tumors but to other organs where it can induce undesirable side effects such as myelosuppression, nauseas and vomiting, or alopecia. Moreover, because of this toxicity the treatment has to be interrupted frequently to allow bone marrow to recover but on the other side this suspension of treatment permits tumor cell repopulation [3]. To prevent such effects and because the drug concentration in the tumor was found to be low after systemic chemotherapy [71,72], nanocarriers were designed for a more targeted drug delivery. Because of the EPR effect previously described, they passively accumulate inside the tumor when intravenously injected [3,5]. But as the EPR effect is based on a typical characteristic of tumor vascularization, it is only limited to oncologic applications, while other diseases may not benefit from this passive targeting [11]. Therefore, considering more particularly lung diseases inhaled nanomedicine could have many advantages.

### *3.2. Inhalation*

#### *3.2.1. Advantages over other administration routes*

As targeted aerosol delivery to the affected lung tissues may both improve therapeutic efficiency and reduce adverse effects, inhaled aerosols are widely used for the treatment of lung

diseases such as asthma, COPD, cystic fibrosis, respiratory infection and, more recently, lung cancer [1,3,7,73].

An aerosol is a stabilized dispersion of solid or liquid droplets suspended in a gaseous vehicle [13]. Different types of aerosol generating systems exist as reviewed later and produce an aerosol inhaled by the patient [74].

Being a non-invasive drug delivery route for drug is a first advantage of inhalation, with a possibility for self-administration, improving the patient compliance [1,7,18].

The respiratory system offers a large surface area (100-140 $m^2$ in humans), with abundantly vascularized thin alveolar epithelium providing a favorable environment for the topical and systemic delivery of drug [1,5,7,13]. Inhalation allows delivery of high concentrations of drug to the target site, at the same time, the systemic drug concentration is low and consequently other organs are less exposed to the drug, thus minimizing side effects [2,5,7,18]. Pulmonary delivery allows reduction of administered dose of drug, for instance a reduction by 10 fold in the treatment of asthma compared to oral route [75].

Furthermore, pulmonary delivery allows an enhanced bioavailability of drugs to lung sites and offers the potential to achieve relatively uniform distribution of drug dose among the alveoli [1,5,18,47].

In case of lung tumors, when nanocarriers are administered via inhalation, they may not accumulate in the tumor through the EPR effect, unlike what happens when they are delivered via systemic administration. However, the drug is delivered directly to the lungs allowing passive targeting and nanocarriers are internalized by cancer cells via endocytosis, which does not occur in the case of solubilized drug. Thus, nanoparticles can increase penetration and accumulation of inhaled drugs in tumor tissues and cells, leading to improved anti-tumor activity compared with the free drug. In addition, due to their small size inhaled nanocarriers can cross the blood barrier and reach the systemic circulation and in a second time accumulate in the lung through the EPR effect [76]. Inhalation can deliver drugs to the tumors creating a favorable drug concentration gradient for diffusion, giving the drug an alternative to vascular access to the tumor, which is the main delivery route in systemic treatments [3]. However, some areas of tumors are poorly if even not vascularized, which renders them hypoxic, making an environment favorable to the development of invasive and resistant cancer cells or clonogenic cells that will subsequently cause tumor cell repopulation [77–79]. In addition, being farther from blood vessels, the cells from these areas are exposed to a much lower drug concentration from systemic routes [3].

Contrary to other routes of administration, inhalation has a rapid onset of action [80]. Last, but not least, pulmonary delivery allows bypassing hepatic first-pass metabolism [1].

The successful pulmonary delivery of drug involves a good understanding of the drug and aerosol characteristics, target pathophysiological lung condition and the aerosol generating devices [7,13]. Indeed, the deposition of inhaled drugs in the respiratory tract highly relies on several parameters, including the physicochemical features of the aerosolized drug particles.

### *3.2.2. Deposition of inhaled therapeutic agents in the respiratory tract - impact of the aerosolized drug particles features*

The size of the aerosolized drug particles, their shape, density, and electrostatic charge will deeply impact their deposition in the respiratory tract [12,13,18,81]. Particle size being the most important characteristic to take into account in order to achieve a deep lung deposition [47]. The size of these droplets is referred to as an aerodynamic diameter. This latter corresponds to the diameter of a sphere of unit density with the same settling velocity as the particle of interest [13]. It has been defined that to be inhalable an aerosol should be with an aerodynamic diameter lower than 10 μm and is classified into coarse particles (> 2 μm), fine particles (0.1-2 μm), and ultrafine particles (< 0.1 μm) fractions [49]. Figure 2 illustrates the influence of particle size in lung deposition (A) and the underlying mechanisms of deposition (B).

Particles with diameters higher than 5 μm tend to deposit in the upper airways by impaction, while particles with diameters in the 1-5 μm range are the most efficient to reach the deep lung by inertial impaction and sedimentation. Particles smaller than 1 μm can reach the alveoli through diffusion and sedimentation mechanisms [2,3,3,13]. In addition, charged particles could be exposed to electrostatic interactions [13].

For optimal aerosol delivery, it is considered that aerosol particles should have an aerodynamic diameter ranging between 0.5 and 5 μm and lung flow rates should be 15-30 L/minute [13,82,83]. By adjusting the particle size, one can achieve targeting to the desired lung region. For instance, particles with diameters of about 500 nm have been reported as ideal to be phagocytized by alveolar macrophages, which could be an interesting target in the treatment of tuberculosis as the bacteria responsible for this disease, *Mycobacterium tuberculosis*, infect alveolar macrophages [12,84]. Indeed, while phagocytosis of nanocarriers by macrophages is often a hurdle to the delivery of the nanomedicine it becomes an advantage in the case of infections such as listeriosis, salmonellosis, leishmaniosis, cryptococcosis, or tuberculosis where micro-organisms live and replicate within macrophages. One could thus take advantage of the nanocarrier phagocytosis to specifically target macrophages. As an example, we can

quote Arikayce, an amikacin liposome inhalation suspension, administered using a nebulizer, the first and only FDA-approved treatment option designed specifically for MAC lung disease. *Mycobacterium avium complex* (MAC) is the most common form of nontuberculous mycobacteria, which are commonly found in the environment. Upon inhalation, the liposomes reach the lungs where they can enter alveolar macrophages where the MAC bacteria live whereas free amikacin has a limited ability to cross the membranes of mammalian cells, reducing its ability to achieve sufficient anti-mycobacterial levels inside the infected cells. The liposomal formulation of amikacin has shown better *in vitro* and *in vivo* efficiency in the delivery of the drug into macrophages, airways, and lung tissue compared to its non-liposomal counterpart [85–87].

Successful aerosolization of various nanoparticle formulations has already been shown, including liposomes [88,89], polymeric nanoparticles [90–92], and even Cu/Zn nanoparticles [93]. However, liposomes are the only ones that have reached clinical development for pulmonary delivery [17]. Regarding inorganic nanoparticles, their potential aerosolization remains to be determined as currently too limited data are available in the literature [32].

The Mass Median Aerodynamic Diameter (MMAD) is a widely used parameter. It is defined as the particle aerodynamic diameter for which half of the aerosol mass is contained in smaller particles and half is contained in larger particle aerodynamic diameters, it is obtained from the cumulative mass distribution [94].

### *3.2.3. Aerosol generating devices*

As mentioned before, for pulmonary administration in human lungs by inhalation, the size range of nanoparticles should not be too large to ensure their deposition and then retention in the deep lung. Indeed, most of inhaled nanoparticles lower than 100 nm would be expelled during exhalation or will remain in the upper airways if they deposited. It is generally acknowledged that the optimal aerosol size for lung targeting is in the 1-5 µm range of aerodynamic diameters [95]. As a result, the delivery of nanoparticles to lungs could be performed: i) by nebulization of nanosuspensions (*e.g.* generating airborne micron-size droplets containing nanoparticles using a medical device such as nebulizer) [96–102], or ii) by aerosolization of solid microparticles containing nanoparticles (*e.g.* the Trojan horse concept) [76,103–105]. Undoubtedly, the easiest way to deliver airborne nanoparticles to the lungs seems to nebulize, as no complex pharmaceutical development is needed. However, some challenges remain, such as the choice of the most adapted aerosol drug delivery systems.

Three types of aerosol drug delivery systems can be distinguished: nebulizers, metered dose inhalers (MDIs), and dry powder inhalers (DPIs) [13,18,46]. These devices use different delivery mechanisms, and hence require different types of drug formulations [46]. The features, advantages and drawbacks of each of them are detailed below. It should be kept in mind that no universal system suitable for all applications exist, and different parameters should be considered when choosing a device such as the active agent, the formulation characteristics, the target site, and the pulmonary pathophysiology [13,73]. Indeed, the site and extent of aerosol deposition in the lung is dependent on particle size, velocity and inertia, patient's inspiratory air flow and the inhalation technique. [7,106]

The principle of aerosol delivery is based on the transformation of an aqueous solution or suspension of drug in an aerosol containing fine droplets in which the drug is dispersed. The latter will be subsequently inhaled and deposited in the lung [73]. Theoretically by tuning the particle size of the drug aerosol, one may control the dose of drug to be delivered and target specific sites within the lung [73]. It is worth noting here that the deposition is dependent on the size of the aerosol particles/droplets (in the micrometer range) not the size of the nano-carrier systems. Accordingly, optimal technologies are required to produce droplets or particles with a Mass Median Aerodynamic Diameter (MMAD) of 1-5 μm to be suitable for deposition in the therapeutically relevant areas of the lung [5,7,106–108].

In pressurized metered dose inhalers (pMDIs) the aerosol is emitted through a nozzle at a high velocity using propellants [5]. The main drawback of this system is that it requires hand-mouth coordination, a lack of such coordination results in a low aerosol deposition in the lung (only 10 to 20% of the emitted aerosol) [109–112].

Dry powder inhalers (DPIs) were designed to overcome poor actuation-inhalation coordination [5]. They can be either single or multi-dose (containing only one or several capsules of dry powder respectively). Their efficiency is highly variable among different devices, the lung deposition ranging from 12 to 40% of the emitted dose and about 20%–25% is retained within the device [5].

In the treatment of respiratory diseases, nebulizers have been widely used. Easy to use, using face mask and not requiring coordination, they are particularly convenient for young or elderly patients. Two main types of nebulizers exist: jet and ultrasonic nebulizers [5,73]. Jet nebulizers are the most used device for inhalation of solutions and suspensions. Their functioning is based on the use of compressed air allowing a liquid in the reservoir cup to be transformed into fine mist. However, they are noisy, less comfortable to carry, need long time to nebulize a solution (10–15 min) and only 10% of the emitted dose is deposited to the lungs [5,73].

Ultrasonic nebulizers operate using piezo-electric crystals vibrating at a high frequency (1–3 mHz) to produce a mist of liquid in the nebulizer. By increasing the frequency, smaller droplets can be produced [5]. Ultrasonic nebulizers are compact, silent, and faster than jet nebulizers, however, they are not adapted to suspensions. Another issue is that the piezoelectric crystal can heat therefore inducing the inactivation of protein-based drugs [73,113].

A new generation of nebulizers consists of vibrating mesh nebulizers and uses vibrating perforated mesh to generate respirable sized droplets [5,73]. Their main assets are their high efficiency, silence, portability, in addition, they exhibit an extremely low residual volume to prevent drug waste. However, they are expensive, and need maintenance and cleaning to prevent colonization by pathogens, buildup of deposits, and blockage of the apertures [5].

The main technical challenge using an aerosol drug delivery system is to generate airborne nanoparticles without changes of chemistry or size and maintaining adequate aerodynamic performance to allow a satisfactory aerosol regional deposition both in terms of drug concentration and targeted areas. Indeed, the aerosol drug delivery system such as nebulization, the technology or the type of medical devices for a same technology could have a strong impact on the features of aerosol generation such as: modification of airborne droplet size, decrease of aerosol output rate, increased nanoparticle aggregation within droplets, modified nanoparticle shape. As an example, several studies have shown that jet nebulization is generally more disruptive for liposomes or polymeric nanoparticles than vibrating-mesh nebulization [57,96–99,101,102]. Compared to liposomes or polymeric nanoparticles, inorganic nanoparticles exhibit a higher stability and a lower fragility (because of their physicochemical properties) [114], making their use promising for jet nebulization.

Beside the choice of the aerosol generating device, some parameters of the aerosolized solution will impact the nebulization process such as pH, viscosity, ionic strength, osmolarity, and surface tension [5]. For example, a reduced drug output and bronchoconstriction, coughing, or irritation can be caused by high drug concentration, extremely low pH, and hyper- or hypo-osmolarity [115,116].

Among the numerous advantages offered by nanoparticle drug formulations compared to traditional aerosol powders and liquid pulmonary dose formulations, one may quote the highly enhanced bioavailability of poorly water-soluble drugs due to the large surface area of drug nanoparticle formulations. Furthermore, nanoparticles can be formulated to offer increased control over the morphology of dry powder drug formulations and the ability to produce structures with both a low-density microstructure for delivery to the deep lung and nanostructure for enhanced dissolution and bioavailability [46].

Submicron aqueous dispersions, were proved to be more readily nebulized than their micronized counterparts [7]. For example, ultrasonic nebulization of beclomethasone dipropionate nanocrystals™ produced a higher respirable dose and 40% lower throat deposition than the commercially available pMDIs containing the micronized drug (Vanceril®) [117]. Similarly, Wiedmann *et al.* reported that a higher respirable fraction of beclmethasone dipropionate was achieved by nebulization of a nanosuspension rather than with a micronized suspension of the drug [118]. Ali *et al.* confirmed that the particle size of the aerosolized drug has an influence on the regional deposition following pulmonary administration as they compared nano and micro-sized formulation of fluticasone propionate. When administered to healthy human volunteers, significantly higher respirable fraction has been achieved by using the nanoparticles (60.3 ± 2.4%) rather than their micronized counterpart (16.4 ± 0.7%). Nanosized fluticasone propionate also showed a better deposition in the alveolar region than the micronized drug, which was primarily deposited in the oropharyngeal region [119].

### *3.2.4. Limitations of pulmonary delivery*

Despite numerous advantages the pulmonary route of administration faces challenges. First, drug particles deposited in the respiratory tract can be cleared by the natural defense system of the body (mucociliary clearance, phagocytosis by alveolar macrophages, degradation by surrounding enzymes…) [2,5,32].

Second, a successful drug deposition in targeted areas will depend on anatomical features. In particular, in respiratory diseases alterations of the architecture of the lung can be observed such as airway constrictions, the presence of a tumor, inflammation, abundant mucus secretion... Such modifications and obstructions alter the lung ventilation, and therefore the aerosol deposition pattern [3,5].

Third, some technical challenges are associated with the inhalation route, especially regarding drug formulation, storage, and delivery [13]. For instance, in the inhalation field the choice of excipients is quite restricted, limiting pharmaceutical developments and formulation strategies [3,17,120]. Still about formulation, the pharmaceutical development of a dry powder for inhalation is more technically challenging than aqueous solution for nebulization [121,122]. In addition, the scale-up capacity can be different from a process to another and should be carefully considered for further development [3,123]. Other challenges are related to the aerosol generating device. Indeed, unlike in the treatment of asthma or COPD, in the case of lung cancer, it is necessary to deliver high doses of drug (*i.e.* one to several tens of mg) which can require a long time of administration by nebulization and consequently represents an issue for

patient compliance [3,65,124]. Delivering high drug doses by nebulization implies to deal with different parameters. First, the drug concentration has to be considered (and consequently the drug solubility in the formulation to be nebulized), second, the efficiency of the nebulizer, especially the nebulization rate which usually ranges from 0.2 to 0.3 mL/min and third, the fraction of the drug dose deposited in the lungs (usually between 10 and 15%) [76,124]. Only nebulizers or DPIs are able to deliver high drug doses. In addition, aerosol formulations are device specific [3,123]. The stability of the formulations is also an issue of paramount importance both during their storage and the nebulization process. For instance, in the case of nanoformulation, the stability of liposomes has been reported to be a concern upon storage and nebulization [2,3,7,17,65]. This instability may lead to the dissociation of surface-modified components subsequently affecting the targeting ability of nano-delivery systems [2]. Furthermore, nanocarrier such as liposomes in particular, can be mechanically-damaged upon nebulization, triggering a leakage of the encapsulated drug [5,17,32]. For instance, Wittgen *et al.* reported a 40-50% loss of cisplatin from liposomes during aerosolization [65]. Because of this uncontrolled release of the drug, the efficiency of this therapeutic approach could be seriously mitigated [7]. However if liposomes stability is a concern, other nanoformulations could be more robust and better resist nebulization such as solid lipid or polymeric nanoparticles [7,32]. Of course they also have their own drawbacks, for example, the degradation rate of PLA nanoparticles was shown to be too low for practical applications (drug release about 15% over a period of 8 days) [125]. And their accumulation following repeated dosing, their biodegradability and toxicity should be examined [17,18]. Also, it has been reported that when passed through a nebulizer, polymeric nanoparticles can highly aggregate within aerosol droplets, especially those highly hydrophobic [32].

Finally, in some specific cases, safety issues could be associated with the inhalation route, representing a limitation. It is exemplified in the treatment of lung cancer where high doses of cytotoxic drugs are necessary and as a large part of the aerosol is lost in the device and in the air during the aerosolization process, systems preventing air contamination should be used [3]. For instance, nebulizers can be equipped with filters collecting exhaled aerosols, or be designed to allow mouth-only inhalation or patients can be located in depressurized ‘tents’ or ‘cabins’ equipped with an air extractor and with activated charcoal and high efficient particulate air (HEPA) filters [124].

## 4. Concrete examples of applications

Despite intensive research and very likely because of the limitations mentioned above, approximately 50 nanopharmaceuticals have been FDA-approved and available for clinical use, a fifth of which having oncologic indications [3]. However, many nanoformulations, still in pre-clinical development show promising perspectives. Few current clinical trials of nanomedicine related to respiratory diseases have been reported, and the clinically known applications of nanocarriers are liposomes [65,126] in non-tuberculous mycobacterial lung disease and lung cancer, and PLGA in pulmonary arterial hypertension [127]. Table 1, far from being exhaustive, presents some examples to illustrate the potential of nano-delivery systems loaded with different agents (antibiotics, anti-inflammatory agents…) for the potential treatment of various respiratory diseases.

Table 1 – Examples of applications of nanoformulations in the treatment of respiratory diseases (in development).

| Respiratory disease | Nano-delivery system | Active agent | Main conclusion | Reference |
|---|---|---|---|---|
| COPD | Solid lipid nanoparticles | Amikacin | Pulmonary delivery of solid lipid nanoparticles of amikacin caused higher drug concentration in lungs than i.v. administration of free drug. | [128] |
| | PLGA nanoparticles | Ibuprofen | Nanoparticles efficiently delivered ibuprofen to neutrophils in murine models of obstructive lung diseases. | [129] |
| Asthma | Nano-salbutamol sulphate dry powder inhalation | Salbutamol | The total deposition of salbutamol nanoparticles in the lungs increased by 2.3 times compared to conventional dry powder inhalation formulations. | [130] |
| | Liposomes | Salbutamol | Nanoformulation can prolong the retention time in the lesion, and its curative effect is better than that of the free drug. | [22] |
| | Liposomes | Budesonide | Liposomal budesonide significantly reduced lung inflammation and the toxicity of inhaled steroid asthma drugs. | [131] |
| Lung fibrosis | Gold nanoparticles | Imatinib | Gold nanoparticles loaded with imatinib could significantly improve the anti-fibrotic efficacy of imatinib, thereby inhibiting the proliferation of fibroblasts and macrophages. | [132] |
| | PLGA nanoparticles | Pirfenidone | Pirfenidone levels in the lungs were much higher following intratracheal administration of pirfenidone nanoparticles than pirfenidone solution. The number of inflammatory cells in BAL was more significantly reduced by the pirfenidone-containing nanoparticles than pirfenidone solution. | [133] |
| Lung tumor | Nanostructured lipid carriers | Doxorubicin or paclitaxel | After inhalation, nanostructured lipid carriers effectively delivered their payload into lung cancer cells leaving healthy lung tissues intact. | [134] |
| | Liposomes | Paclitaxel | Pulmonary delivery of paclitaxel in liposome aerosol formulations was more efficient than intravenous injection in mice. | [135] |
| | Polymeric nanoparticles | Doxorubicin and cisplatin | Drug loaded nanoparticles exhibited higher *in vitro* cytotoxicity than that of the drugs alone. *In vivo*, after pulmonary delivery, nanoparticles accumulated in tumor tissues and showed higher anti-tumor efficiency than that in the single treatment of doxorubicin or cisplatin, while no obvious side effects were observed. | [136] |

| | | | | |
|---|---|---|---|---|
| | PLGA nanoparticles | Paclitaxel | Nanoparticles favor the intracellular uptake of paclitaxel and enhance its antitumor effect in human and murine lung cancer cells. | [137] |
| Fungal infections | Polymeric nanoparticles | Amphotericin B | The killing rate of nanoparticles loaded with amphotericin B administered by aerosol against Aspergillus was > 99%, and lung tumor necrosis factor-α was reduced by 90%. | [138] |
| Lung infections | Liposomes | Ciprofloxacin | When compared to free ciprofloxacin, nebulized liposome ciprofloxacin has a better *in vitro* efficacy against *Mycobacterium avium* and *Mycobacterium abscessus*. | [139] |
| | Liposomes | Ciprofloxacin | Aerosol inhalation of liposome-encapsulated ciprofloxacin provided complete protection to mice against a pulmonary lethal infection model of *F. tularensis*, while ciprofloxacin given in its free form, was ineffective. | [140] |
| Tuberculosis | Silver and zinc oxide nanoparticles | Rifampicin | Efficient uptake of the drug-loaded nanoparticles by *M.tuberculosis*-infected macrophage and increased drug efficiency were observed. | [141] |
| | Silica nanoparticles | Isoniazid | Isoniazid-loaded nanoparticles are avidly ingested by *M. tuberculosis*-infected human macrophages and kill the intracellular bacteria in a dose-dependent manner. | [142] |
| Cystic fibrosis | Nanostructured lipid carriers | Lumacaftor and ivacaftor | The combination of lumacaftor and ivacaftor delivered by lipid nanoparticles directly into the lungs was highly effective in treating lung manifestations of cystic fibrosis. | [143] |
| | PLGA nanoparticles | Ciprofloxacin | Enhanced antibacterial activity with drug loaded nanoparticles. | [144] |
| | PLGA nanoparticles | Tobramycin | The effectiveness against biofilms of *P. aeruginosa* and *B. cepacia* was strongly enhanced by the encapsulation of tobramycin in nanoparticles. | [145] |

It is also of interest to mention that nano-delivery systems could be used for gene delivery. In this context, usually cationic liposomes have been preferred because they are able of self-assembly with DNA through favorable cationic–anionic electrostatic interactions [18,146–154].

Beside the therapeutic aspects, it is worth citing applications in the diagnosis field. For example, among the radiolabelled aerosols clinically used in patients, one of the most convenient is the inhalation of radioactive nanosized aerosol using the so-called Technegas technique. The preparation of this radiolabeled nanoaerosol takes place in a specially designed machine, where a solution of sodium pertechnetate is loaded into a graphite crucible, evaporated until dry, and then heated to 2550°C in an atmosphere of pure argon [155]. The resulting aerosol produced is an ultrafine suspension of carbon particles labeled with $^{99m}$Tc showing a number size distribution of airborne particles below 100 nm [156]. Since 3 decades, this radioactive nanoaerosol is regularly used to perform lung ventilation scintigraphy as a diagnostic technique in nuclear medicine for human application without any toxicological issues [157–160].

## 5. Conclusion and future directions

### *5.1. Features of the ideal nano-delivery system*

Aerosolization allows targeting the lung via a non-invasive route. Successful delivery of therapeutic agents using this mode of administration requires a good understanding and mastering of various parameters including the drug physicochemical characteristics, the formulation, the inhaler device, and the pathophysiologic lung conditions [13].

Some challenges are associated with the development of formulations suitable for pulmonary delivery such as poor loading capacity, loss of stability, degradation by proteolytic enzymes, mucociliary and phagocytic clearance, immunogenicity, and toxicity. In that context, it has been shown that carrier-based delivery systems may help overcoming these challenges [13]. In particular, nanocarriers have raised interest as they show some advantages over their micron-sized counterparts. But their design needs to carefully consider some of their features such as surface properties, particle size and shape, to allow them a better penetration of the pulmonary biological barrier [161]. The ideal features of a nano-delivery system could be defined as follows: small particle size (< 200 nm), with neutral charge surface or coated with PEG, strong targeting ability (can be fostered by active targeting), sufficient loading of active drugs, and low toxicity [2]. Also important are the features of the aerosol: an aerodynamic particle size below 5 μm is crucial for particles reaching the mid and deep lung parenchyma [73]. Combining

nanotechnology and pulmonary delivery represents a promising way to improve the targeting, release, and therapeutic effects of drugs [18].

### *5.2. Perspectives, future developments*

#### *5.2.1. Nano-in-micro approaches*

Drug delivery to the lung through a nanocarrier is limited because of the low efficiency of nanocarriers deposition in the lungs by diffusion, sedimentation or impaction. Indeed, because of their small size the majority of the inhaled dose is exhaled. Therefore, to overcome this issue and improve the aerosol delivery of nanoparticles to the deep lung, micron-sized powder carriers containing nanoparticles or agglomerated nanoparticles were designed and used with MDIs and DPIs [18]. Upon inhalation, these nano-in-microparticles (or nano-embedded microparticles) dissolve to release individualized nanoparticles within the lung parenchyma [3,73,162]. Thus, this system combines the advantages of nano-sized particles (potential for drug targeting) to those of micron-sized particles (better flow and aerolization properties) [73]. However, this approach often requires additional excipients in the formulation, which dilute the final drug content and can induce additional tolerance concerns. Furthermore, the pharmaceutical development is more made complex due to necessary additional production steps [3].

#### *5.2.2. The emergence of drug vaping to generate airborne carrier in the submicron size range*

In the last decade, the development of new aerosol drug delivery systems has benefited from various innovations enabling the generation of smaller airborne particles [163–165] compared to standard medical devices used in clinical practice for aerosol therapy like nebulizers, pMDIs and DPIs. For example, these technological breakthroughs allowed the improvement of the drug delivery via the deep lung for systemic administration (for instance for the delivery of nicotine or insulin). Regarding this latter application, we can mention the example of Afrezza® from MannKind, FDA-approved in 2014 and currently the only inhalable insulin available in the US. It is based on the Technosphere® Insulin (TI) technology consisting of a dry-powder formulation of human insulin delivered from a small and portable inhaler. It allows medication to be delivered efficiently through the lungs, a rapid subsequent passage into the bloodstream allows the control of blood sugar level within minutes of administration [166–168]. Other devices have also been applied in the treatment of obstructive lung diseases such as asthma, cystic fibrosis and COPD [148,149]. Despite this progress, alternative aerosols delivery

technologies still need to be developed for nanodrug delivery to the deep lung. These systems should be inexpensive, convenient and user-friendly for the patient, and allowing the production of small-sized aerosol particles (with aerodynamic diameter ranging from 500 nm to 1 µm) to reach potential gain in terms of aerosol deposition [171].

Smokers of tobacco have implicitly found out that aerosols generated from thermal generation can reach the alveoli and are mostly systemically absorbed upon inhalation. For pharmaceutical purposes, of course smoking cannot be considered as a possible drug delivery system because it is accompanied by the production of carcinogens, and the size of the produced aerosol particles is uncontrolled. In this context, Electronic nicotine delivery systems (ENDS) were launched more than 10 years ago and experienced a growing success (from several thousand users worldwide in 2006 to several million in 2020). They have become a popular tool for smoking reduction or cessation [172,173].

Basically, ENDS are battery-powered personal devices that deliver aerosol that can contain nicotine as active pharmaceutical ingredient. This device contains 3 main components: a battery, an atomizer and a coil heating element. The physical principle shared by all ENDS is an electrically-powered heating element which enables to vaporize a liquid solution (named e-liquid) so that airborne particles are produced for the user to inhale. This e-liquid usually used contains humectants in variable concentrations (vegetal glycerin and propylene glycol), nicotine, water and other ingredients in small quantities such as flavorings.

ENDS can therefore be considered as thermal aerosol generation devices. Indeed, ENDS exhibit a working principle similar to that of some medical devices previously developed and commercialized for different clinical applications such as schizophrenia or bipolar disorder in adults, the delivery of anti-panic or anti-migraine agents [171,174,175]. Since their emergence in the early 2000s, ENDS have experienced continuous development and evolution. The latest models of ENDS have proved their efficiency to deliver very high levels of airborne nicotine [176,177]. Consequently, ENDS could be adapted for clinical purposes, representing a potential promising aerosol device. Indeed, they are user-friendly and could be adapted to the customer need, and most of all, they were proved to be efficient to deliver a drug (*i.e.* nicotine) for systemic administration thanks to small-sized aerosol particles. However, currently ENDS are mainly regulated as general consumer products and not as medical devices. Nevertheless, some very rare exceptions exist, as for example the UK Medicines and Healthcare Products Regulatory Agency (MHRA), which approved and licensed the use of British American Tobacco's e-cigarette e-Voke, in a move that enables doctors to prescribe the vaping device as

a smoking cessation aid [178]. Thus, since a decade, ENDS are mainly consumer products that became a popular alternative to conventional tobacco smoking.

Recently a patent has been applied for an ENDS with face adapter to spray essential oils [179]. In addition to this, in a recent study it was demonstrated that in the treatment of COPD for chronic smokers, employing e-cigarettes with bronchodilators or corticosteroids nanosuspensions; such as Beclomethasone dipropionate might be beneficial [180]. Finally, a work also demonstrated the potential of recent high power ENDS as thermal aerosol generation devices for inhaled bronchodilators such as terbutaline sulfate [181]. This study proved that ENDS could represent a possible alternative aerosol delivery system to be considered for a respiratory pathology requiring inhaled bronchodilators. As a matter of fact, this work showed that new generation high-power ENDS appear to be highly patient-adaptive, and very efficient to generate carrier-droplets in the submicron range containing drug molecules with a constant drug concentration whatever the size-fractions. It also proved that drug vaporization using ENDS can occur without thermal degradation of the terbutaline sulfate whatever the power levels. Finally, a linear correlation was observed between the power level and the delivery of aerosol bronchodilator. All things considered, this shows the growing scientific field and the emerging industrial interest in the development of drug vaping, *i.e.* the use of ENDS capable of aerosolizing active substances other than nicotine.

#### 5.2.3. *Beyond in vivo animal experiments, the need to develop innovative respiratory models to assess regional aerosol deposition of nanoparticles*

In the context of nano-delivery to the lungs, *in vivo* preclinical evaluation in animal models remains essential. Indeed, to overcome patient to patient variability inherent to clinical research, preclinical models have been widely used to characterize the transport of a drug, its deposition, and the biological effects induced. As a result, animal models are of paramount importance to assess the pharmacokinetics as well the possible toxicity of inhaled nano-delivery systems. Two main types of preclinical *in vivo* aerosol studies can be performed: *in vivo* aerosol deposition studies on rodents; and *in vivo* aerosol deposition studies on pigs and non-human primates (animal models most similar to human lungs). However, despite the positive and promising preclinical results of nano-delivery to the lungs (both of diagnostic and therapeutic agents) using *in vivo* animal models, the translation from animals to humans remains challenging and requires further methodological validation steps.

One of the major pitfalls in animal-human transposition lies in the different anatomy of the airways and the different respiratory physiology between rodents and humans [182–184]. For instance, rodents have no lobe divisions in the left lung, similarly, the bronchial divisions of most mammals are very different from those of humans [185]. Furthermore, physiological ventilation is very different: for example, while the respiratory rate is 15 cycles per minute for an adult human at rest, it is approximately 80 cycles per minute for a rat. This limitation specific of rodent models could be partially overcome by using bigger animals such as pigs or non-human primates. Indeed, the anatomy of the airways of these animals are quite similar to that of humans, making them appropriate to perform *in vivo* aerosol deposition investigation [186–188]. As an example, baboons have been used to mimic the respiratory tract of children [163,189,190]. Although very relevant, these studies can however be limited by ethical restrictions, high cost of experiments, and uncontrolled breathing pattern (inspired to expired ratio, frequency, obstruction, etc.) in spontaneous breathing.

Another major pitfall is due to the technology of administration of the nanoparticles in the respiratory tract. In small animal models such as rodents, direct instillation is used (intratracheal administrations) [191–194], using a needle placed in the trachea and an aerosolization process using a syringe generating aerosol from the nanoparticle suspension. However, it has been clearly demonstrated that this mode of administration induced an inhomogeneous deposition of the aerosol, making it difficult to transpose data to human [195]. On the contrary, inhalation of an aerosol generated by a nebulizer resulted in a homogeneous deposition and, thus, an easier extrapolation to human [196]. The fact that instillation and inhalation resulted in different deposition pattern has already been reported in nanotoxicology [197–199] and it has also been observed in mechanically ventilated patients [200].

Therefore, to bridge the existing gap, an important research direction to assess nano-delivery to the lungs is to develop *ex vivo* preclinical models highly relevant for human that are easy to use, less expensive and no subject to ethical restriction. To reach this objective, innovative *ex vivo* respiratory models appear to be an interesting preclinical tool to assess aerosol regional deposition of nano-delivery systems in healthy and pathological-like respiratory tracts [165,201]. Composed of a 3D-printed Ear-Nose-Throat replica connected to *ex vivo* animal respiratory tract in a hypobaric chamber simulating passive ventilation, these models appear as an innovative and promising way to quantitatively assess aerosol deposition into the lungs and to transpose it more easily to human [202,203].

For example, this type of innovative *ex vivo* anatomical model was developed to optimize a process of nebulization of inorganic nanoparticles (in terms of emitted dose and mass median

aerodynamic diameter), but also to determine the lung distribution of nanoparticles [204]. This study is focused on AGuIX® nanoparticles, as a potential theranostic approach by the pulmonary route. AGuIX® nanoparticles act as radiosensitizers under radiotherapy and as MRI positive contrast agent [205–207]. These inorganic nanoparticles are composed of a polysiloxane matrix and allow multimodal imaging and theranostic approach [114,208–212]. These nano-delivery systems were primarily developed for intravenous injection (IV). However their administration to the lungs has been also evaluated in mice [191,192]. These nanoparticles can be delivered passively to lung tumors after administration through the airways [191]. These radio-sensitizing nanoparticles were administered via the intrapulmonary route to the mice, these latter were then exposed to radiotherapy. A 45%-increase of the mean survival time was observed [193]. Within the perspective of a potential clinical translation of nebulized AGuIX® for the treatment of lung diseases in human, innovative *ex vivo* anatomical model was used to determine the performance of a process of nebulization thanks to a commonly clinically used jet nebulizer [204]. Through this work, a multimodal approach thanks to scintigraphy and Magnetic Resonance Imaging (MRI) were performed to qualitatively and quantitatively assess the deposited aerosol on an *ex vivo* respiratory model. Thus two types of labelled aerosols were developed, using molecular tracers ($^{99m}$Tc-DTPA for scintigraphy and gadoterate meglumine for MRI) and nanoparticular tracers ($^{111}$indium chloride chelated on the AGuIX® nanoparticles for scintigraphy and Gd-AGuIX® nanoparticles for MRI). With both imaging techniques, a homogenous pattern of deposition was observed with the nanoparticular aerosol, pattern which is similar to the one obtained with a molecular tracer using the same nebulizer. The quantity of aerosol deposited into the respiratory tract with the nanoparticular aerosol was 2-fold lower compared to that of the molecular tracer aerosol, for both imaging techniques. Despite the limitations of the *ex vivo* model used to assess the regional deposition pattern of the aerosol, this work proved that the aerosolization of a nanoparticulate suspension could be achieved with a clinical nebulizer, and perfectly demonstrated that this type of preclinical respiratory model offers promising findings which could facilitate the transfer of the aerosolized nanoparticles to the clinic.

### *5.2.4. Toward other clinical applications of nano-delivery to the lung by inhalation*

One may wonder "why nano when micro is largely sufficient"? As a matter of fact, micro is sufficient for local delivery of active agents into the lungs. Nano could be helpful to address some of the limits of current pulmonary therapeutic (frequency of administration, loss of dose in not relevant lung regions, ...). However, there are still many challenges to overcome to reach systemic approaches by pulmonary route. Thus, nano could be an interesting tool for such

purposes. Indeed, inhaled nanoparticles can reach the blood circulation through translocation and exert systemic effects. This is why nano-systems for pulmonary delivery of insulin have been designed [213,214].

More recently, a European-funded project, Cupido, has developed a new strategy to treat cardiovascular diseases. They propose to use calcium phosphate nanoparticles loaded with therapeutic molecules, formulated as microparticulate dry powder that once inhaled reach the alveoli and then the heart through blood circulation. Here, the drug cargo is released to cardiac cells where it can exert its therapeutic effect [215].

This example perfectly illustrates how could nano-delivery to the lung be a promising strategy, offering much more potential than the treatment of respiratory diseases. Although further developments and optimizations are still necessary, promising therapeutic perspectives can be expected from such approach.

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**References**

[1] M.-X. Luo, S. Hua, Q.-Y. Shang, Application of nanotechnology in drug delivery systems for respiratory diseases (Review), Mol. Med. Rep. 23 (2021) 325. https://doi.org/10.3892/mmr.2021.11964.

[2] W. Zhong, X. Zhang, Y. Zeng, D. Lin, J. Wu, Recent applications and strategies in nanotechnology for lung diseases, Nano Res. (2021) 1–23. https://doi.org/10.1007/s12274-020-3180-3.

[3] N. Wauthoz, R. Rosière, K. Amighi, Inhaled cytotoxic chemotherapy: clinical challenges, recent developments, and future prospects, Expert Opin. Drug Deliv. 18 (2021) 333–354. https://doi.org/10.1080/17425247.2021.1829590.

[4] World Health Organization, Cancer, World Health Organ. (2021). https://www.who.int/news-room/fact-sheets/detail/cancer (accessed July 28, 2021).

[5] P. Zarogoulidis, E. Chatzaki, K. Porpodis, K. Domvri, W. Hohenforst-Schmidt, E.P. Goldberg, N. Karamanos, K. Zarogoulidis, Inhaled chemotherapy in lung cancer: future concept of nanomedicine, Int. J. Nanomedicine. 7 (2012) 1551–1572. https://doi.org/10.2147/IJN.S29997.

[6] World Health Organization, Chronic obstructive pulmonary disease (COPD), World Health Organ. (2021). https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd) (accessed July 28, 2021).

[7] M.E. Ali, J.T. McConville, A. Lamprecht, Pulmonary delivery of anti-inflammatory agents, Expert Opin. Drug Deliv. 12 (2015) 929–945. https://doi.org/10.1517/17425247.2015.993968.

[8] X. Murgia, B. Loretz, O. Hartwig, M. Hittinger, C.-M. Lehr, The role of mucus on drug transport and its potential to affect therapeutic outcomes, Adv. Drug Deliv. Rev. 124 (2018) 82–97. https://doi.org/10.1016/j.addr.2017.10.009.

[9] F.L. Ramos, J.S. Krahnke, V. Kim, Clinical issues of mucus accumulation in COPD, Int. J. Chron. Obstruct. Pulmon. Dis. 9 (2014) 139–150. https://doi.org/10.2147/COPD.S38938.

[10] European Science Foundation, Nanomedicine, 2004. http://archives.esf.org/fileadmin/Public_documents/Publications/Nanomedicine.pdf.

[11] I. Roy, N. Vij, Nanodelivery in airway diseases: challenges and therapeutic applications, Nanomedicine Nanotechnol. Biol. Med. 6 (2010) 237–244. https://doi.org/10.1016/j.nano.2009.07.001.

[12] A. Costa, M. Pinheiro, J. Magalhães, R. Ribeiro, V. Seabra, S. Reis, B. Sarmento, The formulation of nanomedicines for treating tuberculosis, Adv. Drug Deliv. Rev. 102 (2016) 102–115. https://doi.org/10.1016/j.addr.2016.04.012.

[13] N. Osman, K. Kaneko, V. Carini, I. Saleem, Carriers for the targeted delivery of aerosolized macromolecules for pulmonary pathologies, Expert Opin. Drug Deliv. 15 (2018) 821–834. https://doi.org/10.1080/17425247.2018.1502267.

[14] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25. https://doi.org/10.1016/j.addr.2013.11.009.

[15] L. Salvioni, M.A. Rizzuto, J.A. Bertolini, L. Pandolfi, M. Colombo, D. Prosperi, Thirty Years of Cancer Nanomedicine: Success, Frustration, and Hope, Cancers. 11 (2019) E1855. https://doi.org/10.3390/cancers11121855.

[16] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, Nat. Rev. Cancer. 17 (2017) 20–37. https://doi.org/10.1038/nrc.2016.108.

[17] C. Loira-Pastoriza, J. Todoroff, R. Vanbever, Delivery strategies for sustained drug release in the lungs, Adv. Drug Deliv. Rev. 75 (2014) 81–91. https://doi.org/10.1016/j.addr.2014.05.017.

[18] H.M. Mansour, Y.-S. Rhee, X. Wu, Nanomedicine in pulmonary delivery, Int. J. Nanomedicine. 4 (2009) 299–319. https://doi.org/10.2147/ijn.s4937.

[19] Y. Barenholz, Doxil®--the first FDA-approved nano-drug: lessons learned, J. Control. Release Off. J. Control. Release Soc. 160 (2012) 117–134. https://doi.org/10.1016/j.jconrel.2012.03.020.

[20] G. Chimote, R. Banerjee, In vitro evaluation of inhalable isoniazid-loaded surfactant liposomes as an adjunct therapy in pulmonary tuberculosis, J. Biomed. Mater. Res. B Appl. Biomater. 94B (2010) 1–10. https://doi.org/10.1002/jbm.b.31608.

[21] W.H. Finlay, J.P. Wong, Regional lung deposition of nebulized liposome-encapsulated ciprofloxacin, Int. J. Pharm. 167 (1998) 121–127. https://doi.org/10.1016/S0378-5173(98)00055-6.

[22] X. Chen, W. Huang, B.C. Kwan Wong, L. Yin, Y.F. Wong, M. Xu, Z. Yang, Liposomes prolong the therapeutic effect of anti-asthmatic medication via pulmonary delivery, Int. J. Nanomedicine. 7 (2012) 1139–1148. https://doi.org/10.2147/IJN.S28011.

[23] W.-H. Huang, Z.-J. Yang, H. Wu, Y.-F. Wong, Z.-Z. Zhao, L. Liu, Development of Liposomal Salbutamol Sulfate Dry Powder Inhaler Formulation, Biol. Pharm. Bull. 33 (2010) 512–517. https://doi.org/10.1248/bpb.33.512.

[24] M. Chougule, B. Padhi, A. Misra, Nano-liposomal dry powder inhaler of tacrolimus: Preparation, characterization, and pulmonary pharmacokinetics, Int. J. Nanomedicine. 2 (2007) 675–688.

[25] J.C. Waldrep, J. Arppe, K.A. Jansa, M. Vidgren, Experimental pulmonary delivery of cyclosporin A by liposome aerosol, Int. J. Pharm. 160 (1998) 239–249. https://doi.org/10.1016/S0378-5173(97)00322-0.

[26] C.J. Hitzman, W.F. Elmquist, L.W. Wattenberg, T.S. Wiedmann, Development of a Respirable, Sustained Release Microcarrier for 5-Fluorouracil I: In Vitro Assessment of Liposomes, Microspheres, and Lipid Coated Nanoparticles, J. Pharm. Sci. 95 (2006) 1114–1126. https://doi.org/10.1002/jps.20591.

[27] M. Murata, K. Nakano, K. Tahara, Y. Tozuka, H. Takeuchi, Pulmonary delivery of elcatonin using surface-modified liposomes to improve systemic absorption: Polyvinyl alcohol with a hydrophobic anchor and chitosan oligosaccharide as effective surface

modifiers, Eur. J. Pharm. Biopharm. 80 (2012) 340–346. https://doi.org/10.1016/j.ejpb.2011.10.011.

[28] A. Shahiwala, A. Misra, Pulmonary absorption of liposomal levonorgestrel, AAPS PharmSciTech. 5 (2009) 96. https://doi.org/10.1208/pt050113.

[29] F. Hajos, B. Stark, S. Hensler, R. Prassl, W. Mosgoeller, Inhalable liposomal formulation for vasoactive intestinal peptide, Int. J. Pharm. 357 (2008) 286–294. https://doi.org/10.1016/j.ijpharm.2008.01.046.

[30] F. -y. Liu, Z. Shao, D.O. Kildsig, A.K. Mitra, Pulmonary Delivery of Free and Liposomal Insulin, Pharm. Res. Off. J. Am. Assoc. Pharm. Sci. 10 (1993) 228–232. https://doi.org/10.1023/A:1018934810512.

[31] O.B. Garbuzenko, M. Saad, S. Betigeri, M. Zhang, A.A. Vetcher, V.A. Soldatenkov, D.C. Reimer, V.P. Pozharov, T. Minko, Intratracheal Versus Intravenous Liposomal Delivery of siRNA, Antisense Oligonucleotides and Anticancer Drug, Pharm. Res. 26 (2008) 382. https://doi.org/10.1007/s11095-008-9755-4.

[32] M. Skibba, A. Drelich, M. Poellmann, S. Hong, A.R. Brasier, Nanoapproaches to Modifying Epigenetics of Epithelial Mesenchymal Transition for Treatment of Pulmonary Fibrosis, Front. Pharmacol. 11 (2020) 607689. https://doi.org/10.3389/fphar.2020.607689.

[33] J.-H. Seong, K.-M. Lee, S.T. Kim, S.-E. Jin, C.-K. Kim, Polyethylenimine-based antisense oligodeoxynucleotides of IL-4 suppress the production of IL-4 in a murine model of airway inflammation, J. Gene Med. 8 (2006) 314–323. https://doi.org/10.1002/jgm.848.

[34] K. Wernig, M. Griesbacher, F. Andreae, F. Hajos, J. Wagner, W. Mosgoeller, A. Zimmer, Depot formulation of vasoactive intestinal peptide by protamine-based biodegradable nanoparticles, J. Control. Release Off. J. Control. Release Soc. 130 (2008) 192–198. https://doi.org/10.1016/j.jconrel.2008.06.005.

[35] Z. Ahmad, A. Zahoor, S. Sharma, G.K. Khuller, Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis, Int. J. Antimicrob. Agents. 26 (2005) 298–303. https://doi.org/10.1016/j.ijantimicag.2005.07.012.

[36] R. Pandey, A. Sharma, A. Zahoor, S. Sharma, G.K. Khuller, B. Prasad, Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis, J. Antimicrob. Chemother. 52 (2003) 981–986. https://doi.org/10.1093/jac/dkg477.

[37] S. Kimura, K. Egashira, L. Chen, K. Nakano, E. Iwata, M. Miyagawa, H. Tsujimoto, K. Hara, R. Morishita, K. Sueishi, R. Tominaga, K. Sunagawa, Nanoparticle-mediated delivery of nuclear factor kappaB decoy into lungs ameliorates monocrotaline-induced pulmonary arterial hypertension, Hypertens. Dallas Tex 1979. 53 (2009) 877–883. https://doi.org/10.1161/HYPERTENSIONAHA.108.121418.

[38] S. Azarmi, X. Tao, H. Chen, Z. Wang, W.H. Finlay, R. Löbenberg, W.H. Roa, Formulation and cytotoxicity of doxorubicin nanoparticles carried by dry powder aerosol particles, Int. J. Pharm. 319 (2006) 155–161. https://doi.org/10.1016/j.ijpharm.2006.03.052.

[39] W. Zou, C. Liu, Z. Chen, N. Zhang, Studies on bioadhesive PLGA nanoparticles: A promising gene delivery system for efficient gene therapy to lung cancer, Int. J. Pharm. 370 (2009) 187–195. https://doi.org/10.1016/j.ijpharm.2008.11.016.

[40] A.L. Cortajarena, D. Ortega, S.M. Ocampo, A. Gonzalez-García, P. Couleaud, R. Miranda, C. Belda-Iniesta, A. Ayuso-Sacido, Engineering Iron Oxide Nanoparticles for Clinical Settings, Nanobiomedicine. 1 (2014) 2. https://doi.org/10.5772/58841.

[41] J. Pellico, J. Llop, I. Fernández-Barahona, R. Bhavesh, J. Ruiz-Cabello, F. Herranz, Iron Oxide Nanoradiomaterials: Combining Nanoscale Properties with Radioisotopes for Enhanced Molecular Imaging, Contrast Media Mol. Imaging. 2017 (2017) e1549580. https://doi.org/10.1155/2017/1549580.

[42] F. Rommasi, N. Esfandiari, Liposomal Nanomedicine: Applications for Drug Delivery in Cancer Therapy, Nanoscale Res. Lett. 16 (2021) 95. https://doi.org/10.1186/s11671-021-03553-8.

[43] S. Habib, M. Singh, Recent Advances in Lipid-Based Nanosystems for Gemcitabine and Gemcitabine-Combination Therapy, Nanomater. Basel Switz. 11 (2021) 597. https://doi.org/10.3390/nano11030597.

[44] J. Ahmad, S. Akhter, M. Rizwanullah, S. Amin, M. Rahman, M.Z. Ahmad, M.A. Rizvi, M.A. Kamal, F.J. Ahmad, Nanotechnology-based inhalation treatments for lung cancer: state of the art, Nanotechnol. Sci. Appl. 8 (2015) 55–66. https://doi.org/10.2147/NSA.S49052.

[45] C.L. Ngan, A.A. Asmawi, Lipid-based pulmonary delivery system: a review and future considerations of formulation strategies and limitations, Drug Deliv. Transl. Res. 8 (2018) 1527–1544. https://doi.org/10.1007/s13346-018-0550-4.

[46] M.M. Bailey, C.J. Berkland, Nanoparticle formulations in pulmonary drug delivery, Med. Res. Rev. 29 (2009) 196–212. https://doi.org/10.1002/med.20140.

[47] J.C. Sung, B.L. Pulliam, D.A. Edwards, Nanoparticles for drug delivery to the lungs, Trends Biotechnol. 25 (2007) 563–570. https://doi.org/10.1016/j.tibtech.2007.09.005.

[48] A. Hussain, J.J. Arnold, M.A. Khan, F. Ahsan, Absorption enhancers in pulmonary protein delivery, J. Control. Release Off. J. Control. Release Soc. 94 (2004) 15–24. https://doi.org/10.1016/j.jconrel.2003.10.001.

[49] C. Kleinstreuer, Z. Zhang, J.F. Donohue, Targeted drug-aerosol delivery in the human respiratory system, Annu. Rev. Biomed. Eng. 10 (2008) 195–220. https://doi.org/10.1146/annurev.bioeng.10.061807.160544.

[50] T. Cedervall, I. Lynch, S. Lindman, T. Berggård, E. Thulin, H. Nilsson, K.A. Dawson, S. Linse, Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2050–2055. https://doi.org/10.1073/pnas.0608582104.

[51] V. Forest, CHAPTER 2:Biological Significance of the Nanoparticles Protein Corona, in: Nanoparticle–Protein Corona, 2019: pp. 31–60. https://doi.org/10.1039/9781788016308-00031.

[52] V. Forest, J. Pourchez, Preferential binding of positive nanoparticles on cell membranes is due to electrostatic interactions: A too simplistic explanation that does not take into account the nanoparticle protein corona, Mater. Sci. Eng. C Mater. Biol. Appl. 70 (2017) 889–896. https://doi.org/10.1016/j.msec.2016.09.016.

[53] N. Vij, Nano-based theranostics for chronic obstructive lung diseases: challenges and therapeutic potential, Expert Opin. Drug Deliv. 8 (2011) 1105–1109. https://doi.org/10.1517/17425247.2011.597381.

[54] E. Rytting, J. Nguyen, X. Wang, T. Kissel, Biodegradable polymeric nanocarriers for pulmonary drug delivery, Expert Opin. Drug Deliv. 5 (2008) 629–639. https://doi.org/10.1517/17425247.5.6.629.

[55] B.S. Schuster, J.S. Suk, G.F. Woodworth, J. Hanes, Nanoparticle diffusion in respiratory mucus from humans without lung disease, Biomaterials. 34 (2013) 3439–3446. https://doi.org/10.1016/j.biomaterials.2013.01.064.

[56] J.V. Fahy, B.F. Dickey, Airway mucus function and dysfunction, N. Engl. J. Med. 363 (2010) 2233–2247. https://doi.org/10.1056/NEJMra0910061.

[57] L.A. Dailey, T. Kissel, New poly(lactic-co-glycolic acid) derivatives: Modular polymers with tailored properties, Drug Discov. Today Technol. 2 (2005) 7–13. https://doi.org/10.1016/j.ddtec.2005.05.017.

[58] Y. Xu, C.-S. Kim, D.M. Saylor, D. Koo, Polymer degradation and drug delivery in PLGA-based drug-polymer applications: A review of experiments and theories, J. Biomed. Mater. Res. B Appl. Biomater. 105 (2017) 1692–1716. https://doi.org/10.1002/jbm.b.33648.

[59] S.K. Sahoo, J. Panyam, S. Prabha, V. Labhasetwar, Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake, J. Control. Release Off. J. Control. Release Soc. 82 (2002) 105–114. https://doi.org/10.1016/s0168-3659(02)00127-x.

[60] W.-S. Cho, R. Duffin, C.A. Poland, A. Duschl, G.J. Oostingh, W. Macnee, M. Bradley, I.L. Megson, K. Donaldson, Differential pro-inflammatory effects of metal oxide nanoparticles and their soluble ions in vitro and in vivo; zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lungs, Nanotoxicology. 6 (2012) 22–35. https://doi.org/10.3109/17435390.2011.552810.

[61] W.-S. Cho, R. Duffin, F. Thielbeer, M. Bradley, I.L. Megson, W. Macnee, C.A. Poland, C.L. Tran, K. Donaldson, Zeta potential and solubility to toxic ions as mechanisms of lung inflammation caused by metal/metal oxide nanoparticles, Toxicol. Sci. Off. J. Soc. Toxicol. 126 (2012) 469–477. https://doi.org/10.1093/toxsci/kfs006.

[62] W.-S. Cho, R. Duffin, C.A. Poland, S.E.M. Howie, W. MacNee, M. Bradley, I.L. Megson, K. Donaldson, Metal oxide nanoparticles induce unique inflammatory footprints in the lung: important implications for nanoparticle testing, Environ. Health Perspect. 118 (2010) 1699–1706. https://doi.org/10.1289/ehp.1002201.

[63] L.K. Limbach, P. Wick, P. Manser, R.N. Grass, A. Bruinink, W.J. Stark, Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress, Environ. Sci. Technol. 41 (2007) 4158–4163. https://doi.org/10.1021/es062629t.

[64] C.F. Verschraegen, B.E. Gilbert, E. Loyer, A. Huaringa, G. Walsh, R.A. Newman, V. Knight, Clinical evaluation of the delivery and safety of aerosolized liposomal 9-nitro-20(s)-camptothecin in patients with advanced pulmonary malignancies, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 10 (2004) 2319–2326. https://doi.org/10.1158/1078-0432.ccr-0929-3.

[65] B.P.H. Wittgen, P.W.A. Kunst, K. van der Born, A.W. van Wijk, W. Perkins, F.G. Pilkiewicz, R. Perez-Soler, S. Nicholson, G.J. Peters, P.E. Postmus, Phase I study of aerosolized SLIT cisplatin in the treatment of patients with carcinoma of the lung, Clin.

Cancer Res. Off. J. Am. Assoc. Cancer Res. 13 (2007) 2414–2421. https://doi.org/10.1158/1078-0432.CCR-06-1480.

[66] A. Sharma, U.S. Sharma, Liposomes in drug delivery: Progress and limitations, Int. J. Pharm. 154 (1997) 123–140. https://doi.org/10.1016/S0378-5173(97)00135-X.

[67] V.S. Koster, P.F.M. Kuks, R. Lange, H. Talsma, Particle size in parenteral fat emulsions, what are the true limitations?, Int. J. Pharm. 134 (1996) 235–238. https://doi.org/10.1016/0378-5173(95)04409-4.

[68] W. Yang, J.I. Peters, R.O. Williams, Inhaled nanoparticles--a current review, Int. J. Pharm. 356 (2008) 239–247. https://doi.org/10.1016/j.ijpharm.2008.02.011.

[69] K.A. Foster, M. Yazdanian, K.L. Audus, Microparticulate uptake mechanisms of in-vitro cell culture models of the respiratory epithelium, J. Pharm. Pharmacol. 53 (2001) 57–66. https://doi.org/10.1211/0022357011775190.

[70] J. Davda, V. Labhasetwar, Characterization of nanoparticle uptake by endothelial cells, Int. J. Pharm. 233 (2002) 51–59. https://doi.org/10.1016/s0378-5173(01)00923-1.

[71] A.I. Minchinton, I.F. Tannock, Drug penetration in solid tumours, Nat. Rev. Cancer. 6 (2006) 583–592. https://doi.org/10.1038/nrc1893.

[72] J.H. Schiller, D. Harrington, C.P. Belani, C. Langer, A. Sandler, J. Krook, J. Zhu, D.H. Johnson, Eastern Cooperative Oncology Group, Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer, N. Engl. J. Med. 346 (2002) 92–98. https://doi.org/10.1056/NEJMoa011954.

[73] E. de Pablo, R. Fernández-García, M.P. Ballesteros, J.J. Torrado, D.R. Serrano, Nebulised antibiotherapy: conventional versus nanotechnology-based approaches, is targeting at a nano scale a difficult subject?, Ann. Transl. Med. 5 (2017) 448. https://doi.org/10.21037/atm.2017.09.17.

[74] R.K. Verma, M. Ibrahim, L. Garcia-Contreras, Lung Anatomy and Physiology and Their Implications for Pulmonary Drug Delivery, in: Pulm. Drug Deliv., John Wiley & Sons, Ltd, 2015: pp. 1–18. https://doi.org/10.1002/9781118799536.ch1.

[75] J.L. Rau, The inhalation of drugs: advantages and problems, Respir. Care. 50 (2005) 367–382.

[76] S. Mangal, W. Gao, T. Li, Q.T. Zhou, Pulmonary delivery of nanoparticle chemotherapy for the treatment of lung cancers: challenges and opportunities, Acta Pharmacol. Sin. 38 (2017) 782–797. https://doi.org/10.1038/aps.2017.34.

[77] K. Graham, E. Unger, Overcoming tumor hypoxia as a barrier to radiotherapy, chemotherapy and immunotherapy in cancer treatment, Int. J. Nanomedicine. 13 (2018) 6049–6058. https://doi.org/10.2147/IJN.S140462.

[78] J.J. Kim, I.F. Tannock, Repopulation of cancer cells during therapy: an important cause of treatment failure, Nat. Rev. Cancer. 5 (2005) 516–525. https://doi.org/10.1038/nrc1650.

[79] H.H. Popper, Progression and metastasis of lung cancer, Cancer Metastasis Rev. 35 (2016) 75–91. https://doi.org/10.1007/s10555-016-9618-0.

[80] J.S. Patton, C.S. Fishburn, J.G. Weers, The lungs as a portal of entry for systemic drug delivery, Proc. Am. Thorac. Soc. 1 (2004) 338–344. https://doi.org/10.1513/pats.200409-049TA.

[81] G.A. Ferron, Aerosol properties and lung deposition, Eur. Respir. J. 7 (1994) 1392–1394. https://doi.org/10.1183/09031936.94.07081392.

[82] F. Depreter, G. Pilcer, K. Amighi, Inhaled proteins: challenges and perspectives, Int. J. Pharm. 447 (2013) 251–280. https://doi.org/10.1016/j.ijpharm.2013.02.031.

[83] A. Ray, A. Mandal, A.K. Mitra, Recent Patents in Pulmonary Delivery of Macromolecules, Recent Pat. Drug Deliv. Formul. 9 (2015) 225–236. https://doi.org/10.2174/1872211309666150729122231.

[84] P. Muttil, C. Wang, A.J. Hickey, Inhaled drug delivery for tuberculosis therapy, Pharm. Res. 26 (2009) 2401–2416. https://doi.org/10.1007/s11095-009-9957-4.

[85] J. van Ingen, M. Obradovic, M. Hassan, B. Lesher, E. Hart, A. Chatterjee, C.L. Daley, Nontuberculous mycobacterial lung disease caused by Mycobacterium avium complex - disease burden, unmet needs, and advances in treatment developments, Expert Rev. Respir. Med. 15 (2021) 1387–1401. https://doi.org/10.1080/17476348.2021.1987891.

[86] Arikayce, Arikayce - Amikacin liposome inhalation suspension, (n.d.). https://www.arikayce.com/about-arikayce/.

[87] E.K. DIMITROVA, Arikayce liposomal, Eur. Med. Agency. (2020). https://www.ema.europa.eu/en/medicines/human/EPAR/arikayce-liposomal (accessed January 7, 2022).

[88] D. Arora, A. K. Goyal, S. R. Paliwal, B. Khurana, S. P. Vyas, Oral Mucosal Immunization: Recent Advancement and Future Prospects, Curr. Immunol. Rev. 6 (2010) 234–259. https://doi.org/10.2174/157339510791823637.

[89] M. Zaru, C. Sinico, A. De Logu, C. Caddeo, F. Lai, M.L. Manca, A.M. Fadda, Rifampicin-loaded liposomes for the passive targeting to alveolar macrophages: in vitro

and in vivo evaluation, J. Liposome Res. 19 (2009) 68–76. https://doi.org/10.1080/08982100802610835.

[90] M. Beck-Broichsitter, J. Gauss, T. Gessler, W. Seeger, T. Kissel, T. Schmehl, Pulmonary targeting with biodegradable salbutamol-loaded nanoparticles, J. Aerosol Med. Pulm. Drug Deliv. 23 (2010) 47–57. https://doi.org/10.1089/jamp.2009.0759.

[91] T.V.P. Doan, J.C. Olivier, Preparation of rifampicin-loaded PLGA microspheres for lung delivery as aerosol by premix membrane homogenization, Int. J. Pharm. 382 (2009) 61–66. https://doi.org/10.1016/j.ijpharm.2009.08.008.

[92] F. Ungaro, R. d'Emmanuele di Villa Bianca, C. Giovino, A. Miro, R. Sorrentino, F. Quaglia, M.I. La Rotonda, Insulin-loaded PLGA/cyclodextrin large porous particles with improved aerosolization properties: in vivo deposition and hypoglycaemic activity after delivery to rat lungs, J. Control. Release Off. J. Control. Release Soc. 135 (2009) 25–34. https://doi.org/10.1016/j.jconrel.2008.12.011.

[93] E.G. Langenback, J.M. Davis, C. Robbins, N. Sahgal, R.J. Perry, S.R. Simon, Improved pulmonary distribution of recombinant human Cu/Zn superoxide dismutase, using a modified ultrasonic nebulizer, Pediatr. Pulmonol. 27 (1999) 124–129. https://doi.org/10.1002/(sici)1099-0496(199902)27:2<124::aid-ppul9>3.0.co;2-e.

[94] W.H. Finlay, C. Darquenne, Particle Size Distributions, J. Aerosol Med. Pulm. Drug Deliv. 33 (2020) 178–180. https://doi.org/10.1089/jamp.2020.29028.whf.

[95] M. Paranjpe, C.C. Müller-Goymann, Nanoparticle-mediated pulmonary drug delivery: a review, Int. J. Mol. Sci. 15 (2014) 5852–5873. https://doi.org/10.3390/ijms15045852.

[96] M. Beck-Broichsitter, M.-C. Knuedeler, N. Oesterheld, W. Seeger, T. Schmehl, Boosting the aerodynamic properties of vibrating-mesh nebulized polymeric nanosuspensions, Int. J. Pharm. 459 (2014) 23–29. https://doi.org/10.1016/j.ijpharm.2013.11.040.

[97] M. Beck-Broichsitter, M.-C. Knuedeler, T. Schmehl, W. Seeger, Following the concentration of polymeric nanoparticles during nebulization, Pharm. Res. 30 (2013) 16–24. https://doi.org/10.1007/s11095-012-0819-0.

[98] M. Beck-Broichsitter, P. Kleimann, T. Schmehl, T. Betz, U. Bakowsky, T. Kissel, W. Seeger, Impact of lyoprotectants for the stabilization of biodegradable nanoparticles on the performance of air-jet, ultrasonic, and vibrating-mesh nebulizers, Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV. 82 (2012) 272–280. https://doi.org/10.1016/j.ejpb.2012.07.004.

[99] P.A. Bridges, K.M. Taylor, An investigation of some of the factors influencing the jet nebulisation of liposomes, Int. J. Pharm. 204 (2000) 69–79. https://doi.org/10.1016/s0378-5173(00)00477-4.

[100] L.A. Dailey, T. Schmehl, T. Gessler, M. Wittmar, F. Grimminger, W. Seeger, T. Kissel, Nebulization of biodegradable nanoparticles: impact of nebulizer technology and nanoparticle characteristics on aerosol features, J. Control. Release Off. J. Control. Release Soc. 86 (2003) 131–144. https://doi.org/10.1016/s0168-3659(02)00370-x.

[101] A.M.A. Elhissi, M. Faizi, W.F. Naji, H.S. Gill, K.M.G. Taylor, Physical stability and aerosol properties of liposomes delivered using an air-jet nebulizer and a novel micropump device with large mesh apertures, Int. J. Pharm. 334 (2007) 62–70. https://doi.org/10.1016/j.ijpharm.2006.10.022.

[102] M.M. Gaspar, O. Gobbo, C. Ehrhardt, Generation of liposome aerosols with the Aeroneb Pro and the AeroProbe nebulizers, J. Liposome Res. 20 (2010) 55–61. https://doi.org/10.3109/08982100903085150.

[103] N. Anton, A. Jakhmola, T.F. Vandamme, Trojan Microparticles for Drug Delivery, Pharmaceutics. 4 (2012) 1–25. https://doi.org/10.3390/pharmaceutics4010001.

[104] X. Li, N. Anton, T.M.C. Ta, M. Zhao, N. Messaddeq, T.F. Vandamme, Microencapsulation of nanoemulsions: novel Trojan particles for bioactive lipid molecule delivery, Int. J. Nanomedicine. 6 (2011) 1313–1325. https://doi.org/10.2147/IJN.S20353.

[105] N. Tsapis, D. Bennett, B. Jackson, D.A. Weitz, D.A. Edwards, Trojan particles: large porous carriers of nanoparticles for drug delivery, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 12001–12005. https://doi.org/10.1073/pnas.182233999.

[106] J. Haughney, D. Price, N.C. Barnes, J.C. Virchow, N. Roche, H. Chrystyn, Choosing inhaler devices for people with asthma: current knowledge and outstanding research needs, Respir. Med. 104 (2010) 1237–1245. https://doi.org/10.1016/j.rmed.2010.04.012.

[107] N.R. Labiris, M.B. Dolovich, Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications, Br. J. Clin. Pharmacol. 56 (2003) 588–599. https://doi.org/10.1046/j.1365-2125.2003.01892.x.

[108] W. Stahlhofen, J. Gebhart, J. Heyder, K. Philipson, P. Camner, Intercomparison of regional deposition of aerosol particles in the human respiratory tract and their long-term elimination, Exp. Lung Res. 2 (1981) 131–139. https://doi.org/10.3109/01902148109052309.

[109] W.D. Bennett, G.C. Smaldone, Human variation in the peripheral air-space deposition of inhaled particles, J. Appl. Physiol. Bethesda Md 1985. 62 (1987) 1603–1610. https://doi.org/10.1152/jappl.1987.62.4.1603.

[110] G.K. Crompton, Problems patients have using pressurized aerosol inhalers, Eur. J. Respir. Dis. Suppl. 119 (1982) 101–104.

[111] M. Dolovich, G. Ryan, M.T. Newhouse, Aerosol penetration into the lung; influence on airway responses, Chest. 80 (1981) 834–836.

[112] S.P. Newman, Therapeutic inhalation agents and devices. Effectiveness in asthma and bronchitis, Postgrad. Med. 76 (1984) 194–203, 206–207. https://doi.org/10.1080/00325481.1984.11698763.

[113] R.W. Niven, A.Y. Ip, S. Mittelman, S.J. Prestrelski, T. Arakawa, Some factors associated with the ultrasonic nebulization of proteins, Pharm. Res. 12 (1995) 53–59. https://doi.org/10.1023/a:1016282502954.

[114] A. Mignot, C. Truillet, F. Lux, L. Sancey, C. Louis, F. Denat, F. Boschetti, L. Bocher, A. Gloter, O. Stéphan, R. Antoine, P. Dugourd, D. Luneau, G. Novitchi, L.C. Figueiredo, P.C. de Morais, L. Bonneviot, B. Albela, F. Ribot, L. Van Lokeren, I. Déchamps-Olivier, F. Chuburu, G. Lemercier, C. Villiers, P.N. Marche, G. Le Duc, S. Roux, O. Tillement, P. Perriat, A top-down synthesis route to ultrasmall multifunctional Gd-based silica nanoparticles for theranostic applications, Chem. Weinh. Bergstr. Ger. 19 (2013) 6122–6136. https://doi.org/10.1002/chem.201203003.

[115] W.L. Eschenbacher, H.A. Boushey, D. Sheppard, Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone, Am. Rev. Respir. Dis. 129 (1984) 211–215.

[116] A. Weber, G. Morlin, M. Cohen, J. Williams-Warren, B. Ramsey, A. Smith, Effect of nebulizer type and antibiotic concentration on device performance, Pediatr. Pulmonol. 23 (1997) 249–260. https://doi.org/10.1002/(sici)1099-0496(199704)23:4<249::aid-ppul2>3.0.co;2-h.

[117] K.D. Ostrander, H.W. Bosch, D.M. Bondanza, An in-vitro assessment of a NanoCrystal beclomethasone dipropionate colloidal dispersion via ultrasonic nebulization, Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV. 48 (1999) 207–215. https://doi.org/10.1016/s0939-6411(99)00049-1.

[118] T.S. Wiedmann, L. DeCastro, R.W. Wood, Nebulization of NanoCrystals: production of a respirable solid-in-liquid-in-air colloidal dispersion, Pharm. Res. 14 (1997) 112–116. https://doi.org/10.1023/a:1012024021511.

[119] R. Ali, G. Mittal, R. Ali, M. Kumar, R. Kishan Khar, F.J. Ahmad, A. Bhatnagar, Development, characterisation and pharmacoscintigraphic evaluation of nano-fluticasone propionate dry powder inhalation as potential antidote against inhaled toxic gases, J. Microencapsul. 30 (2013) 546–558. https://doi.org/10.3109/02652048.2013.764937.

[120] G. Pilcer, K. Amighi, Formulation strategy and use of excipients in pulmonary drug delivery, Int. J. Pharm. 392 (2010) 1–19. https://doi.org/10.1016/j.ijpharm.2010.03.017.

[121] T.G. O'Riordan, Formulations and nebulizer performance, Respir. Care. 47 (2002) 1305–1312; discussion 1312-1313.

[122] M.J. Telko, A.J. Hickey, Dry powder inhaler formulation, Respir. Care. 50 (2005) 1209–1227.

[123] T.C. Carvalho, S.R. Carvalho, J.T. McConville, Formulations for pulmonary administration of anticancer agents to treat lung malignancies, J. Aerosol Med. Pulm. Drug Deliv. 24 (2011) 61–80. https://doi.org/10.1089/jamp.2009.0794.

[124] R. Rosière, T. Berghmans, P. De Vuyst, K. Amighi, N. Wauthoz, The Position of Inhaled Chemotherapy in the Care of Patients with Lung Tumors: Clinical Feasibility and Indications According to Recent Pharmaceutical Progresses, Cancers. 11 (2019) E329. https://doi.org/10.3390/cancers11030329.

[125] M.M. El-Baseir, M.A. Phipps, I.W. Kellaway, Preparation and subsequent degradation of poly(l-lactic acid) microspheres suitable for aerosolisation: a physico-chemical study, Int. J. Pharm. 2 (1997) 145–153. https://doi.org/10.1016/S0378-5173(96)04873-9.

[126] K.N. Olivier, D.E. Griffith, G. Eagle, J.P. McGinnis, L. Micioni, K. Liu, C.L. Daley, K.L. Winthrop, S. Ruoss, D.J. Addrizzo-Harris, P.A. Flume, D. Dorgan, M. Salathe, B.A. Brown-Elliott, R. Gupta, R.J. Wallace, Randomized Trial of Liposomal Amikacin for Inhalation in Nontuberculous Mycobacterial Lung Disease, Am. J. Respir. Crit. Care Med. 195 (2017) 814–823. https://doi.org/10.1164/rccm.201604-0700OC.

[127] K. Nakamura, H. Matsubara, S. Akagi, T. Sarashina, K. Ejiri, N. Kawakita, M. Yoshida, T. Miyoshi, A. Watanabe, N. Nishii, H. Ito, Nanoparticle-Mediated Drug Delivery System for Pulmonary Arterial Hypertension, J. Clin. Med. 6 (2017) E48. https://doi.org/10.3390/jcm6050048.

[128] J. Varshosaz, S. Ghaffari, S.F. Mirshojaei, A. Jafarian, F. Atyabi, F. Kobarfard, S. Azarmi, Biodistribution of amikacin solid lipid nanoparticles after pulmonary delivery, BioMed Res. Int. 2013 (2013) 136859. https://doi.org/10.1155/2013/136859.

[129] N. Vij, T. Min, M. Bodas, A. Gorde, I. Roy, Neutrophil targeted nano-drug delivery system for chronic obstructive lung diseases, Nanomedicine Nanotechnol. Biol. Med. 12 (2016) 2415–2427. https://doi.org/10.1016/j.nano.2016.06.008.

[130] null Bhavna, F.J. Ahmad, G. Mittal, G.K. Jain, G. Malhotra, R.K. Khar, A. Bhatnagar, Nano-salbutamol dry powder inhalation: a new approach for treating broncho-constrictive conditions, Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV. 71 (2009) 282–291. https://doi.org/10.1016/j.ejpb.2008.09.018.

[131] K.S. Konduri, S. Nandedkar, D.A. Rickaby, N. Düzgüneş, P.R.J. Gangadharam, The use of sterically stabilized liposomes to treat asthma, Methods Enzymol. 391 (2005) 413–427. https://doi.org/10.1016/S0076-6879(05)91023-9.

[132] V. Codullo, E. Cova, L. Pandolfi, S. Breda, M. Morosini, V. Frangipane, M. Malatesta, L. Calderan, M. Cagnone, C. Pacini, L. Cavagna, H. Recalde, J.H.W. Distler, M. Giustra, D. Prosperi, M. Colombo, F. Meloni, C. Montecucco, Imatinib-loaded gold nanoparticles inhibit proliferation of fibroblasts and macrophages from systemic sclerosis patients and ameliorate experimental bleomycin-induced lung fibrosis, J. Control. Release Off. J. Control. Release Soc. 310 (2019) 198–208. https://doi.org/10.1016/j.jconrel.2019.08.015.

[133] R. Trivedi, E.F. Redente, A. Thakur, D.W.H. Riches, U.B. Kompella, Local delivery of biodegradable pirfenidone nanoparticles ameliorates bleomycin-induced pulmonary fibrosis in mice, Nanotechnology. 23 (2012) 505101. https://doi.org/10.1088/0957-4484/23/50/505101.

[134] O. Taratula, A. Kuzmov, M. Shah, O.B. Garbuzenko, T. Minko, Nanostructured lipid carriers as multifunctional nanomedicine platform for pulmonary co-delivery of anticancer drugs and siRNA, J. Control. Release Off. J. Control. Release Soc. 171 (2013) 349–357. https://doi.org/10.1016/j.jconrel.2013.04.018.

[135] N.V. Koshkina, J.C. Waldrep, L.E. Roberts, E. Golunski, S. Melton, V. Knight, Paclitaxel liposome aerosol treatment induces inhibition of pulmonary metastases in murine renal carcinoma model, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 7 (2001) 3258–3262.

[136] C. Xu, Y. Wang, Z. Guo, J. Chen, L. Lin, J. Wu, H. Tian, X. Chen, Pulmonary delivery by exploiting doxorubicin and cisplatin co-loaded nanoparticles for metastatic lung cancer therapy, J. Control. Release Off. J. Control. Release Soc. 295 (2019) 153–163. https://doi.org/10.1016/j.jconrel.2018.12.013.

[137] J. Jiménez-López, M.M. El-Hammadi, R. Ortiz, M.D. Cayero-Otero, L. Cabeza, G. Perazzoli, L. Martin-Banderas, J.M. Baeyens, J. Prados, C. Melguizo, A novel nanoformulation of PLGA with high non-ionic surfactant content improves in vitro and in vivo PTX activity against lung cancer, Pharmacol. Res. 141 (2019) 451–465. https://doi.org/10.1016/j.phrs.2019.01.013.

[138] K. Shirkhani, I. Teo, D. Armstrong-James, S. Shaunak, Nebulised amphotericin B-polymethacrylic acid nanoparticle prophylaxis prevents invasive aspergillosis, Nanomedicine Nanotechnol. Biol. Med. 11 (2015) 1217–1226. https://doi.org/10.1016/j.nano.2015.02.012.

[139] M. Doroudian, R. MacLoughlin, F. Poynton, A. Prina-Mello, S.C. Donnelly, Nanotechnology based therapeutics for lung disease, Thorax. 74 (2019) 965–976. https://doi.org/10.1136/thoraxjnl-2019-213037.

[140] J.P. Wong, H. Yang, K.L. Blasetti, G. Schnell, J. Conley, L.N. Schofield, Liposome delivery of ciprofloxacin against intracellular Francisella tularensis infection, J. Control. Release Off. J. Control. Release Soc. 92 (2003) 265–273. https://doi.org/10.1016/s0168-3659(03)00358-4.

[141] T. Ellis, M. Chiappi, A. García-Trenco, M. Al-Ejji, S. Sarkar, T.K. Georgiou, M.S.P. Shaffer, T.D. Tetley, S. Schwander, M.P. Ryan, A.E. Porter, Multimetallic Microparticles Increase the Potency of Rifampicin against Intracellular Mycobacterium tuberculosis, ACS Nano. 12 (2018) 5228–5240. https://doi.org/10.1021/acsnano.7b08264.

[142] A.A. Hwang, B.-Y. Lee, D.L. Clemens, B.J. Dillon, J.I. Zink, M.A. Horwitz, pH-Responsive Isoniazid-Loaded Nanoparticles Markedly Improve Tuberculosis Treatment in Mice, Small Weinh. Bergstr. Ger. 11 (2015) 5066–5078. https://doi.org/10.1002/smll.201500937.

[143] O.B. Garbuzenko, N. Kbah, A. Kuzmov, N. Pogrebnyak, V. Pozharov, T. Minko, Inhalation treatment of cystic fibrosis with lumacaftor and ivacaftor co-delivered by nanostructured lipid carriers, J. Control. Release Off. J. Control. Release Soc. 296 (2019) 225–231. https://doi.org/10.1016/j.jconrel.2019.01.025.

[144] N. Günday Türeli, A. Torge, J. Juntke, B.C. Schwarz, N. Schneider-Daum, A.E. Türeli, C.-M. Lehr, M. Schneider, Ciprofloxacin-loaded PLGA nanoparticles against cystic fibrosis P. aeruginosa lung infections, Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV. 117 (2017) 363–371. https://doi.org/10.1016/j.ejpb.2017.04.032.

[145] J. Ernst, M. Klinger-Strobel, K. Arnold, J. Thamm, A. Hartung, M.W. Pletz, O. Makarewicz, D. Fischer, Polyester-based particles to overcome the obstacles of mucus and biofilms in the lung for tobramycin application under static and dynamic fluidic conditions, Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik EV. 131 (2018) 120–129. https://doi.org/10.1016/j.ejpb.2018.07.025.

[146] E.W. Alton, P.G. Middleton, N.J. Caplen, S.N. Smith, D.M. Steel, F.M. Munkonge, P.K. Jeffery, D.M. Geddes, S.L. Hart, R. Williamson, Non-invasive liposome-mediated gene delivery can correct the ion transport defect in cystic fibrosis mutant mice, Nat. Genet. 5 (1993) 135–142. https://doi.org/10.1038/ng1093-135.

[147] S.L. Chadwick, H.D. Kingston, M. Stern, R.M. Cook, B.J. O'Connor, M. Lukasson, R.P. Balfour, M. Rosenberg, S.H. Cheng, A.E. Smith, D.P. Meeker, D.M. Geddes, E.W. Alton, Safety of a single aerosol administration of escalating doses of the cationic lipid GL-67/DOPE/DMPE-PEG5000 formulation to the lungs of normal volunteers, Gene Ther. 4 (1997) 937–942. https://doi.org/10.1038/sj.gt.3300481.

[148] C.L. Densmore, T.H. Giddings, J.C. Waldrep, B.M. Kinsey, V. Knight, Gene transfer by guanidinium-cholesterol: dioleoylphosphatidyl-ethanolamine liposome-DNA complexes in aerosol, J. Gene Med. 1 (1999) 251–264. https://doi.org/10.1002/(SICI)1521-2254(199907/08)1:4<251::AID-JGM43>3.0.CO;2-Z.

[149] H. Eliyahu, A. Joseph, J.P. Schillemans, T. Azzam, A.J. Domb, Y. Barenholz, Characterization and in vivo performance of dextran-spermine polyplexes and DOTAP/cholesterol lipoplexes administered locally and systemically, Biomaterials. 28 (2007) 2339–2349. https://doi.org/10.1016/j.biomaterials.2006.09.001.

[150] A. Gautam, C.L. Densmore, J.C. Waldrep, Pulmonary cytokine responses associated with PEI-DNA aerosol gene therapy, Gene Ther. 8 (2001) 254–257. https://doi.org/10.1038/sj.gt.3301369.

[151] N. Oudrhiri, J.P. Vigneron, M. Peuchmaur, T. Leclerc, J.M. Lehn, P. Lehn, Gene transfer by guanidinium-cholesterol cationic lipids into airway epithelial cells in vitro and in vivo, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1651–1656. https://doi.org/10.1073/pnas.94.5.1651.

[152] R. Pillai, K. Petrak, P. Blezinger, D. Deshpande, V. Florack, B. Freimark, G. Padmabandu, A. Rolland, Ultrasonic nebulization of cationic lipid-based gene delivery systems for airway administration, Pharm. Res. 15 (1998) 1743–1747. https://doi.org/10.1023/a:1011964813817.

[153] B. Pitard, N. Oudrhiri, O. Lambert, E. Vivien, C. Masson, B. Wetzer, M. Hauchecorne, D. Scherman, J.L. Rigaud, J.P. Vigneron, J.M. Lehn, P. Lehn, Sterically stabilized BGTC-based lipoplexes: structural features and gene transfection into the mouse airways in vivo, J. Gene Med. 3 (2001) 478–487. https://doi.org/10.1002/jgm.211.

[154] R. Stribling, E. Brunette, D. Liggitt, K. Gaensler, R. Debs, Aerosol gene delivery in vivo, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 11277–11281. https://doi.org/10.1073/pnas.89.23.11277.

[155] W. Möller, K. Felten, J. Seitz, K. Sommerer, S. Takenaka, P. Wiebert, K. Philipson, M. Svartengren, W.G. Kreyling, A generator for the production of radiolabelled ultrafine carbonaceous particles for deposition and clearance studies in the respiratory tract, J. Aerosol Sci. 37 (2006) 631–644. https://doi.org/10.1016/j.jaerosci.2005.05.010.

[156] J. Pourchez, I.M.D. Albuquerque-Silva, M. Cottier, A. Clotagatide, L. Vecellio, M. Durand, F. Dubois, Generation and characterization of radiolabelled nanosized carbonaceous aerosols for human inhalation studies, J. Aerosol Sci. 55 (2013) 1–11. https://doi.org/10.1016/j.jaerosci.2012.07.011.

[157] J. Jögi, B. Jonson, M. Ekberg, M. Bajc, Ventilation-perfusion SPECT with 99mTc-DTPA versus Technegas: a head-to-head study in obstructive and nonobstructive disease, J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 51 (2010) 735–741. https://doi.org/10.2967/jnumed.109.073957.

[158] T.J. Senden, K.H. Moock, J.F. Gerald, W.M. Burch, R.J. Browitt, C.D. Ling, G.A. Heath, The physical and chemical nature of technegas, J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 38 (1997) 1327–1333.

[159] J.J. Lloyd, R.A. Shields, C.J. Taylor, R.S. Lawson, J.M. James, H.J. Testra, Technegas and Pertechnegas particle size distribution, Eur. J. Nucl. Med. 22 (1995) 473–476. https://doi.org/10.1007/BF00839062.

[160] W.M. Burch, P.J. Sullivan, C.J. McLaren, Technegas--a new ventilation agent for lung scanning, Nucl. Med. Commun. 7 (1986) 865–871. https://doi.org/10.1097/00006231-198612000-00003.

[161] J.A. Champion, Y.K. Katare, S. Mitragotri, Particle shape: a new design parameter for micro- and nanoscale drug delivery carriers, J. Control. Release Off. J. Control. Release Soc. 121 (2007) 3–9. https://doi.org/10.1016/j.jconrel.2007.03.022.

[162] P. Muralidharan, M. Malapit, E. Mallory, D. Hayes, H.M. Mansour, Inhalable nanoparticulate powders for respiratory delivery, Nanomedicine Nanotechnol. Biol. Med. 11 (2015) 1189–1199. https://doi.org/10.1016/j.nano.2015.01.007.

[163] I. Albuquerque-Silva, L. Vecellio, M. Durand, J. Avet, D. Le Pennec, M. de Monte, J. Montharu, P. Diot, M. Cottier, F. Dubois, J. Pourchez, Particle deposition in a child respiratory tract model: in vivo regional deposition of fine and ultrafine aerosols in baboons, PloS One. 9 (2014) e95456. https://doi.org/10.1371/journal.pone.0095456.

[164] L. Leclerc, J. Pourchez, G. Aubert, S. Leguellec, L. Vecellio, M. Cottier, M. Durand, Impact of airborne particle size, acoustic airflow and breathing pattern on delivery of nebulized antibiotic into the maxillary sinuses using a realistic human nasal replica, Pharm. Res. 31 (2014) 2335–2343. https://doi.org/10.1007/s11095-014-1330-6.

[165] S. Perinel, L. Leclerc, N. Prévôt, A. Deville, M. Cottier, M. Durand, J.-M. Vergnon, J. Pourchez, Micron-sized and submicron-sized aerosol deposition in a new ex vivo preclinical model, Respir. Res. 17 (2016) 78. https://doi.org/10.1186/s12931-016-0395-7.

[166] A. Leclercq, Afrezza® : le retour des insulines à inhaler ?, UPJV - Université de Picardie Jules Verne, 2015. https://dumas.ccsd.cnrs.fr/dumas-01379443 (accessed January 7, 2022).

[167] MannKind, MannKind Presents Data at 21st Annual Diabetes Technology Meeting, (2021). https://www.globenewswire.com/news-release/2021/11/05/2328421/29517/en/MannKind-Presents-Data-at-21st-Annual-Diabetes-Technology-Meeting.html.

[168] P.C. Richardson, A.H. Boss, Technosphere insulin technology, Diabetes Technol. Ther. 9 Suppl 1 (2007) S65-72. https://doi.org/10.1089/dia.2007.0212.

[169] M.B. Dolovich, R.C. Ahrens, D.R. Hess, P. Anderson, R. Dhand, J.L. Rau, G.C. Smaldone, G. Guyatt, American College of Chest Physicians, American College of Asthma, Allergy, and Immunology, Device selection and outcomes of aerosol therapy: Evidence-based guidelines: American College of Chest Physicians/American College of Asthma, Allergy, and Immunology, Chest. 127 (2005) 335–371. https://doi.org/10.1378/chest.127.1.335.

[170] M.W. Sims, Aerosol therapy for obstructive lung diseases: device selection and practice management issues, Chest. 140 (2011) 781–788. https://doi.org/10.1378/chest.10-2068.

[171] M. Ibrahim, R. Verma, L. Garcia-Contreras, Inhalation drug delivery devices: technology update, Med. Devices Auckl. NZ. 8 (2015) 131–139. https://doi.org/10.2147/MDER.S48888.

[172] https://www.gov.uk/government/publications/e-cigarettes-an-evidence-update, E-cigarettes: an evidence update, Httpswwwgovukgovernmentpublicationse-Cigar.--Evid.-

Update. (n.d.). https://www.gov.uk/government/publications/e-cigarettes-an-evidence-update (accessed November 3, 2021).

[173] K. Fagerstrom, J.-F. Etter, J.B. Unger, E-cigarettes: a disruptive technology that revolutionizes our field?, Nicotine Tob. Res. Off. J. Soc. Res. Nicotine Tob. 17 (2015) 125–126. https://doi.org/10.1093/ntr/ntu240.

[174] J.D. Rabinowitz, P.M. Lloyd, P. Munzar, D.J. Myers, S. Cross, R. Damani, R. Quintana, D.A. Spyker, P. Soni, J.V. Cassella, Ultra-fast absorption of amorphous pure drug aerosols via deep lung inhalation, J. Pharm. Sci. 95 (2006) 2438–2451. https://doi.org/10.1002/jps.20694.

[175] J.D. Rabinowitz, M. Wensley, P. Lloyd, D. Myers, W. Shen, A. Lu, C. Hodges, R. Hale, D. Mufson, A. Zaffaroni, Fast onset medications through thermally generated aerosols, J. Pharmacol. Exp. Ther. 309 (2004) 769–775. https://doi.org/10.1124/jpet.103.062893.

[176] K.E. Farsalinos, N. Yannovits, T. Sarri, V. Voudris, K. Poulas, Protocol proposal for, and evaluation of, consistency in nicotine delivery from the liquid to the aerosol of electronic cigarettes atomizers: regulatory implications, Addict. Abingdon Engl. 111 (2016) 1069–1076. https://doi.org/10.1111/add.13299.

[177] K.E. Farsalinos, A. Spyrou, K. Tsimopoulou, C. Stefopoulos, G. Romagna, V. Voudris, Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices, Sci. Rep. 4 (2014) 4133. https://doi.org/10.1038/srep04133.

[178] Medicines and Healthcare Products Regulatory Agency, UKPAR e-Voke 10 & 15mg Electronic Inhaler, 2015. https://mhraproductsprod.blob.core.windows.net/docs-20200420/56f25daab2a2968139bc37075e194d1a5f12b33f.

[179] I. Banoun, United States Patent Application: 0190054261, 20190054261, 2019. https://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=2&f=G&l=50&co1=AND&d=PG01&s1=Banoun&OS=Banoun&RS=Banoun (accessed November 3, 2021).

[180] L. Casula, C. Sinico, D. Valenti, E. Pini, R. Pireddu, M. Schlich, F. Lai, A. Maria Fadda, Delivery of beclomethasone dipropionate nanosuspensions with an electronic cigarette, Int. J. Pharm. 596 (2021) 120293. https://doi.org/10.1016/j.ijpharm.2021.120293.

[181] J. Pourchez, F. de Oliveira, S. Perinel-Ragey, T. Basset, J.-M. Vergnon, N. Prévôt, Assessment of new-generation high-power electronic nicotine delivery system as thermal aerosol generation device for inhaled bronchodilators, Int. J. Pharm. 518 (2017) 264–269. https://doi.org/10.1016/j.ijpharm.2017.01.009.

[182] E.D. Kuempel, C.L. Tran, V. Castranova, A.J. Bailer, Lung dosimetry and risk assessment of nanoparticles: evaluating and extending current models in rats and humans, Inhal. Toxicol. 18 (2006) 717–724. https://doi.org/10.1080/08958370600747887.

[183] R.A. Parent, Comparative Biology of the Normal Lung, Academic Press, 2015.

[184] O.V. Sjaastad, K. Hove, O. Sand, Physiology of Domestic Animals, Scan. Vet. Press, 2010.

[185] K.E. Pinkerton, L.S. Van Winkle, C. Plopper, S. Smiley-Jewell, E.C. Covarrubias, J.T. McBride, Architecture of the Tracheobronchial Tree, in: Comp. Biol. Norm. Lung Second Ed., Elsevier Inc., 2015: pp. 33–51. https://doi.org/10.1016/B978-0-12-404577-4.00004-7.

[186] W.R. Ford, A.E. Blair, R.L. Evans, E. John, J.J. Bugert, K.J. Broadley, E.J. Kidd, Human parainfluenza type 3 virus impairs the efficacy of glucocorticoids to limit allergy-induced pulmonary inflammation in guinea-pigs, Clin. Sci. Lond. Engl. 1979. 125 (2013) 471–482. https://doi.org/10.1042/CS20130130.

[187] M. Awadalla, S. Miyawaki, M.H. Abou Alaiwa, R.J. Adam, D.C. Bouzek, A.S. Michalski, M.K. Fuld, K.J. Reynolds, E.A. Hoffman, C.-L. Lin, D.A. Stoltz, Early Airway Structural Changes in Cystic Fibrosis Pigs as a Determinant of Particle Distribution and Deposition, Ann. Biomed. Eng. (2013). https://doi.org/10.1007/s10439-013-0955-7.

[188] M.D.I. Manunta, R.J. McAnulty, A. McDowell, J. Jin, D. Ridout, J. Fleming, S.E. Bottoms, L. Tossici-Bolt, G.J. Laurent, L. Biassoni, C. O'Callaghan, S.L. Hart, Airway deposition of nebulized gene delivery nanocomplexes monitored by radioimaging agents, Am. J. Respir. Cell Mol. Biol. 49 (2013) 471–480. https://doi.org/10.1165/rcmb.2013-0030OC.

[189] A.L. Patra, Comparative anatomy of mammalian respiratory tracts: the nasopharyngeal region and the tracheobronchial region, J. Toxicol. Environ. Health. 17 (1986) 163–174. https://doi.org/10.1080/15287398609530813.

[190] A.L. Patra, A. Gooya, M.G. Ménache, A morphometric comparison of the nasopharyngeal airway of laboratory animals and humans, Anat. Rec. 215 (1986) 42–50. https://doi.org/10.1002/ar.1092150107.

[191] A. Bianchi, S. Dufort, F. Lux, A. Courtois, O. Tillement, J.-L. Coll, Y. Crémillieux, Quantitative biodistribution and pharmacokinetics of multimodal gadolinium-based

nanoparticles for lungs using ultrashort TE MRI, Magma N. Y. N. 27 (2014) 303–316. https://doi.org/10.1007/s10334-013-0412-5.

[192] A. Bianchi, F. Lux, O. Tillement, Y. Crémillieux, Contrast enhanced lung MRI in mice using ultra-short echo time radial imaging and intratracheally administrated Gd-DOTA-based nanoparticles, Magn. Reson. Med. 70 (2013) 1419–1426. https://doi.org/10.1002/mrm.24580.

[193] S. Dufort, A. Bianchi, M. Henry, F. Lux, G. Le Duc, V. Josserand, C. Louis, P. Perriat, Y. Crémillieux, O. Tillement, J.-L. Coll, Nebulized gadolinium-based nanoparticles: a theranostic approach for lung tumor imaging and radiosensitization, Small Weinh. Bergstr. Ger. 11 (2015) 215–221. https://doi.org/10.1002/smll.201401284.

[194] N. Tassali, A. Bianchi, F. Lux, G. Raffard, S. Sanchez, O. Tillement, Y. Crémillieux, MR imaging, targeting and characterization of pulmonary fibrosis using intra-tracheal administration of gadolinium-based nanoparticles, Contrast Media Mol. Imaging. 11 (2016) 396–404. https://doi.org/10.1002/cmmi.1703.

[195] L. Yang, R. Gradl, M. Dierolf, W. Möller, D. Kutschke, A. Feuchtinger, L. Hehn, M. Donnelley, B. Günther, K. Achterhold, A. Walch, T. Stoeger, D. Razansky, F. Pfeiffer, K.S. Morgan, O. Schmid, Multimodal Precision Imaging of Pulmonary Nanoparticle Delivery in Mice: Dynamics of Application, Spatial Distribution, and Dosimetry, Small Weinh. Bergstr. Ger. 15 (2019) e1904112. https://doi.org/10.1002/smll.201904112.

[196] L. Yang, A. Feuchtinger, W. Möller, Y. Ding, D. Kutschke, G. Möller, J.C. Schittny, G. Burgstaller, W. Hofmann, T. Stoeger, null Daniel Razansky, A. Walch, O. Schmid, Three-Dimensional Quantitative Co-Mapping of Pulmonary Morphology and Nanoparticle Distribution with Cellular Resolution in Nondissected Murine Lungs, ACS Nano. 13 (2019) 1029–1041. https://doi.org/10.1021/acsnano.8b07524.

[197] G. Oberdörster, C. Cox, R. Gelein, Intratracheal Instillation Versus Intratracheal Inhalation of Tracer Particles for Measuring Lung Clearance Function, Exp. Lung Res. 23 (1997) 17–34. https://doi.org/10.3109/01902149709046045.

[198] N. Shinohara, T. Nakazato, M. Tamura, S. Endoh, H. Fukui, Y. Morimoto, T. Myojo, M. Shimada, K. Yamamoto, H. Tao, Y. Yoshida, J. Nakanishi, Clearance Kinetics of Fullerene C60 Nanoparticles from Rat Lungs after Intratracheal C60 Instillation and Inhalation C60 Exposure, Toxicol. Sci. 118 (2010) 564–573. https://doi.org/10.1093/toxsci/kfq288.

[199] R. Landsiedel, U.G. Sauer, L. Ma-Hock, J. Schnekenburger, M. Wiemann, Pulmonary toxicity of nanomaterials: a critical comparison of published in vitro assays and in vivo

inhalation or instillation studies, Nanomed. 9 (2014) 2557–2585. https://doi.org/10.2217/nnm.14.149.

[200] M. Klockare, A. Dufva, A.-M. Danielsson, R. Hatherly, S. Larsson, H. Jacobsson, M. Mure, Comparison between direct humidification and nebulization of the respiratory tract at mechanical ventilation: distribution of saline solution studied by gamma camera, J. Clin. Nurs. 15 (2006) 301–307. https://doi.org/10.1111/j.1365-2702.2006.01300.x.

[201] Y. Montigaud, S. Périnel-Ragey, L. Plantier, L. Leclerc, C. Goy, A. Clotagatide, N. Prévôt, J. Pourchez, Development of an ex vivo preclinical respiratory model of idiopathic pulmonary fibrosis for aerosol regional studies, Sci. Rep. 9 (2019) 17949. https://doi.org/10.1038/s41598-019-54479-2.

[202] Y. Crémillieux, Y. Montigaud, C. Bal, N. Pinaud, V. Pham, S. Perinel, M. Natuzzi, F. Lux, O. Tillement, N. Ichinose, B. Zhang, J. Pourchez, Three-dimensional quantitative MRI of aerosolized gadolinium-based nanoparticles and contrast agents in isolated ventilated porcine lungs, Magn. Reson. Med. 83 (2020) 1774–1782. https://doi.org/10.1002/mrm.28041.

[203] Y. Montigaud, Q. Georges, J. Pourchez, L. Leclerc, C. Goy, A. Clotagatide, N. Prevot, S. Perinel-Ragey, Aerosol delivery during invasive mechanical ventilation: development of a preclinical ex vivo respiratory model for aerosol regional deposition, Sci. Rep. 9 (2019) 17930. https://doi.org/10.1038/s41598-019-54480-9.

[204] Y. Montigaud, J. Pourchez, L. Leclerc, O. Tillement, A. Clotagatide, C. Bal, N. Pinaud, N. Ichinose, B. Zhang, S. Perinel, F. Lux, Y. Crémillieux, N. Prevot, Nebulised Gadolinium-Based Nanoparticles for a Multimodal Approach: Quantitative and Qualitative Lung Distribution Using Magnetic Resonance and Scintigraphy Imaging in Isolated Ventilated Porcine Lungs, Int. J. Nanomedicine. 15 (2020) 7251–7262. https://doi.org/10.2147/IJN.S260640.

[205] F. Lux, V.L. Tran, E. Thomas, S. Dufort, F. Rossetti, M. Martini, C. Truillet, T. Doussineau, G. Bort, F. Denat, F. Boschetti, G. Angelovski, A. Detappe, Y. Crémillieux, N. Mignet, B.-T. Doan, B. Larrat, S. Meriaux, E. Barbier, S. Roux, P. Fries, A. Müller, M.-C. Abadjian, C. Anderson, E. Canet-Soulas, P. Bouziotis, M. Barberi-Heyob, C. Frochot, C. Verry, J. Balosso, M. Evans, J. Sidi-Boumedine, M. Janier, K. Butterworth, S. McMahon, K. Prise, M.-T. Aloy, D. Ardail, C. Rodriguez-Lafrasse, E. Porcel, S. Lacombe, R. Berbeco, A. Allouch, J.-L. Perfettini, C. Chargari, E. Deutsch, G. Le Duc, O. Tillement, AGuIX® from bench to bedside—Transfer of an ultrasmall theranostic

gadolinium-based nanoparticle to clinical medicine, Br. J. Radiol. 92 (2018) 20180365. https://doi.org/10.1259/bjr.20180365.

[206] C. Verry, L. Sancey, S. Dufort, G.L. Duc, C. Mendoza, F. Lux, S. Grand, J. Arnaud, J.L. Quesada, J. Villa, O. Tillement, J. Balosso, Treatment of multiple brain metastases using gadolinium nanoparticles and radiotherapy: NANO-RAD, a phase I study protocol, BMJ Open. 9 (2019). https://doi.org/10.1136/bmjopen-2018-023591.

[207] G. Bort, F. Lux, S. Dufort, Y. Crémillieux, C. Verry, O. Tillement, EPR-mediated tumor targeting using ultrasmall-hybrid nanoparticles: From animal to human with theranostic AGuIX nanoparticles, Theranostics. 10 (2020) 1319–1331. https://doi.org/10.7150/thno.37543.

[208] A. Bianchi, S. Dufort, F. Lux, P.-Y. Fortin, N. Tassali, O. Tillement, J.-L. Coll, Y. Crémillieux, Targeting and in vivo imaging of non-small-cell lung cancer using nebulized multimodal contrast agents, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 9247–9252. https://doi.org/10.1073/pnas.1402196111.

[209] D. Kryza, J. Taleb, M. Janier, L. Marmuse, I. Miladi, P. Bonazza, C. Louis, P. Perriat, S. Roux, O. Tillement, C. Billotey, Biodistribution study of nanometric hybrid gadolinium oxide particles as a multimodal SPECT/MR/optical imaging and theragnostic agent, Bioconjug. Chem. 22 (2011) 1145–1152. https://doi.org/10.1021/bc1005976.

[210] F. Lux, A. Mignot, P. Mowat, C. Louis, S. Dufort, C. Bernhard, F. Denat, F. Boschetti, C. Brunet, R. Antoine, P. Dugourd, S. Laurent, L. Vander Elst, R. Muller, L. Sancey, V. Josserand, J.-L. Coll, V. Stupar, E. Barbier, C. Rémy, A. Broisat, C. Ghezzi, G. Le Duc, S. Roux, P. Perriat, O. Tillement, Ultrasmall rigid particles as multimodal probes for medical applications, Angew. Chem. Int. Ed Engl. 50 (2011) 12299–12303. https://doi.org/10.1002/anie.201104104.

[211] I. Miladi, G.L. Duc, D. Kryza, A. Berniard, P. Mowat, S. Roux, J. Taleb, P. Bonazza, P. Perriat, F. Lux, O. Tillement, C. Billotey, M. Janier, Biodistribution of ultra small gadolinium-based nanoparticles as theranostic agent: application to brain tumors, J. Biomater. Appl. 28 (2013) 385–394. https://doi.org/10.1177/0885328212454315.

[212] E. Miot-Noirault, A. Vidal, J. Morlieras, P. Bonazza, P. Auzeloux, S. Besse, M.-M. Dauplat, C. Peyrode, F. Degoul, C. Billotey, F. Lux, F. Rédini, O. Tillement, J.-M. Chezal, D. Kryza, M. Janier, Small rigid platforms functionalization with quaternary ammonium: targeting extracellular matrix of chondrosarcoma, Nanomedicine Nanotechnol. Biol. Med. 10 (2014) 1887–1895. https://doi.org/10.1016/j.nano.2014.06.011.

[213] F. Andrade, P. Fonte, A. Costa, C.C. Reis, R. Nunes, A. Almeida, D. Ferreira, M. Oliva, B. Sarmento, Pharmacological and toxicological assessment of innovative self-assembled polymeric micelles as powders for insulin pulmonary delivery, Nanomed. 11 (2016) 2305–2317. https://doi.org/10.2217/nnm-2016-0045.

[214] C. Klingler, B.W. Müller, H. Steckel, Insulin-micro- and nanoparticles for pulmonary delivery, Int. J. Pharm. 377 (2009) 173–179. https://doi.org/10.1016/j.ijpharm.2009.05.008.

[215] Cupido, Cupido, Https://Www.Cupidoproject.Eu/. (2021). https://www.cupidoproject.eu/.

**Figure 1**

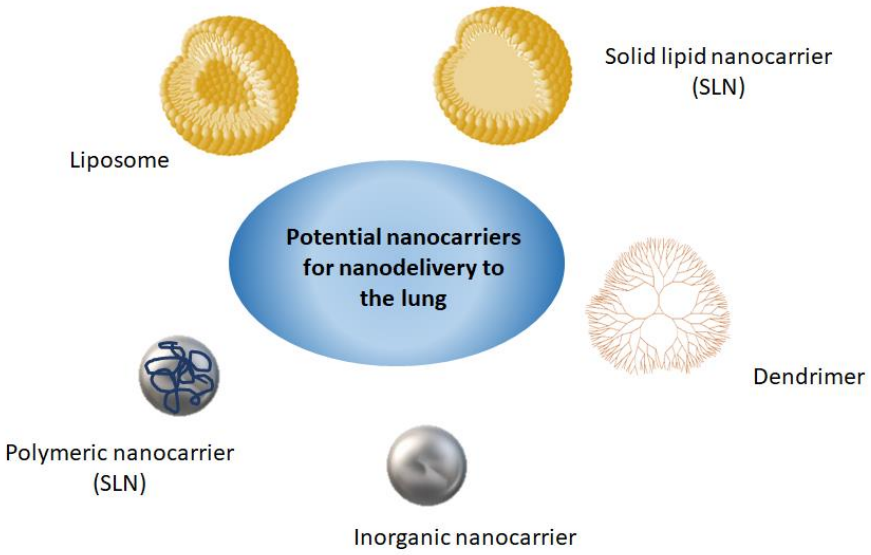
Liposome
Solid lipid nanocarrier
(SLN)
Potential nanocarriers
for nanodelivery to
the lung
Dendrimer
Polymeric nanocarrier
(SLN)
Inorganic nanocarrier

**Figure 2**

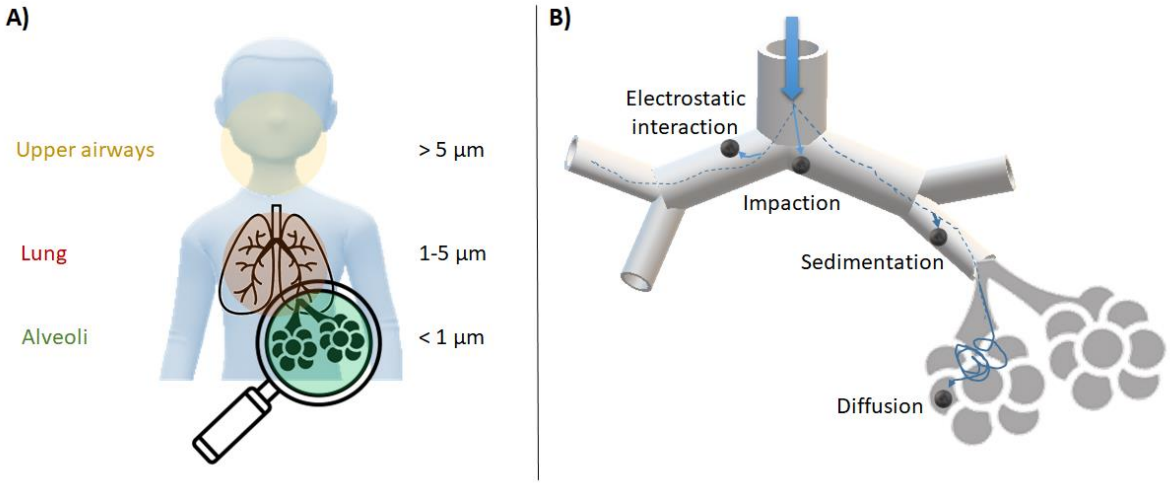
A)
Upper airways
Lung
Alveoli
> 5 µm
1-5 µm
< 1 µm
B)
Electrostatic interaction
Impaction
Sedimentation
Diffusion

**Figure captions**

**Figure 1** – Potential nanocarriers for nano-delivery to the lung (not exhaustive).

**Figure 2** – A) Influence of particle size on lung deposition. B) Deposition mechanisms.