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Nanotechnology approaches for inhalation treatment of lung diseases

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ABSTRACT

Local administration of therapeutics by inhalation for treatment of lung diseases has the ability to deliver drugs, nucleic acids and peptides specifically to the site of their action and therefore enhance the efficacy of the treatment, limit the penetration of nebulized therapeutic agent(s) into the bloodstream and consequently decrease adverse systemic side effects of the treatment. Nanotechnology allows for a further enhancement of the treatment efficiency. The present review analyzes modern therapeutic approaches of inhaled nanoscale-based pharmaceutics for the detection and treatment of various lung diseases.

1. Introduction

Lungs represent an attractive alternative route of drug delivery. They possess a large area for the deposition of therapeutics and high vascularization for the systemic delivery of various pharmaceutical agents. Inhalation lung delivery prevents the degradation of active components in the gastrointestinal tract and first pass metabolism in the liver. Despite these attractive advantages, systemic inhalation delivery of therapeutics is still not used widely. Possible high lung toxicity of drugs and their degradation by lung macrophages, the risk of drug-induced lung injury and occupational exposure of health care workers to nebulized drugs limit enthusiasm for the inhalation route for the systemic drug delivery. On the other hand, the use of systemic delivery of pharmaceuticals for treating lung diseases in most cases demonstrates a low efficiency and potentially severe adverse side effects on other organs. To enhance the efficacy of the treatment of various lung diseases and limit exposure of healthy organs to potentially toxic drugs, it seems natural to deliver therapeutics directly to the lungs by inhalation. An ideal drug formulation and inhalation delivery method should provide a local inhalation delivery specifically to the diseased cells, limit the exposure of healthy lung cells and restrict drug penetration into the circulation. While several efficient inhalation drug delivery devices were already developed, tested and implemented in clinical practice, the formulation of efficient drug delivery systems for local targeted inhalation delivery of various therapeutic modalities is still in the developmental stage.

Recent advances in nanotechnology open a door for enhancing the efficacy of inhalation treatment of different lung diseases. The application of nanotechnology to the design of drug delivery systems for effective delivery of therapeutics specifically to tissues and cells affected by the disease allows for enhanced treatment outcomes and prevention of severe adverse side effects upon tissues and cells, including those in the lungs, as well as entire organs.

The present review analyzes modern nanotechnology approaches for the local inhalation delivery of various drugs. It describes advantages and potential limitations of local inhalation drug delivery and discusses different types of drug delivery systems suitable for inhalation with a special emphasis on nanoparticle-based delivery vehicles. The review also analyzes modern therapeutic applications of inhalation delivery with an accent on the treatment of lung cancer and metastases. In addition this review briefly describes modern patents related to the inhalation drug delivery and recent clinical trials of therapeutics for treatment of lung diseases. Finally, it also describes future directions in nanotechnology approaches for inhalation treatment of lung diseases.

2. Inhalation local delivery

The term inhalation local delivery is used in most cases to denote inhalation route of delivering therapeutics or other exogenous entities directly to the lungs with their preferential accumulation in the specific lung areas or cells and limited penetration into the blood circulation. This type of inhalation delivery is especially useful for treating various lung diseases. It is expected that such delivery method will transport

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an active component(s) to the place of its action and retain it there for the required time. The efficiency of inhalation local delivery mainly depends on lung aerodynamics, breathing conditions, particle size, inhalation methods and devices used [1–8]. To be inhaled, a delivered liquid or solid should be suspended in a gaseous medium to form an aerosol [4,5,9–17]. In the present review, we will not discuss these factors as well as delivery devices and will instead focus our attention on the engineering of nanotechnology-based delivery systems that are being used for the local inhalation delivery of therapeutics to the lungs. For the detailed discussion of the influence of different types of aerosols, particle size, lung aerodynamics and inhalation devices on the efficacy of inhalation lung delivery, the reader is referred to the above cited manuscripts.

2.1. Advantages of inhalation local delivery

Major advantages of inhalation route of drug delivery locally to the lungs in order to treat lung diseases include direct delivery of active components to the diseased organs and cells, prevention of potentially toxic therapeutics from entering the bloodstream and therefore limiting possible adverse effects upon other healthy organs (Fig. 1). Moreover, if the inhaled delivery system is specifically targeted to diseased cells (*e.g.* cancer cells), then healthy lung cells will also be protected from the drug or other inhaled exogenous substances. An efficient delivery system specifically designed for the local inhalation lung delivery should therefore retain in the lungs or even preferably, specifically in the diseased cells for the required treatment period and not penetrate in its active form into the bloodstream in order to protect the rest of the body from the potentially toxic exposure.

2.1.1. Organ distribution

Local inhalation delivery of therapeutics in most cases substantially changes the organ distribution of delivery system and its active components in the organisms when compared with oral or parenteral delivery [17–24]. Although various carriers have different body distributions, a general tendency in the body distribution of nanocarriers with size less than 1 μm consists of the following [19]. After intravenous (systemic) delivery, the majority of the injected particles is accumulated in the liver, kidney and spleen (Fig. 2) [18]. A certain amount of nanoparticles (often about one quarter of an injected dose) accumulates in the lungs. Therefore, about 75% of the injected dose will most likely be lost for the treatment of lung diseases. This will not only reduce treatment efficacy, but will require higher doses to compensate an unfavorable drug distribution and potentially can induce severe adverse side effects on other organs. In contrast, some types of nanoparticles (liposomes, nanostructured lipid carriers, mesoporous silica nanoparticles, anisotropic polymer/lipid "Janus" particles, *etc.*) preferentially accumulate in the lungs after inhalation (Fig. 2) [18,19,21–25]. Consequently, even if nanocarriers are not targeted specifically to the lung cancer cells, so-called passive targeting [26] (in our case by a local inhalation delivery directly to the lungs) will enhance organ distribution. It should however be stressed, that targeting a drug specifically to lung cells (or cancer cells in the case of lung cancer) will change the distribution of intravenously injected nanoparticles toward their preferential accumulation in the lungs. However, local inhalation delivery of targeted nanoparticles will further improve the distribution profile, and therefore still will have advantages over the systemic delivery.

2.1.2. Pharmacokinetics

Local inhalation delivery of pharmaceutics directly into the lungs in most cases improves pharmacokinetics of the delivered agent(s). Generally, it increases the retention of the delivered drug in the lungs [17,19]. The area under the curve for overall drug retention in the lungs, half-life and maximal concentration of the drug in the lungs and lung tissue was increased greater for a local inhalation lung delivery when compared with systemic exposure (Fig. 3) [14]. Taken together, the improvements in the drug organ distribution and pharmacokinetics allow for decreasing the overall drug concentration needed for achieving the maximal therapeutic efficiency and substantially limiting adverse side effects upon healthy organs and tissues.

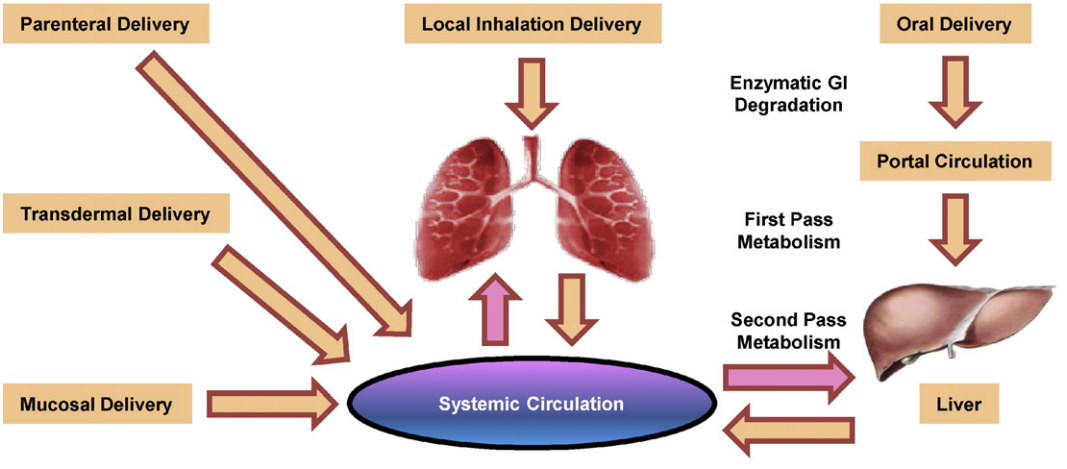

Fig. 1. Advantages and challenges of pulmonary drug delivery.

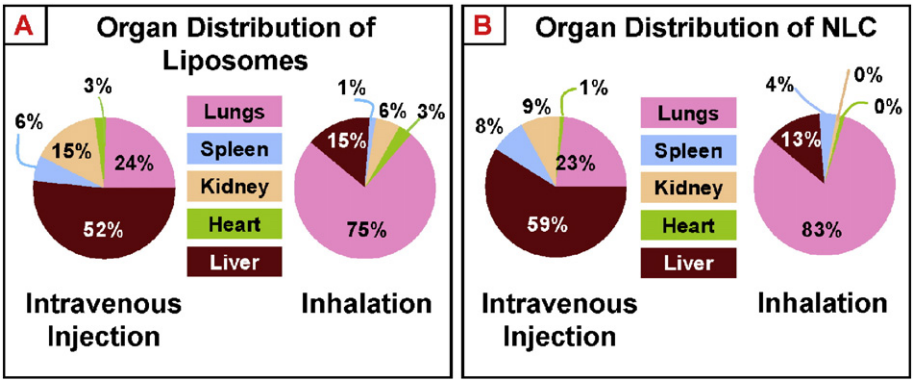

Fig. 2. Advantages of inhalation drug delivery: improved organ distribution. Organ distribution of liposomes (A) and nanostructured lipid carriers (NLC, B) after intravenous and inhalation delivery.
Modified from Ref. [18].

2.2. Potential limitations

Despite marked advantages of inhalation delivery for the treatment of lung diseases, some factors can potentially limit the practical implementation of this approach in the clinic. These limitations include several technological challenges associated with inhalation [27] and more general concerns related to the approach itself [4]. The later challenges are briefly discussed below.

2.2.1. Lung toxicity of drugs

When a drug or other biologically active substance is delivered *via* inhalation, the lungs are inherently exposed to its action and open a possibility of side effects. This is especially important in case of highly toxic anticancer drugs. Consequently, a major obstacle for systemic inhalation chemotherapy includes a fear that inhalation of highly toxic anticancer drug(s) may induce severe undesirable secondary effects on the lungs. Such a problem is less important when the cancer resides inside the lungs and therefore chemotherapeutic toxic effect is applied directly to the site of its action. In this case, the delivery of toxic chemotherapeutic substances directly into the lungs with limited penetration into the bloodstream may even protect the rest of the body from undesirable toxic effects of the treatment. However, when the toxic substance used for treatment distributed equally *via* the lung parenchyma, both diseased (*e.g.* cancer) and healthy lung cells potentially are exposed to the drug. In order to avoid this undesirable exposure of healthy cells, chemotherapeutic agent(s) or other drug(s) can be specifically targeted to the diseased cells.

2.2.2. Drug induced lung disease

Contemporary drugs usually are very potent. This not only enhances the efficacy of treatment of different diseases, but also increases the risk of undesirable side effects on healthy tissues. Being delivered by inhalation, some drugs can potentially induce lung damage, provoke the development of lung disease or increase the severity of existing ones. It was found that about 400 drugs can potentially induce different lung diseases [28]. Again, this problem is especially important in the case of lung cancer and associated inhalation chemotherapy. In many cases lung cancer or/and lung metastases are accompanied by other lung disturbances. Consequently, many patients with lung cancer have impaired pulmonary functions due to smoking and/or chemotherapy. Inhalation of toxic chemotherapeutic agents potentially can affect and further increase these complications. As was mentioned above, targeting of inhaled drug(s) specifically to diseased cells can help alleviate the problem.

2.2.3. Occupational exposure

Healthcare workers involved in providing inhalation therapy can be potentially exposed to the nebulized drug(s). In addition to systemic treatment, where personnel can also be exposed during the preparation, injection and disposal of the toxic agent, exhalation of residual amounts of aerosol represents a serious threat to the health of healthcare workers that routinely perform the treatment procedures. Fortunately, recently developed filters and air cleaning systems substantially minimized such risk [4]. Nevertheless, the possibility of exposure of health workers to the dangerous aerosols should be considered as a hazard during inhalation therapy and minimized by corresponding measures.

2.2.4. Lung defense mechanisms

The lung represents an organ in the human body that is systematically exposed to different and often damaging parenchyma substances. Naturally during evolution, many defensive mechanisms were developed in the lungs in order to limit exposure to potentially dangerous substances and minimize the damage caused to the lung structures

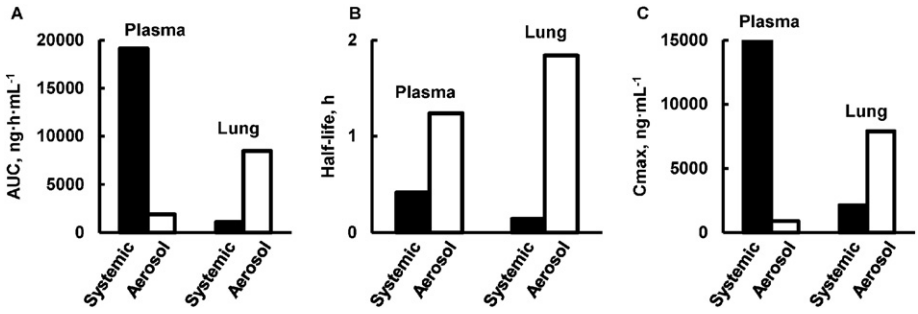

Fig. 3. Advantages of inhalation drug delivery: improved pharmacokinetics. The area under the curve (AUC, A), half-life (B) and maximal concentration (Cmax, C) of delivered anticancer drug (5-azacytidine) in the blood and lungs after systemic and aerosol delivery.
Plotted based on data from Ref. [14].

and cells. Major lung defensive mechanisms include beating cilia, mucus, macrophages, transporters and enzymes [11,29]. In addition, manifestations of lung disease(s) and impaired pulmonary function can potentially interfere with inhalation therapy and prevent deposition of aerosols into the desired regions of the lungs or cellular uptake of the therapeutics. Consequently, inhalation devices, regimens and delivery systems should be designed regarding these obstacles. It should be stressed however, that lung defensive mechanisms much less affect the local inhalation treatment of lung diseases when compared with systemic inhalation delivery. Nevertheless, lung defense mechanisms must be taken into account when designing delivery systems for inhalation therapy. In addition to drug(s), such complex systems may include additional components that help to overcome or suppress lung defensive mechanisms. For example, in order to defeat cellular drug efflux transporters that pumps out drugs from the lung cells, suppressors of corresponding proteins (*e.g.* nucleic acids or small molecules) can be included into a complex delivery system [20,24]. Similarly, suppressors of cellular anti-apoptotic resistance can also be included in the system in order to enhance cell death induction by anticancer drug(s) [22,23]. It should also be taken into account, that the complexity of the delivery system substantially increases its cost and makes the production substantially more complicated.

2.2.5. Drug stability

A therapeutic agent should not drop its activity during the process of nebulization and its travel to the site of action [30]. The later seems to be less important when drug is delivered directly to the lungs for treating of lung diseases. Drug destruction during the nebulization depends on the type of nebulizer and regimen of inhalation. Special constructions of nebulizers were developed in order to protect the integrity of drug delivery system and prevent drug degradation during aerosol formation [17,30]. A detail discussion of such devices is out of the limits of the present review.

2.3. Drug targeting

Generally, two types of targeting approaches are distinguished: passive and active targeting [26]. In case of cancer, the so-called Enhanced Permeability and Retention (EPR) effect is often used for tumor targeting when a drug is delivered using systemic administration [31–33]. Enhanced permeability of tumor blood vessels and poor lymphatic drainage leads to the preferential accumulation of high molecular weight substances in solid tumors. It is understandable, that the EPR effect cannot play a substantial role in passive tumor targeting when an anticancer drug is delivered by a high molecular weight carrier *via* inhalation. However, the fact of delivering drug(s) directly into the lungs provides a passive targeting to this organ. In addition, the selection of a right size of delivered aerosol particles helps to target a specific region of the lungs. It is generally believed that aerosol particles with mean diameter of 5–10 μm are preferentially deposited into oropharynx and large conducting airways [4]. In contrast, smaller particles with diameter less than 1–5 μm are deposited in the small airways and alveoli. Consequently, based on the primary location of the diseased cells inside the lungs, the optimal selection of particle size may potentially help target certain regions of the lungs. However, it is unlikely that particles with size of 1 μm and higher can be effectively taken by lung cells. It was found that particles with the size of 100–150 nm internalized 8–9 times better by cancer cells when compared with similar particles with the size of 3–5 μm [34]. Consequently, when large particles are used as drug carriers, the drug must be released after their accumulation in a close proximity to the targeted cells.

The application of so-called "active targeting" [26] methods may help in targeting delivery system and/or drug(s) specifically to the diseased or cancer cells. In particular targeting cell surface receptors, intracellular organelles and molecules may be useful for active targeting in case of inhalation delivery of pharmaceutics. Many different active targeting approaches were developed during the last two decades and tested in experiments and in clinics [26,35–41]. One of such active targeting approaches was developed and evaluated in or laboratory and is based on targeting luteinizing hormone releasing hormone (LHRH) receptors [22,38,42–46]. We found that LHRH receptors overexpressed in the plasma membrane of various cancer cells (including lung cancer cells) while their expression in healthy cells from visceral organs normally does not exceed the detection level of modern polymerase chain reaction method. Consequently, when the LHRH peptide in its native or modified form is added to the delivery system, the entire system and delivered drug is accumulated predominantly in cancer cells leaving healthy ones intact. Such targeting approach was successfully applied for the inhalation delivery of nanoparticle-based drugs and nucleic acids specifically to lung cancer cells (Fig. 4) [23]. It was found that non-targeted nanostructured lipid carriers (NLC) were relatively uniformly distributed within the lungs including healthy lung tissues. In contrast, LHRH-targeted NLC predominately accumulated in lung tumor nodules with minimal accumulation of the particles and drugs in healthy tissues.

3. Delivery systems

Many different types of dosage forms have been developed for different routes of drug delivery. Not all systems are suitable for inhalation delivery. However, several types of drug delivery systems were developed and tested for the administration of different drugs by inhalation. The majority of these types of delivery systems include colloidal dispersions, different microparticles and nanoparticles. These inhalation delivery systems are briefly described below.

3.1. Colloidal dispersions

Colloidal system or colloidal dispersion for drug delivery represents a heterogeneous system which consists of a dispersed phase (solid or liquid drug) homogenously distributed within the dispersion medium (usually water). The simplest way to produce aerosols of water insoluble drugs is a dispersion of the solid or liquid hydrophobic drug in water using probe sonication. The resulting colloidal dispersion can be used to produce aerosols by different methods and then delivered into the lungs by inhalation. An example of such system is tacrolimus dispersion designed for nebulization and inhalation delivery to the lung transplanted rodent model [47,48]. An average aerodynamic diameter of the resulted nebulized drug dispersion was 4 μm. The developed system was successfully used to deliver tacrolimus locally to the lungs and was successfully tested using a lung transplanted rodent model. The experimental results demonstrated effectiveness of such a colloidal system in the prevention of lung allograft rejection in lung transplant recipients and limitation of systemic adverse side effects.

3.2. Microparticles

The term "microparticle" in drug delivery applications generally refers to a particle with one or several micrometers in size. According to the International Union of Pure and Applied Chemistry (IUPAC), a nanoparticle represents a particle with dimensions between 1×10^{-7} and 1×10^{-4} m [50]. However, it is stressed that the lower limit of the distinguishing between micro- and nanoparticles is still debatable. In our opinion, in terms of usefulness for the delivery of drugs, the particles with dimensions lower than 0.5 μm should be referred to as nanoparticles (Fig. 4) [49]. Microparticles of many materials including ceramics, glass, polymers, and metals are currently commercially available. However, polymer and metal microparticles are being mainly used for the drug delivery purposes. Paclitaxel loaded alginate microparticles represent a typical example of microparticles designed for inhalation delivery and built using a natural polymer–alginic acid [51]. Alginate represents a biocompatible, biodegradable and mucoadhesive polymer that effectively binds to

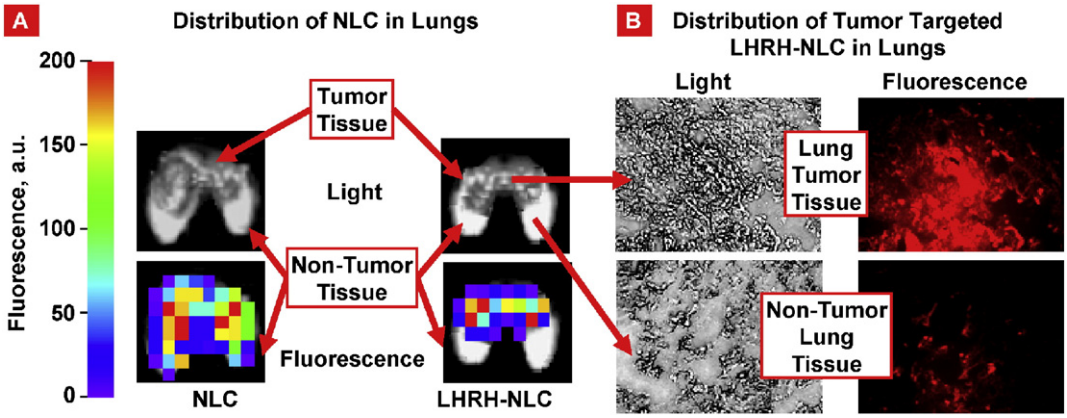

Fig. 4. Distribution of fluorescently labeled (Cy5.5) non-targeted and LHRH-tumor targeted nanostructured lipid carriers (NLC) in mouse lungs bearing human lung cancer. NLC were delivered by inhalation. (A) Representative fluorescence imaging using IVIS system of excised entire lungs. (B) Representative bright field and fluorescence microscope images of frozen lung slices. Red color represents distribution of NLC-LHRH in tumor and non-tumor lung tissues.
Modified from Ref. [23].

mucosal tissue in the lungs. These microparticles were fabricated by an emulsification technique, loaded with paclitaxel and characterized. Their size ranged from 3 to 10 μm. Microparticles with a mean volume diameter of 3 ± 0.7 μm, mass median aerodynamic diameter of 5.9 ± 0.33 μm, and drug encapsulation efficiency of 61 ± 4% were found to be the best suitable for pulmonary delivery.

3.3. Nanoparticles

In general, nanoparticle represents particles with sizes at least in one direction smaller than 500 μm (Fig. 5) [49,50]. They normally form a stable colloidal dispersion. Currently, nanoparticles are widely used for drug transport *via* various delivery routes, including inhalation. During inhalation delivery, nanoparticles can form droplets or aggregates with higher micrometer size. However, a major behavior of these particles after the deposition inside the lungs depends on their nanoscale range dimensions.

The composition, size and shape of nanoparticles significantly influences their retention in the lungs and targeting properties [19]. However, recently we showed that specific active targeting of various nanoparticles to cancer cells (and possibly to other diseased cells) diminishes the differences between various nanocarriers as drug delivery vehicles [38].

3.3.1. Polymers

Polymers of different composition represent a major part of nanotherapeutics that are used for drug delivery *via* various routes,

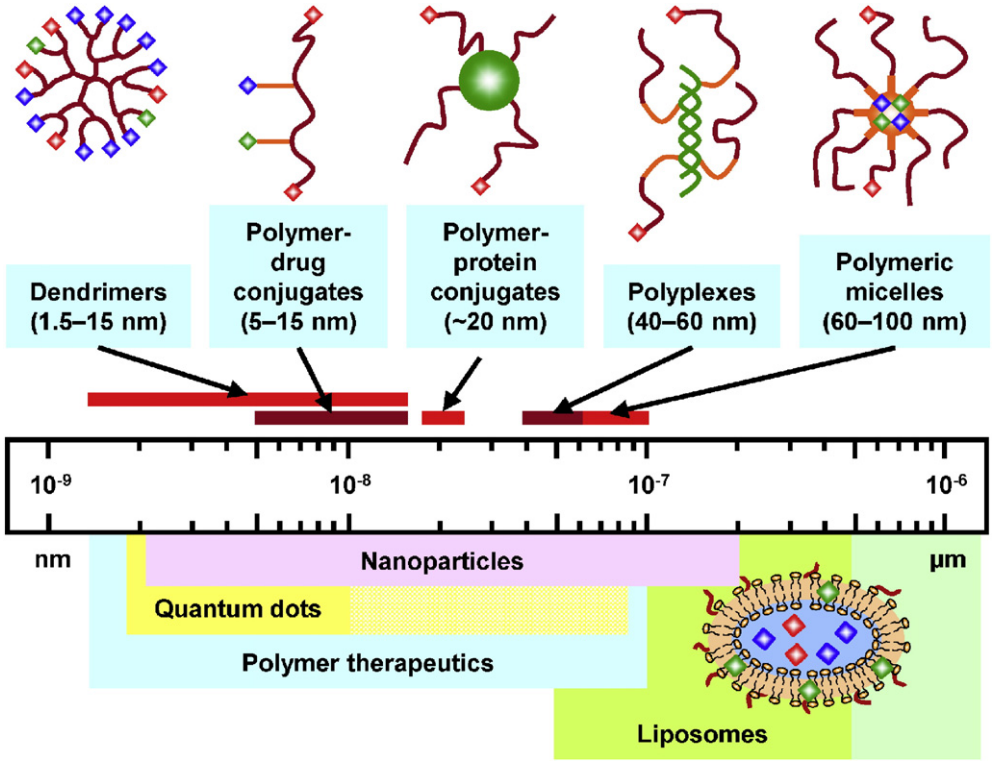

Fig. 5. Classes of nanoscale drug delivery systems (bottom panel) and examples of polymeric drug delivery systems polymer molecules are represented by dark red color curved lines.
Modified from Ref. [49].

including inhalation. In most cases, many different types of nanoparticles included polymers (Fig. 6). Consequently, it is hard to distinguish between different forms of polymer-based delivery systems. Traditionally, we consider as a "polymeric" drug delivery system compositions that have a linear polymer conjugated with active components of the system (drugs, targeting moieties, *etc.*) directly or *via* spacers of different architecture (Fig. 6A).

Many different types of polymers are used for creating delivery systems. However, only a limited number of them are suitable for local pulmonary inhalation delivery. The examples of polymeric systems for inhalation delivery of drugs include poly(lactic-co-glycolic) and poly(ethylene glycol)-co-poly(sebacic acid) aerosol dry powder formulations [52,53]. Because of the relatively small size, carriers comprised from a "pure" linear polymer can easily penetrate into the systemic circulation and open a door for adverse side effects. In addition, an administration to the organism of exogenous polymeric materials can potentially cause adverse effects. Consequently, polymers are mainly used in a complex with other molecules (*e.g.* nucleic acids or lipids) to form nanoparticle-like structures (Figs. 5, 6).

3.3.2. Dendrimers

The term dendrimer (from a Greek word Dendron — tree) usually denote a highly branched structure (Fig. 6B). The size of most dendrimers used as drug carriers varies from 4 to 20 nm. While this size provides for an efficient internalization by different targeted cells, dendrimers usually rapidly penetrate into the circulation minimizing their retention in the lungs. For instance, doxorubicin was conjugated to a 56 kDa PEGylated poly-lysine dendrimer and studied for anticancer efficacy [54]. It was found that the dendrimer was rapidly removed from the lungs (within 24 h) after intratracheal instillation. Finally, only around 15% of the instilled drug was retained in the lungs. However, even with this relatively unfavorable pharmacokinetics, intratracheally administered drug (twice per week) led to almost complete regression of the lung tumor. In contrast, intravenously delivered doxorubicin solution reduced tumor by only 30–50%. However, dendrimers are often used as a component of more complex delivery systems resulting in the formation of larger nanoparticles that potentially can be employed for the inhalation delivery [45,55–61]. For instance, surface or internally charged dendrimers were successfully employed in our laboratory for delivery of nucleic acids [55–57,60,62].

3.3.3. Lipid-based nanoparticles

Lipid-based nanoparticles are used extensively for various drug delivery applications. These nanocarriers allow for easy incorporation of lipophilic drugs in its lipid core/membrane (Fig. 6C, D). The amphiphilic nature of many lipids allows them to form various structures and incorporate hydrophilic drug molecules as well (Fig. 6C). In addition, lipid carriers can be made from biocompatible lipids similar to those that comprise cell membrane. This not only limits toxicity of lipid carriers, but allows them to easily penetrate inside different cells. Moreover, lipophilic nature of the carriers permits the crossing of the blood–brain-barrier under certain conditions allowing brain drug delivery. In terms of inhalation lung delivery, lipid particles can be easily aerosolized and usually are well taken by the lungs providing for a prolonged retention of carriers and drugs in the lungs [19].

3.3.3.1. Lipid nanoparticles. It seems natural to choose lipids similar to those contained in lung surfactants in order to form carriers for inhalation lung delivery. Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine-methoxy(polyethyleneglycol) (DPPE-PEG) are often used for these purposes. For instance, a micro/nanoparticle DPPC/DPPE-PEG system was used for dry powder inhalation delivery of the anticancer drug paclitaxel [63,64]. Different spray-dried micro/nanoparticles with a size of 0.6–3.4 μm were prepared and characterized. These delivery systems demonstrated a satisfactory drug loading capacity and potential for inhalation chemotherapy.

3.3.3.2. Liposomes. Liposomes probably are the most widely used and best characterized lipid-based drug carriers. In most cases, a typical liposome consists of a single bilayer lipid membrane (unilamellar liposomes) (Fig. 6C) or several bilayer lipid membranes (multilamellar liposomes). The outer surface of liposomes is often modified by polymers (mainly poly(ethylene glycol), PEG). Such coating performs several functions. It adds STEALTH properties to the liposomes and allows for conjugating additional components of the delivery system (*e.g.* targeting moiety) to the distal end of the polymer [65–68]. In most cases liposomes suitable for drug delivery have a size range of 50–500 nm, while larger size liposomes are also been employed (Fig. 5) [49]. It also should be stressed, that by varying a composition of liposome membrane(s), a neutral, negatively charged and cationic liposomes may be created. The later liposomes can be used to form complexes with negatively charged nucleic acids [69].

Liposomes were extensively tested as vehicles for the inhalation delivery of drugs, vitamins and nucleic acids for treatment of several disease and pathological conditions [4,5,19–21,67,68,70–75]. Many known and new techniques have been tested for nebulization of liposomes and delivering them as a dry powder [4,5,17,71]. In most cases, after carefully selecting liposome composition and methods of liposome nebulization, liposomes preserve their size, payload and do not aggregate after aerosolization. They demonstrate a preferential accumulation in the lungs (Fig. 2A), suitable lung retention for an extended period, penetration into lung cells after inhalation (Fig. 7A), and release of active payload inside the cells (Fig. 7B) [21,74]. In most cases, no significant adverse side effects were registered after the application of neutral or slightly negatively charged liposomes. However, cationic liposomes were found to be toxic to human cells and potentially can introduce genetic aberrations [58]. Moreover, adverse side effects of cationic liposomes significantly increased with an increase of positive charge of the particles. However, because cationic liposomes normally are used for the formation of almost neutral complexes with negatively charged nucleic acids, such modification of cationic carriers usually prevents adverse effects on the cells [58].

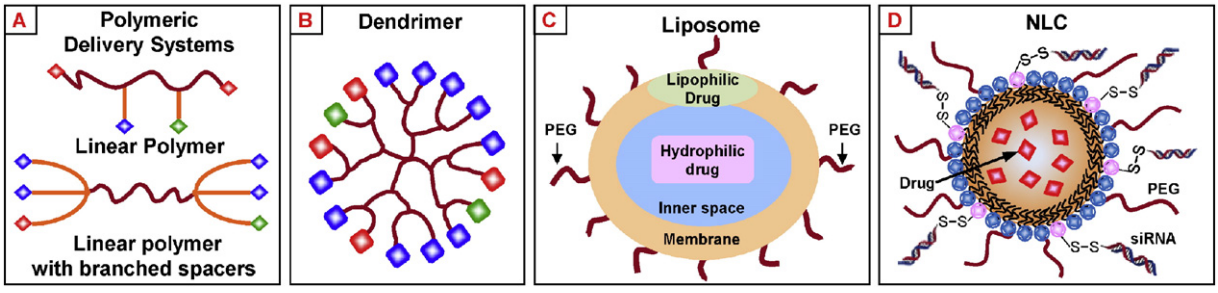

Fig. 6. Examples of different architecture of delivery systems (polymer molecules are represented by dark red color curved lines). Modified from Refs. [18,49].

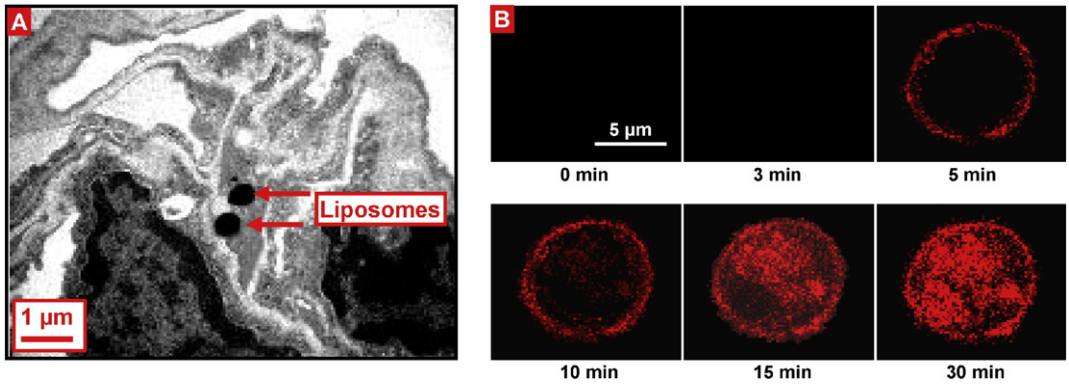

Fig. 7. Localization of liposomes (A) after the inhalation delivery and the release of doxorubicin (B) in the mouse lung cells. Liposomes were labeled by osmium tetroxide and visualized by electron transmission microscopy. Doxorubicin was visualized using fluorescence microscopy.
Modified from Refs. [21,74].

3.3.3.3. Lipid–polymer hybrid nanoparticles. In addition to modification of different types of lipid nanoparticles, polymers can be used to form lipid–polymer hybrid nanoparticles as an alternative to other lipid-based nanocarriers including liposomes [15,16]. Poly(lactic-co-glycolic acid) nanoparticles enveloped by phosphatidylcholine (PC) or PC-stearylamine layers were tested as lipid–polymer hybrid nanoparticles for inhalation delivery. The resulting nanoparticles were spherical in the shape and were adsorbed onto the carrier chitosan particles. It was suggested, that the resulting particles should have an aerodynamic diameter between 1 and 5 μm because nanoparticles smaller than 1 μm could be exhaled back while particles larger than 5 μm could be deposited in the mouth and throat regions, instead of the lungs. However, the suggestion that nanoparticles can be exhaled and not deposited in the lung tissues and cells contradict an extensive literature data showing that lipid-based nanoparticles with the size smaller than 1 μm are successfully deposited and retained in the lung tissues and also penetrate into the lung cells (Figs. 2, 7 and cited above references).

Another hybrid polymer/lipid nanoparticles, suitable for inhalation lung delivery so called "Janus" nanoparticles were developed in our laboratory (Fig. 8) [25]. These nanoparticles have two distinct phases—polymeric phase, which can be loaded with water-soluble drug(s) (*e.g.* doxorubicin), and lipid phase, that can be loaded with lipophilic drug(s) (*e.g.* curcumin). These nanoparticles preserved their shape, size distribution and drug loading during nebulization, and were highly effective in treatment of orthotopic murine model of lung cancer.

3.3.3.4. Nanostructured lipid carriers. Nanostructured lipid carriers (NLC) represent a new generation of lipid nanoparticles suitable for inhalation delivery of different drugs and siRNA (Fig. 6D) [23]. NLC are prepared from mixtures of solid (*e.g.* Precirol ATO 5) and spatially incompatible liquid lipids (*e.g.* Squalene) by melt-emulsification. Lipophilic drug can be loaded into the inner core of NLC. These particles can be made positively charged by using a cationic lipid(s) (*e.g.*N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate, DOTAP) for their fabrication. Such cationic nanoparticles can form complexes with negatively charged nucleic acid molecules. Alternatively, thiol-modified DNA or RNA molecules (*e.g.* small interfering RNS, siRNA) can be conjugated to the surface of NLC *via* biodegradable disulfide (S–S) bonds (Fig. 6D). In addition, the surface of the NLC can be modified with PEG polymer conjugated with targeting moieties (*e.g.* LHRH peptide for cancer cell targeting). Testing of NLC containing paclitaxel for cell death induction and siRNAs targeted to MRP1 and BCL2 mRNAs as suppressors of cancer cell resistance and anti-apoptotic defense for inhalation chemotherapy of lung cancer showed their exceptional therapeutic efficacy [23].

3.3.4. Nanospheres

A novel class of synthetic carriers designed mainly for gene delivery was proposed [76]. These nanospheres were synthesized using four poly-ethyleneoxide/polypropylenoxide blocks centered on an ethylenediamine moiety. The nanoparticles had a positive charge and easily formed complexes with nucleic acids/plasmids. Later, these nanospheres were successfully used for inhalation delivery of the chemokine fractalkine as a cancer chemotherapeutic agent [77]. *In vivo* testing

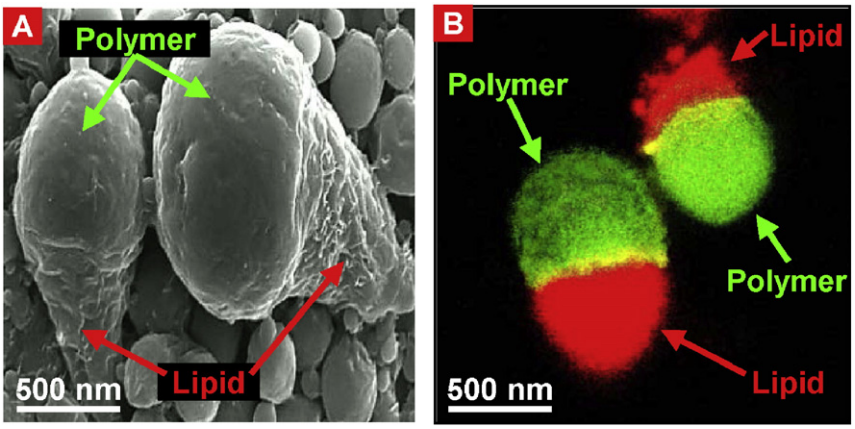

Fig. 8. Anisotropic polymer/lipid "Janus" nanoparticles Representative scanning electron microscopy (A) and fluorescence (B) images of polymer/lipid "ice cream cone" shaped nanoparticles. Polymeric phase of nanoparticles was labeled with FITC (green fluorescence); lipid phase was labeled with DiR (red fluorescence).
Modified from Ref. [25].

using experimental lung metastasis model of mouse colon carcinoma and osteosarcoma supported the use of these nanospheres as promising immunotherapeutic approach.

3.3.5. Complexes with nucleic acids

Low stability of nucleic acid in the plasma and other body fluids and tissues as well as its poor penetration inside the cells *via* the plasma membrane substantially limits the application of free nucleic acids. This is especially important for inhalation delivery of nuclei acids where the destructive influence of nebulization on the fragile nucleic acid molecules aggravates its damage and destruction. Two generic approaches are usually used for delivery of nucleic acids using non-viral vectors. The first approach includes a complex formation between negatively charged nucleic acid molecules and positively charged carrier materials. As the result of the complex formation, positively charged nanoparticles are formed. The second approach for the delivery of nucleic acids comprises a direct conjugation of a modified nucleic acid molecule to the carrier *via* a biodegradable chemical bond.

Polyethylenimine (PEI) and its derivatives as well as DOTAP are most widely used carriers for gene delivery. However, PEI and DOTAP are cytotoxic [12,58]. Consequently, other alternatives (*e.g.* PAE-poly(amino ester) based on glycerol propoxylate triacrylate and spermine) gene carriers have been proposed as PEI and DOTAP alternatives [12]. In addition, practically all types of cationic carriers are suitable for complexation with nuclei acids and their inhalation delivery [10,12,13,22,23,55–57,78–82]. In particular, glucosylated polyethylenimine, chitosan, spermine-alt-poly(ethylene glycol) polyspermin, polypropylene Imine (PPI), silica and other substances have been successfully used to form nanoparticle carriers for inhalation delivery of nucleic acids.

In most cases, cationic carriers used to form complexes with nucleic acid molecules have positive charges on their surface. In contrast, we proposed nanoparticles for DNA complexation with inner positive charges and modified surface for the effective protected delivery of nucleic acids in order to cover them (at least in part) with dendrimers [55]. In addition, a special caging of nanoparticle-DNA/RNA complexes has been proposed in order to protect them from harsh actions of the environment [59]. Mesoporous silica nanoparticles have also been tested for simultaneous inhalation delivery of anticancer drug(s) and siRNA [22]. The advantages of these nanoparticles include development of internal sealed pores that can protect a drug from degradation and conjugation of modified nucleic acid on their surface.

3.3.6. Magnetic nanoparticles

Magnetic nanoparticles can be used for imaging and drug delivery. When such particles accumulate in the targeted site they can be used as contrast agents for magnetic resonance and other contrast imaging. These nanoparticles can be targeted to the site of action by an external magnetic field. In addition, the application of high intensity external magnetic fields raises the temperature of such nanoparticles comprised mainly of metals. High temperature can be used for killing targeted cells (*e.g.* foci of bacterial infection or tumor nodules). The application of magnetic nanoparticles for inhalation local lung delivery has also been proposed and evaluated [83–85]. A simple superparamagnetic iron-oxide nanoparticles (SPIONs), or more complex surface coated of Fe_3O_4 magnetic nanoparticles with polymer poly(lactic-co-glycolic acid) and lipid-coated SPIONs have been used for these purposes.

4. Therapeutic applications of inhalation delivery

As was described above, the delivery of therapeutics to the lungs by inhalation demonstrates a high potential for treatment of lung diseases. In this case, local pulmonary delivery of drugs and/or other active components directly to the site of disease enhances therapeutic efficacy of the drugs, allows for a decrease of drug concentration, and limits drug penetration into the circulation preventing severe adverse side effects upon healthy organs and tissues. Below we discuss major therapeutic applications of inhalation delivery.

4.1. Asthma and chronic obstructive pulmonary disease (COPD)

Treatment and management of asthma and COPD is the most known application of inhalation delivery of therapeutic agents. Various types of β agonists, anticholinergics, corticosteroids anti-inflammatory drugs are effectively delivered by inhalation [8,86–91]. In the present review, we will focus on the application of inhalation delivery for diseases other than asthma and COPD.

4.2. Lung hypoxia and edema

Tissue hypoxia accompanies many lung diseases (lung edema, pneumonia, fibrosis, *etc.*), aggravates the primary disorder, and causes additional cell damage [69,70,92]. Consequently, remediation of cellular hypoxia may help in treatment of primary disease. Despite relatively large number of anti-hypoxic preparations, only limited attempts were made to deliver them by inhalation. In our lab, we developed a liposomal form of α tocopherol that can be delivered to the lungs by intratracheal injection or by inhalation [70]. It was found that the major mechanisms of anti-hypoxic action of liposomal α tocopherol included the following (Fig. 9) [92]. It should be stressed that liposomes used in this study were comprised with phosphatidylcholine — a major component of lung surfactant system. Consequently, delivery of this phospholipid normalized at least in part its deficiency caused by severe hypoxia. This in turn improved lung biomechanics, breathing pattern and increased oxygen consumption. Inhibition of lipid peroxidation by the supplied vitamin E decreased hypoxic damage of air–blood barrier and also limited cellular damage caused by oxygen reactive species. Taken together all these factors limited hypoxic cellular damage, lactate-acidosis, and prevented cell death from apoptosis and necrosis. Consequently, the resistance of an entire organism against acute severe hypoxia significantly increased. As a result, the mortality of animals with hypoxia was substantially decreased after the treatment with liposomal α tocopherol.

4.3. Lung injury

Potentially, inhalation delivery of drugs can help to minimize lung injury caused by the damaging environmental impacts. For instance, it was shown that inhalation delivery of kinase-deficient Akt1 gene that encodes one of serine/threonine-protein kinases attenuates injury of secretory Clara cells induced by naphthalene [13]. These types of cells function in innate defense and epithelial repair. Consequently, limiting of their damage potentially can be useful for minimization of adverse effects caused by some type of treatments (*e.g.* chemotherapy) or damaging impacts of various environmental factors.

4.4. Lung transplantation

The rejection of lung allograft after the transplantation represents a major problem. Immunosuppressive therapy demonstrated a potential on increasing the success rate of transplantation of several organs and bone marrow. In most cases, immunosuppressive agents are delivered systemically, mostly by parenteral injections. However, in case of lung transplantation, it is logical to deliver such agents directly to the lungs in order to suppress lung transplant rejection and limit adverse side effects on the overall body immunity. Several delivery systems were proposed and tested for inhalation delivery of tacrolimus [47,48,93]. In these studies, tacrolimus powder reconstituted in deionized water and amorphous or crystalline nanoparticles of the drug produced by the ultra-rapid freezing method were used. The results showed a high potential of inhalation form of tacrolimus in limiting the rejection rate of lung transplants.

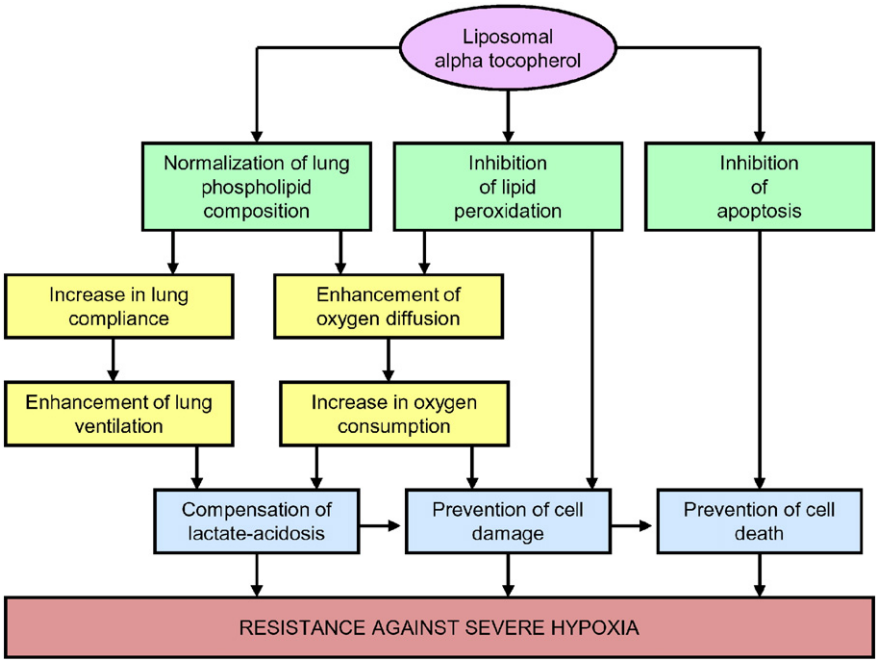

Fig. 9. Correction of hypoxic lung damage by liposomal α tocopherol.
Modified from Ref. [92].

4.5. Fungal infections

The risk of lung fungal infections increased in patients undergoing chemotherapy, organ and cell transplantation or treated in intensive care units. However, pulmonary infections often poorly respond to the systemic treatments due to the low accumulation of antifungal drugs in the lungs [94]. Several attempts were made in order to deliver antifungal drugs *via* inhalation. A 2-hydroxypropyl-β-cyclodextrin (HPβCD) solubilized itraconazole (ITZ) solution (*i.e.*, HPβCD-ITZ) and colloidal dispersion of ITZ were created and delivered as aerosol to mouse lungs [95]. The analysis of pharmacokinetics and distribution pattern showed that both formulations are suitable for local inhalation delivery to the lungs. In another independent study, aerosolized commercially available voriconazole solution was tested for the prevention of invasive pulmonary aspergillosis [96]. The results showed clear advantages of voriconazole delivered *via* inhalation over amphotericin B deoxycholate delivered intraperitoneally (Fig. 10). In fact, mice treated with the inhaled drug had fewer signs of invasive disease.

4.6. Pulmonary fibrosis and inflammation

Idiopathic pulmonary fibrosis (IPF) often accompanied by inflammation is a devastative lung disease that is often associated with mortality. Treatment of this disease is difficult because an effective therapy is not available yet. We proposed to use liposomal form of prostaglandin E2 for treatment of IPF *via* inhalation [21]. The formulation was tested on experimental mouse model of IPF and inflammation caused by intratracheal administration of bleomycin. It was found that treatment of animals with such a liposomal formulation decreased the signs of IPF, blocked overexpression of many proteins involved in the development of IPF, inflammation and fibrotic lung injury and finally prevented animal mortality.

4.7. Lung cancer and metastases

Lung tumor represents a one of the most deadly and poorly treated cancer diseases. In addition, many other types of cancers often result in the development of lung metastases in the advanced stage of the disease. Most existing systemic therapies (administered by intravenous or oral routes) are not very effective for the treatment of primary lung cancer and metastases and/or induce severe adverse side effects. Inhalation (local drug delivery) would be an important part of combination therapy together with systemic or local treatment of lung cancer, especially of its metastases to other organs [97]. Consequently, local pulmonary inhalation delivery of anticancer agents potentially can improve the outcome of lung cancer therapy.

4.7.1. Rationale and limitations

The rationales for inhalation cancer treatment are essentially similar to pulmonary therapeutics for treatment of other pulmonary diseases [8,98,99]. The delivery of highly toxic anticancer drug(s) locally to the lungs allows for decreasing the total drug concentration and preventing its penetration into the bloodstream and therefore limiting adverse side effects of chemotherapy [100]. Although the first attempts of inhalation chemotherapy was reported almost 50 years ago [99] and despite clear advantages of this delivery route for the treatment of primary lung cancer and metastases, inhalation chemotherapy still is not widely used for treatment. Toxicity of the inhaled drug to normal lung cells and the entire respiratory system after inhalation, penetration of inhaled anticancer drug into the bloodstream and associated high systemic toxicity as well as already mentioned safety concerns for the occupational exposure of healthcare workers that provide inhalation therapy represent major concerns and potential limitations for the inhalation of chemotherapy. While occupational exposure can be prevented by using modern filters, the problem of limiting the penetration of inhaled drug into the systemic circulation and preventing lung toxicity does not have an effective solution. The development of tumor-targeted delivery system that mostly retains in the lungs and does not enter a systemic circulation is an important task for inhalation chemotherapy of lung cancer. Below we briefly describe major active components along with delivery vehicles that were already tested for inhalation treatment of lung cancer.

4.7.2. Active components

Many different types of active components have been recently tested for inhalation chemotherapy of lung cancer. They ranged from

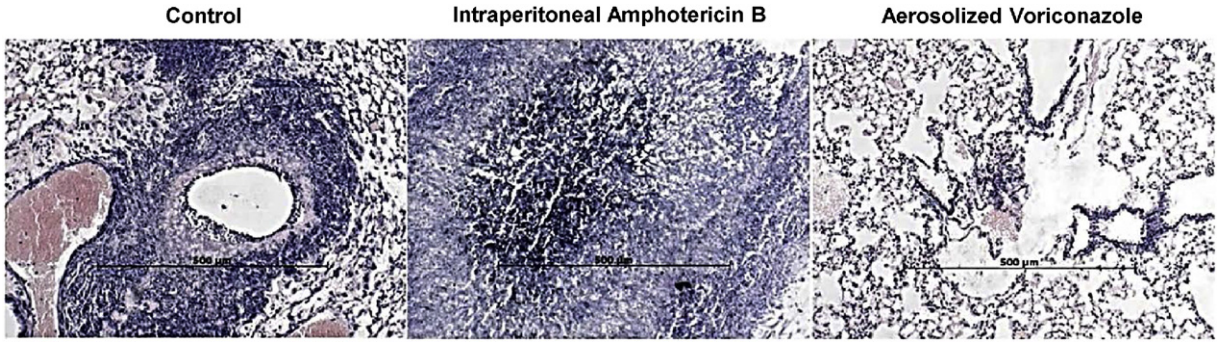

Fig. 10. Representative histopathology images of immunosuppressed mice eight days after inoculation of lungs with *Aspergillus fumigatus*. Mice were treated daily with substances indicated. Control mice received aerosolized sulfobutyl ether-β-cyclodextrin sodium. Lung sections were stained with hematoxylin and eosin and viewed by light microscopy at ×20 magnification.
Modified from Ref. [96].

conventional anticancer drugs in forms that are ready for aerosolization to various types of antibodies and nucleic acids, drug that activates cellular immune response, delivery systems for hyperthermia and radiotherapy, adjuvant inhalation chemotherapy and combinational therapy.

4.7.2.1. Antibodies. Antibodies represent an attractive alternative to traditional chemotherapy with anticancer drugs. By their nature, they are targeted to the specific site of the action and potentially can provide a more effective treatment and fewer side effects. Several monoclonal antibodies including cetuximab and bevacizumab targeted to the epidermal growth factor receptor and vascular endothelial growth factor receptor, respectively, have been approved for treatment of lung cancer. However, systemic delivery of these antibodies represents challenges because of relatively low accumulation of antibodies in the lungs, high possibility of their inactivation in the plasma, liver, spleen and other organs. Consequently, attempts have been made to deliver antibodies *via* inhalation in order to treat lung cancer.

An innovative Respite™ system that employs surface acoustic waves (SAW) was developed and tested for inhalation delivery of monoclonal antibodies against the epidermal growth factor receptor[2]. It was found that a portable SAW nebulizer was able to generate an aerosol and did not cause antibody fragmentation or their specific activity. Aerosolized cetuximab was also tested in nude mouse model of lung cancer sensitive to this drug [101]. The lung cancer was initiated by intratracheal instillation of A431 cancer cells with EDTA. It was found that almost 80% of inhaled antibodies were retained in the lungs, where the rest of them were almost equally divided between the mouth and stomach (Fig. 11). In contrast, free drug was found primarily in the liver and no free drug was detected in the lungs. It was also found that the inhaled cetuximab substantially decreased the size of lung tumor.

4.7.2.2. Nucleic acids. Nucleic acids are currently widely used for the treatment of various diseases, including lung cancer. Viral and non-viral vectors are being used to suppress oncogenes and/or genes responsible for the progression of tumor growth or to overcome cancer cells resistance to chemotherapy. The suppression of oncogenes usually is not very efficient in terms of the suppression of tumor growth. Alternatively, the suppression of genes responsible for the tumor growth and proliferation already generated positive results. In addition, the suppression of genes/proteins responsible for multidrug resistance of cancer cells can be helpful in enhancing the efficacy of chemotherapy and therefore should be used together with chemotherapy.

Inhalation gene therapy was studied mainly for the delivery of tumor suppressor genes, anti-vascular endothelial growth factor (VEGF), epidermal growth factor suppressor (EGF), K-Ras, and immuno-therapy [100]. The most commonly used approach for the inhalation delivery of nucleic acids is the formation of complexes with cationic carriers. Polyethyleneimine (PEI) and its derivatives as linear or branched polymers are frequently used for these purposes. The advantages of PEI include efficient attachments to the airway epithelial cells and introduction of nucleic acids in the cells and their nuclei, protection of nucleic acid molecules from degradation and sheer forces during nebulization [100]. Sometimes PEI-DNA complexes are modified with PEG. Liposomes and other polymers also are frequently used for DNA complexation to form stable nanoparticles [22–24,56,57,60,62,73,81,102–105].

Concerning the inhalation delivery of nucleic acids, it should be stressed that the following delivery systems were used: poly(amino ester) (PAE) based on glycerol propoxylatetriacrylate (GPT) and spermine (SPE) copolymer for the delivery of an entire gene in plasmid or short hairpin RNA (shRNA) [12,13]; complexes of PEI with BC-819 — a novel plasmid DNA which encodes for the A-fragment of Diphtheria toxin [10] and siRNA targeted to the Wilms' tumor gene [106]; tetrafunctional block copolymers nanospheres containing chemokine fractalkine [77]; different nanoparticles containing a combination of various anticancer drugs and suppressors of drug efflux transporters (ABCA, MRP-1, BCL-2) overexpressed in lung cancer cells in order to simultaneously induce cell death and suppress multidrug resistance [11,18,20,22–24].

4.7.2.3. Anticancer drugs. Several anticancer drugs have been successfully delivered into the lungs by inhalation. Most of them include paclitaxel, cisplatin, doxorubicin, gemcitabine, camptothecin, azacytidine and fluorouracil (Fig. 12).

4.7.2.3.1. Paclitaxel. Paclitaxel represents a natural plant-borne anticancer drug first isolated from the bark of the Pacific yew, *Taxus brevifolia* [107]. The generic name of this drug is “paclitaxel” with trademarks defined as Taxol and Abraxane. Paclitaxel targets tubulin interfering with mitotic spindle function, chromosome segregation, and cell division [108,109]. Several nontechnology-based carriers were used for the inhalation delivery of paclitaxel. Liposome-encapsulated formulations of paclitaxel and 9-nitrocamptothecin (9-NC) were proposed and tested in patients with primary and metastatic lung cancer [4]. Inhalable lung surfactant-based carriers composed of synthetic phospholipids similar to lung surfactants or other phospholipids were also used for the dry powder delivery of paclitaxel [63,64,110]. The attempts to use biodegradable microparticles were also undertaken [51,111]. The results showed a clear perspective of inhalation nanoparticle-based delivery of paclitaxel. However, signs of drug toxicity to the upper and lower respiratory was also registered.

4.7.2.3.2. Cisplatin. Cisplatin, cisplatinum, platamin, neoplatin, cismaplat or cis-diamminedichloroplatinum(II) (CDDP) is a member of platinum-containing anti-cancer drugs which also includes carboplatin and oxaliplatin. These anticancer drugs cause crosslinking of DNA, which ultimately triggers cell death by apoptosis. Feasibility and effectiveness of inhaled cisplatin analog carboplatin was evaluated in patients with non-small cell lung cancer cells [99]. This study revealed a high

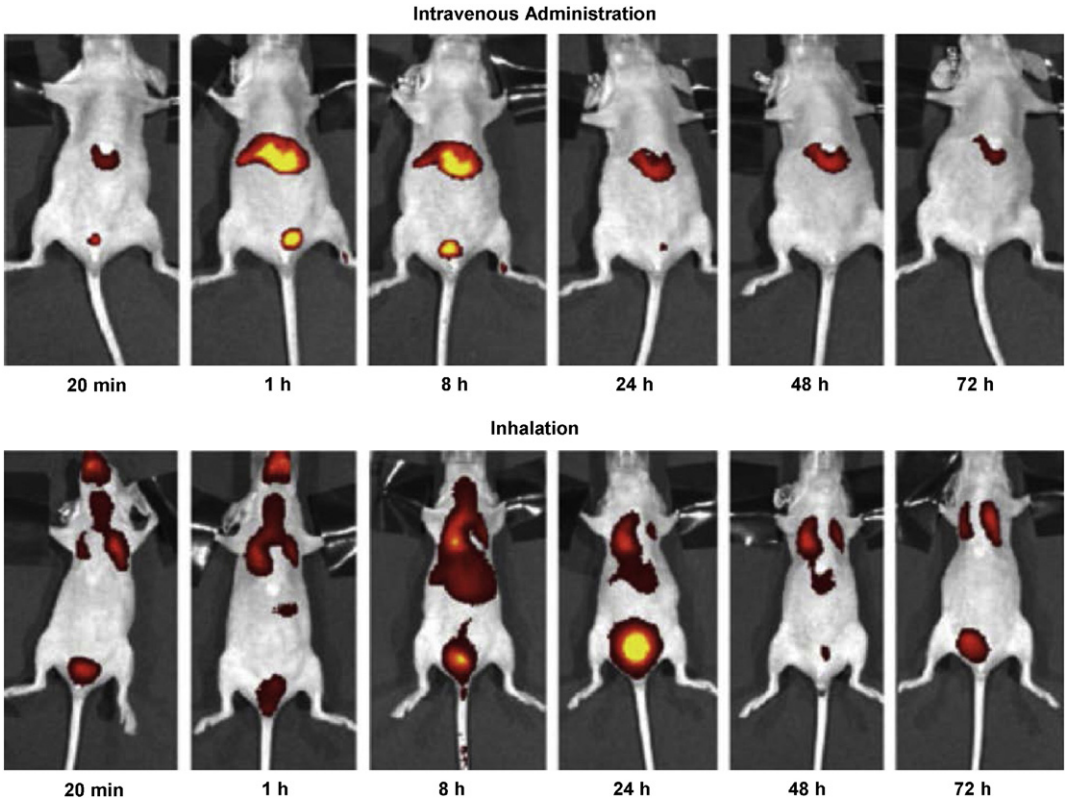

Fig. 11. Distribution of cetuximab administered *via* different routes in mice bearing A431 lung tumors. Representative near infrared images of Xenofluor750™-cetuximab. Modified from Ref. [101].

potential of inhalation delivery of carboplatin and also pointed out some adverse effects of inhalation delivery of non-targeted anticancer drugs.

4.7.2.3.3. Doxorubicin. Doxorubicin (trade name Adriamycin), also known as hydroxydaunorubicin and hydroxydaunomycin, is an anthracycline antitumor antibiotic. Its main action includes intercalating DNA and inhibition of macromolecular biosynthesis [112]. Doxorubicin is widely used for treatment of different cancers. The liposomal form of this drug (Doxil) has also been proposed that potentially is ready and was used for inhalation delivery to the lungs [113,114]. Other carriers were also used of aerosol delivery of doxorubicin [4] including dendrimers, n-butylcyanoacrylate, and dextran nanoparticles [54,115]. In addition, complex delivery systems used for the delivery of doxorubicin in combination with other active chemotherapeutic agents were employed for inhalation therapy of lung cancer [19,20,24]. It was shown that inhalation delivery of doxorubicin enhances drug exposure to primary lung tumors and metastases and improves cancer therapy.

4.7.2.3.4. Camptothecin. Camptothecin represents one of cytotoxic quinoline alkaloids which inhibits topoisomerase I and therefore interferes in the process of DNA replication preventing tumor cell proliferation [116]. It was first isolated from the bark and stem of *Camptotheca acuminate*. Several Camptothecin analogs and modifications were synthesized including topotecan (hycamtin), irinotecan (CPT-11, camptosar), DB 67 (AR67), BNP 1350, exatecan, lurtotecan, ST 1481, and CKD 602. In addition, camptothecin was linked to the cyclodextrin-based polymer to for a liposomal anti-cancer drug CRLX101 [117]. Liposome-encapsulated formulations of 9-nitrocamptothecin as well as the carboxylate form of hydroxycamptothecin were also tested [4,118].

4.7.2.3.5. Gemcitabine. Gemcitabine is a nucleoside analog of cytidine in which the hydrogen atom on the 2′ carbon of deoxycytidine is replaced by a fluorine atom. It is marketed as Gemzar by Eli Lilly and Co. As other analogs of DNA, it replaces a corresponding analog of an amino acid (in case of gemcitabine–cytidine) and arrests cell division causing apoptosis and therefore arrests tumor growth [119]. Several approaches were used for inhalation delivery of gemcitabine [4,120].

4.7.2.3.6. Azacytidine. Azacytidine (trade name Vidaza) as well as its deoxyderivative decitabine represents another nucleoside analog of cytidine with similar to gemcitabine's mechanism of action [121]. It was found that inhalation delivery of azacytidine substantially improved pharmacokinetics of the drug and enhanced apoptosis induction in lung tumors [14].

4.7.2.3.7. Fluorouracil (5-FU). Fluorouracil or 5-FU (trademarked as Adrucil, Carac, Efudex and Efudix) is a pyrimidine analog that acts primarily as a thymidylate synthase inhibitor to block synthesis of the pyrimidine thymidine and therefore interferes with DNA replication and inhibits cancer growth [122]. It was found that inhalation delivery of 5-FU by nebulized aerosols (a mixture with the drug Bisolvon vaporized by a supersonic nebulizer) enhanced its accumulation in trachea, bronchi and regional lymph nodes [123]. In addition, the supercritical antisolvent process was utilized for the production of 5-fluorouracil (5-FU) nanoparticles [124].

4.7.2.4. Induction of natural killer cell proliferation. Activation of natural human immune defense system in order to kill cancer cells represents a promising alternative to chemotherapy. It was found that aerosol interleukin-2 induces natural killer cell proliferation in the lung and improved the survival of mice with osteosarcoma lung metastasis [9]. This method of induction of natural killer cells demonstrates advantages over the transfusion of natural killer lymphocytes where infused cells only temporary reside in the lungs. Two hours after infusion they predominately accumulated in the liver, spleen, and bone marrow [125].

4.7.2.5. Hyperthermia. An induction of hyperthermia specifically in tumor cells denotes another alternative to chemotherapy for the treatment of cancer. A main idea of such therapy includes the delivery to the tumor vicinity substances (in most cases metal nanoparticles)

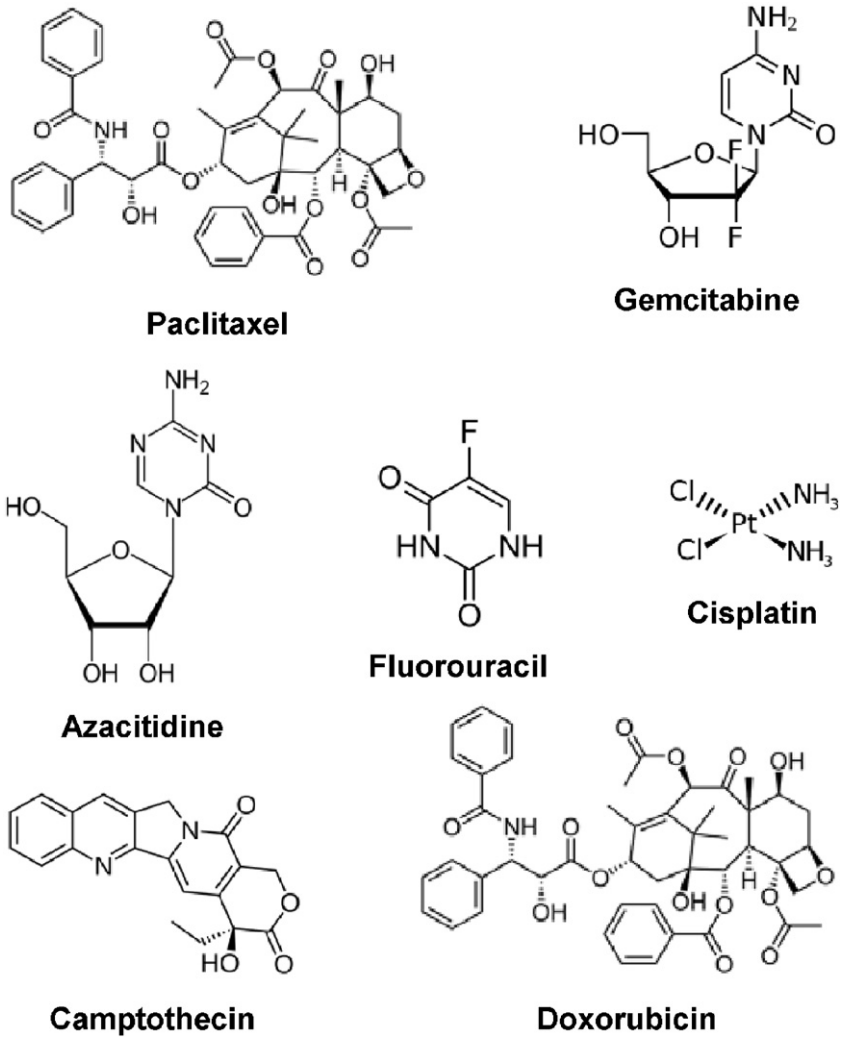

Fig. 12. Chemical structures of the most frequently used anticancer drugs for inhalation chemotherapy.

capable of increasing their temperature in order to kill cancer cells. Magnetic nanoparticles and magnetized thermo-responsive lipid vehicles were used to: (1) target delivery to the tumor cells by an external magnetic field and (2) treat tumors by increasing temperature of internalized nanoparticles under the action of high magnitude of this magnetic field [59,61,84,126]. However, the fear of decomposition of the treated tumors under the action of hyperthermia, invasion of cancer cells into the bloodstream and development of metastases limit the enthusiasm to this approach.

4.7.2.6. Combination therapy. A combination of several methods of killing cancer cells has advantages over a "simple" cell death induction by one anticancer agent. Several complex approaches were developed for treatment of different cancers [26,49]. Some of such approaches were implemented for inhalation treatment of lung cancer. For instance, a combination of intravenously injected human natural killer cells and interleukin-2 delivered as aerosol displayed a synergic effect and substantially enhanced a survival of mice with osteosarcoma lung metastases [9]. A combination of gene therapy expressing ABC10 protein with aerosol delivery of cisplatin was also investigated [11]. Another complex multifunctional approach was developed in our laboratory for treatment of different cancers including their drug resistant variants [43,44,72,73,105,127]. The approach was based on the simultaneous targeted delivery of anticancer drug(s) for apoptosis induction and suppressor(s) of cancer cell resistance to chemotherapy. The application of such approach to inhalation treatment of lung cancer showed its high efficacy accompanied with low adverse side effects upon healthy organs, tissues and cells [19,20,22–25].

4.7.3. Lung tumor imaging and radiosensitization

Nanotechnology approaches have been successfully used to improve tumor imaging and radiosensitization. When imaging is combined with cancer therapy, the agent that allows for this combination is usually called a theranostic (or theragnostic) agent that combines the abilities to detect/image cancers with therapeutic effects [128]. Most of such methods are based on the delivery of contrast agents or carriers labeled with easily detected dyes specifically to lung tumors, visualization of this agent by various types of tumor imaging, and, if the delivered theranostic system contains anticancer drug(s), killing the tumor cells, and real-time monitoring of the therapeutic outcome. Gadolinium-based nanoparticles were developed as a theranostic agent for the detection and radiosensitization of lung tumors after their inhalation delivery [129]. It was shown that the use of these nanoparticles localized in tumor nodules of experimental animals and dramatically increased the survival of animals after radiation treatment. Another approach for a targeted delivery of a contrast agent directly to lung tumor cells was developed in our laboratory [126]. The method is based on the use of PEGylated water soluble Mn_3O_4 nanoparticles and nanostructured lipid carriers targeted to the lung tumor cells by the LHRH peptide. *In vivo* experiments carried out on mice bearing an orthotopic model of

lung cancer showed that the proposed approach significantly enhanced the imaging of lung tumors and open possibilities for a simultaneous targeted treatment of the disease (Fig. 13).

5. Clinical trials and patents of inhaled therapeutics

5.1. Clinical trials

5.1.1. Inhaled granulocyte macrophage colony-stimulating factor (GM-CSF)

GM-CSF is a growth factor capable of stimulating the differentiation of hematopoietic cells to increase the production of neutrophils, macrophages, and dendritic cells. It can also activate established granulocytes and macrophages [42]. Through its immune activating effects, it is believed GM-CSF could be used to effectively combat tumor growth. A number of early phase clinical trials have reported the use of inhaled GM-CSF in the treatment of lung metastases. The majority of the patients in the early trials had primary diagnoses of melanoma, renal cell carcinoma, or osteosarcoma [130–133].

P. Anderson et al. reported one of the first dose escalation studies using recombinant GM-CSF or sargramostim. They used a PARI LC PLUS nebulizer set (PARI Respiratory Equipment, Inc.) and a standard air compressor with air flow 3.5–8 L/min. The first administration was performed at the clinic with subsequent doses administered at home along with at home pulmonary function test (PFT) monitoring. The patients were dosed at 60 μg twice daily for 7 days at the level 1; 120 μg twice daily for 7 days at the level 2; 240 μg twice daily for 7 days at the level three with one week of rest between every dose escalation. A small number of patients continued the level three dosing with 7 days of rest between cycles for an additional 2–6 months. Minimal pulmonary toxicities were registered in patients who completed dose escalation. Four of the patients showed stable disease for greater than 6 months with one patient having a complete response [130].

R. Rao et al. reported the results of inhaled sargramostim in 45 patients with metastatic lung disease [133]. The patients had the following primary diagnoses: melanoma (14 patients), renal cell carcinoma (12 patients), various types of sarcoma (13 patients), and other primary malignancies (6 patients). The majority of the patients had previously received treatment for their diseases with twenty one patients having received four or more therapies. The average duration of treatment was 4.6 months. A disease progression was the most common reason for discontinuation of therapy. Of all the patients evaluated, three showed partial regression of lung tumors while twenty one patients showed stable disease. The patients who responded showed benefits of therapy for an average of 10 months. Overall, the patients tolerated the treatment very well with twenty eight patients (62%) reporting no toxicities. Fourteen patients (31%) in the study complained of mild pulmonary symptoms such as dyspnea, cough, and wheezing, however, some of these effects could be contributed to the presence of lung metastases. To study the mechanism of action of GM-CSF, one patient who had previously received no therapy and was diagnosed with

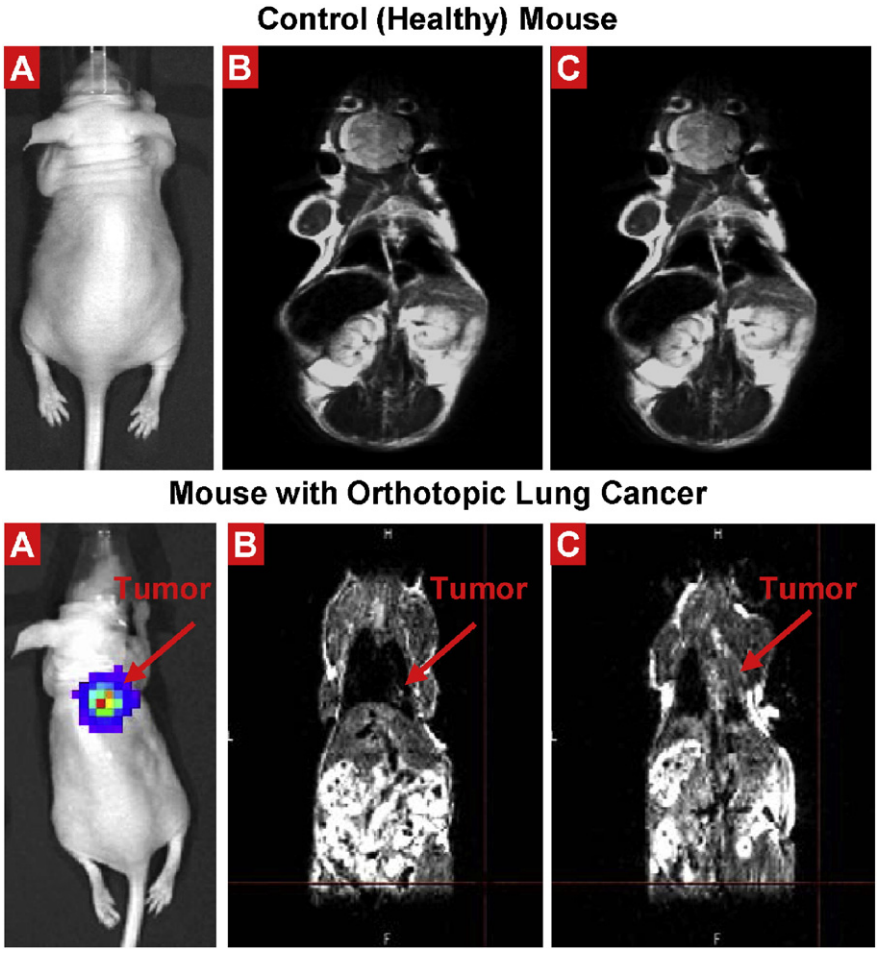

Fig. 13. Enhancement in MRI sensitivity and specificity by cancer-targeted Mn_3O_4 nanoparticles. (A) Representative light or bioluminescence IVIS optical imaging. Lung tumor was created in nude mice by the intratracheal instillation of A549 human lung cancer cells transfected with luciferase. (B–C) Representative magnetic resonance imaging. (B) MRI without a contrast agent. (C) MRI after injection of biocompatible cancer-targeted Mn_3O_4 nanoparticles.
Modified from Ref. [126].

malignant melanoma was selected for evaluation of melanoma specific cytotoxic T lymphocytes in peripheral blood. After two months of treatment, this patient showed an increase in melanoma specific cytotoxic T-lymphocytes (CTL) [133]. Encouraged by the safety data and the increase in cytotoxic T-lymphocytes in the patient that was evaluated, investigators began a second dose escalation study to determine the optimal dosing regimen required to induce a robust immunologic response in a majority of patients with acceptable side effects.

Markovic et al. report the results of forty patients with metastatic melanoma treated with escalating doses of GM-CSF ranging from 500 to 2000 μg twice daily with doses increasing at 250 μg intervals [132]. The patients were treated in 28 day cycles with dosing on days 1–7 and days 15–21. Patients' blood samples were evaluated for cytotoxic T-lymphocytes (CTL) before treatment, after two cycles of treatment, and every other cycle thereafter if therapy was continued. If a patient had detectable anti-melanoma CTL before treatment, a fivefold increase in CTL after the first two cycles of treatment was considered a positive immune response. If the CTL were not detectable, confirmation of CTL was considered a positive immune response. At a given dose level if 3/5 patients showed an immune response, another 5 patients would be enrolled at that dose. If 7/10 patients had an immune response, that would be defined as an immunologically effective dose. Among those treated with the 1250 μg dose or lower, two patients exhibited a positive immune response where they developed detectable anti-melanoma CTL. In those treated with doses 1500 μg or higher, three patients had a positive immune response by showing detectable CTL to specific melanoma antigens. In the two highest dose groups, 6/11 patients developed a 2–4 fold increase in previously detectable CTL. These results did not establish an immunogenic effective dose, but the investigators also did not reach a maximum tolerated dose. Of the patients treated, two patients (1750 μg, 2000 μg dose) experienced grade 3 or higher toxicities requiring discontinuation of therapy. One patient in the 1000 μg dose level experienced grade 3 fatigue and grade 2 dyspnea but could not continue at a lower dose due to disease progression. A number of lower grade toxicities such as cough, anemia, fatigue, dizziness, and nausea were also reported. Pulmonary function tests were monitored for all patients and there were no significant changes requiring discontinuation of therapy [132]. These results imply that the use of inhaled GM-CSF may have some utility in developing anti-melanoma CTL in patients, however, further evaluation of the appropriate dose and the treatment schedule is required.

5.1.2. Inhaled recombinant human interleukin-2 (rhIL-2)

rhIL-2 is a chemokine approved for the treatment of metastatic renal cell carcinoma and metastatic melanoma. It is believed that this interleukin can enhance lymphocyte cytotoxicity, increase the effects of lymphocyte activated and natural killer cells, and increase interferon gamma production [33]. Inhaled rhIL-2 used for the treatment of renal cell carcinoma patients with lung metastases has been studied by a number of investigators [134–137]. Huland et al. report the comparison of inhaled IL-2 combined with low dose sub-cutaneous (SC) IL-2 *versus* historical controls receiving standard dose IL-2 therapy. The inhalation arm treatment consisted of 3.3 MIU rhIL-2 SC once daily with 6.5 MIU rhIL-2 administered *via* nebulizer. Standard therapy included cycles of intravenous therapy at 18 MIU/m^2/day or cycles of SC therapy administered at 3.6–18 MIU per day. The results showed an overall response rate of 45% in the inhalation arm vs 33% in the systemic treatment arm. The inhalation arm also showed better survival rates at the 1, 3, and 5 year marks. The side effects profile for the inhalation group appeared more favorable compared to systemic therapy. The most common side effect noted in the inhaled rhIL-2 was cough which was most commonly reported after the last inhalation of the day. Patients in both groups also reported constipation, diarrhea, nausea, and fatigue [135]. Overall, inhalation treatment was shown to provide better stabilization of disease with a more tolerable side effect profile as compared to systemic IL-2.

Merimsky et al. reported their clinical experience with a similar dosing regimen of 0.9–1.8 MIU SC daily combined with 6 MIU inhaled three times a day (18 MIU daily). The SC injections were discontinued after the first 10 patients refused systemic therapy due to side effects. One of forty patients (2.5%) had a partial response while twenty two patients (55%) showed stable disease with the median time until progression of 8.7 months. The side effect profile was similar to that previously described with cough and weakness reported as the most common side effects with dyspnea, fever, sleepiness, asthenia, decreased appetite, and abdominal pain reported by less than three patients each [136]. Although the investigators did not see a large number of patients with a reduction in tumor size, stabilization in a large portion of patients with limited side effects could be seen as a success when compared to the natural course of disease.

The studies of inhaled rhIL-2 in metastatic renal cell carcinoma have shown the feasibility of the treatment with limited toxicity as compared to high dose systemic therapy. A limited benefit with inhalation therapy was seen although the few trials have shown varying levels of response in the patients studied. The administration of the treatment is convenient as the therapy can be administered at home by the patient as compared with traditional chemotherapy. However, the administration of the aerosols three to five times per day may be problematic for some patients.

Recently, Posch et al. reported the results of rhIL-2 inhalation in combination with dacarbazine treatment in stage IV malignant melanoma patients as well as the use of prophylactic rhIL-2 dosing to prevent recurrence post resection of lung metastases. The study enrolled 15 patients into the treatment arm and 5 into the prophylaxis arm. Although the number of patients was too small for statistical analysis, four (27%) patients showed a partial response and five (33%) had stabilization of disease. In the prophylaxis group, 4 patients received treatment for a median of 24.5 months with no recurrence of lung metastases, however, the patients eventually experienced disease recurrence at other sites requiring systemic therapy [137]. Overall, the inhaled rhIL-2 trials have shown to be effective in prolonging survival in metastatic lung disease. There is still a need to determine the optimal dosing schedule as the trials have used varying dosing regiments with or without systemic therapy. It is also unclear whether it is best used in combination with systemic therapy, as single agent therapy, or as prophylaxis post-surgical resection of lung tumors. These questions, along with efficacy data in more patients, need to be answered before inhaled IL-2 enters a mainstay of lung carcinoma treatment.

5.1.3. Inhaled chemotherapy solutions

Inhalation chemotherapy was first described in the late 1960s and has since been explored as a means to target lung malignancy in the hope of reducing systemic side effects [100]. In the past ten years, there have been a number of clinical trials that have evaluated the effectiveness of nebulized doxorubicin, gemcitabine, and carboplatin [99,138–140].

G. Otterson et al. reported the results of a dose escalation study using doxorubicin nebulized *via* an OncoMyst device which consisted of a Pari LC Plus nebulizer (PARI Respiratory Equipment, Inc.) contained within a system to capture stray aerosols. The droplet size produced by this system was reported to be 2–3 μm in size allowing for effective deep lung delivery [140]. The patients were dosed with doxorubicin solution *via* inhalation once every three weeks at doses ranging from 0.4 mg/m^2 to 9.4 mg/m^2. A total of 53 patients were enrolled in the study at varying dosing levels. Limited pharmacokinetic analysis showed some drug was absorbed through the lungs, although the C_{max} was well below the levels achieved after IV administration. The investigators report at a dose of 9.4 mg/m^2 two of four patients developed grade 3 or higher toxicities. At the 7.5 mg/m^2 dose, only one patient of eleven experienced a dose limiting toxicity leading the investigators to designate this as the maximum safe dose of inhaled doxorubicin solution. Grade 1/2 cough was reported in 27/53 patients. Other tolerable side effects such as

dyspnea, chest pain, hoarseness, sore throat, fatigue, taste disturbances, and others were reported [140]. Based on these results, the investigators further studied the use of inhaled doxorubicin in combination with standard doses of cisplatin and docetaxel. Doses of 6 mg/m^2 and 7.5 mg/m^2 were evaluated with the primary objectives of the study was to determine the appropriate dose to use in combination with systemic therapy as well as achieving an overall response rate (complete + partial response) greater than 35%. At completion of the trial, 24 patients were evaluated with seven patients showing positive response to treatment. This number did not meet the predefined response rate, and the investigators recommended against progressing to a phase III study of this regimen [139].

E. Lemarie et al. demonstrated a feasibility of inhaled gemcitabine in eleven patients. Dose escalation showed a maximum tolerated dose of 3 mg/kg gemcitabine used in the nebulizer [138]. Scintigraphic analysis using ^{99}mTc-diethylene triamino pentaacetic acid mixed with gemcitabine during the first inhalation of treatment showed on average 43% of the inhaled gemcitabine reached the lung tissues while on average 47% was detected in the upper airways and stomach. Toxicities reported included one patient (3 mg/kg dose) with grade 4 bronchospasm, and 8 patients were noted to have cough during and after nebulization. Other side effects reported included fatigue, nausea, anorexia, and others. Pharmacokinetic analysis showed limited absorption of drug which correlated with the total drug exposure to the lung, however, there were no severe systemic side effects reported [138]. This small trial confirmed the ability of nebulized chemotherapy to reach the lung with limited systemic side effects.

P. Zarogoulidis et al. reported the use of inhaled carboplatin in combination with 100 mg/m^2 IV docetaxel [99]. Groups A, B and C consisted of IV carboplatin, 1/3 dose inhalation carboplatin and 2/3 dose IV, and full dose inhaled carboplatin, respectfully, combined with docetaxel. Toxicity data for each group showed a wide range of adverse side effects in all three groups. Neutropenia occurred less frequently with the inhalation treatments as compared to the control group with only 1/20 patients in group C experiencing grade 3 neutropenia. When looking at pulmonary side effects, their incidence increased with increasing inhaled carboplatin, however, some such as cough, resolved a few days after completion of the inhalations. Gastrointestinal side effects also seemed to increase in occurrence in the inhalation treatment groups. Median survival benefit was seen in groups B and C with survival benefits of 64 and 25 days, respectfully, as compared to the control group [99]. These results show promise that inhaled chemotherapy can be useful in helping patients with lung malignancies. However, an extensive investigation needs to be done in order to choose the correct dose and formulation of inhaled therapy as well as manage toxicity profiles distinct to the inhalation treatments.

5.1.4. Inhaled drug-loaded nanoparticles

There have been relatively few clinical trials evaluating the use of inhaled nanoparticle systems for the treatment of lung malignancies. The few trials that have been reported have focused on the use of either liposomal cisplatin or 9-nitro-20-camphothecin [141–143]. C. Verschraegen et al. reported a dose escalation trial of inhaled liposomal 9-nitro-20-camphothecin (9NC) in 24 patients. Liposomes were composed of dilauroylphosphatidylcholine (DLPC) and contained 2% 9NC. Initial dosing began at 6.7 μg/kg/day of 9NC and reached maximum of 26.6 μg/kg/day 9NC. 13.3 μg/kg/day 9NC was considered a safe dosing level for future studies as all patients tolerated the treatment well. The majority of side effects across all dosing levels were grade 1 or 2 with cough, nausea, and fatigue occurring most frequently. Although efficacy was seen as partial remission in two patients, this study only established that liposomal 9NC could be safely administered to patients with lung carcinoma [142].

B. Wittgen et al. evaluated liposomal cisplatin in a dose escalation study in patients with pulmonary carcinoma [143]. Liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and cholesterol were loaded with cisplatin. The doses ranged from 1.5 mg/m^2 to 60 mg/m^2 with a total of 17 patients evaluated. The most commonly reported side effects included fatigue, nausea, vomiting, and pulmonary changes although most of these were either grade 1 or 2 [143]. In this study, 12 patients experienced stable disease. Based on the relative safety of this cisplatin formulation, A. Chou et al. reported its use in the inhalation treatment of osteosarcoma metastatic to the lungs [141]. A total of 19 patients were treated with 24 mg/m^2 or 36 mg/m^2 doses of liposomal cisplatin. As seen in previous trials, pulmonary side effects were most commonly reported followed by gastrointestinal side effects. The results of this trial showed that three patients had a complete response after surgical resection post inhalation treatment while one patient had a partial response to treatment [141]. These results show that inhaled liposomal cisplatin is safe and tolerable to patients and can provide a benefit to patients with metastatic lung disease.

5.1.5. Inhalation cystic fibrosis (CF) therapy

Cystic fibrosis is a disease cause by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) which can affect multiple organ systems leading to the buildup of thick mucus, inflammation, and injury in the lung. Some small molecule drugs that improve the symptoms in certain CF mutations were developed, however, they are only effective for a small percentage of patients [144]. This opens the need for the development of a treatment approach that can be used in all patients. Gene therapy was researched extensively for these purposes. Both viral and non-viral delivery systems were investigated. Two clinical trials of adeno-associated virus (AAV) carrying cystic fibrosis transmembrane conductance regulator (CFTR) cDNA have shown safety but limited efficacy data [145,146]. Early trials using cationic liposomes and DNA plasmids delivered to nasal epithelium showed mixed results [147–149]. One trial reported that nasal delivery was safe, however, did not lead to positive transfection of collected samples [149]. Hyde et al. reported no inflammatory response to the liposome/DNA complex as well as on average 6/8 patients after each dose positive for transfection with the plasmid [148]. Alton et al. achieved successful transfection using cationic liposome system for plasmid delivery to the lung and nasal epithelium. Upon lung delivery of the formulation, 7/8 patients developed mild flu like symptoms which resolved within two days of therapy. Ruiz et al. also reported an inflammatory response to inhalation treatment with cationic lipid DNA complexes [150]. The early trials have established feasibility of transfection as well as safety concern for future trials. Recently, a large randomized placebo controlled phase IIb trial was undertaken to determine the efficacy of inhalation cationic liposome plasmid DNA formulation. The subjects were treated with once monthly doses of 13.5 mg of plasmid DNA complexed with 75 mg liposomes for one year. The primary end point is the change in predicted FEV1 [151]. This trial hopefully help to determine the clinical benefits of non-viral mediated CFTR gene therapy delivered *via* inhalation.

5.2. Patents

Recently, there have been a number of patent applications for nanoparticle delivery systems. The systems claim a variety of diverse applications and advantages over traditional drug delivery methods. Table 1, shows some typical examples of patents or patent applications for the inhalation treatment of diseases. The patents/applications were identified using a Google patent search for “drug loaded nanoparticles for lung delivery” and limited to those submitted in 2013, 2014, or 2015. The search also focused on technologies designed specifically for lung therapy excluding those patents that made very broad claims for the application of their inventions.

Table 1
Recent patent and patent applications for nanoparticle platforms for inhalation delivery of therapeutics.

Patent/application number	Nanoparticle platform	Particle size or molecular weight	Therapeutic agent(s)	Possible applications
US20140186290	Polystyrene	500 nm	Empty particles	Dispersal of thick mucus produced by various lung diseases and diseases at other mucosal surfaces [152]
US20150126589	Polyethyleneimine (PEI)	1–1000 kDa	mRNA for various target genes	Treatment of diseases due to defective or insufficient production of proteins and other diseases targeted by gene therapy [153]
US20150038556	Polyethylimine (PEI), protamine, poly-L-lysine (PLL), cationic lipids	Varies with delivery platform	CFTR mRNA	Correction of CFTR gene function in cystic fibrosis patients [154]
US8440231	Variable biodegradable polymer types that swell in size upon delivery to the lungs	Mass median aerodynamic diameter less than 5 μm	Any number of therapeutics directed to treat disease	Treatment of any number of pulmonary diseases [155]
US20130273164	Liposomes or nanostructured lipid carriers	1–1000 nm	Lipid soluble small molecule drugs, peptides, or siRNA's	Inhalation treatment of a number of inflammatory pulmonary diseases [156]
WO2014145606	Silica particles	1–25 nm	Fluorescent molecules, radionuclides, targeting peptides, oligonucleotides, and any number of small molecule drugs	Formulation may be delivered by any number of routes for the treatment and imaging of various diseases [157]
WO2014144285	Poly(ethylene glycol)–poly(propylene oxide)–poly(ethylene glycol) triblock co-polymer	50 nm–1000 nm composed of varying molecular weight polymers	Meropenem derivatives	Inhalation treatment of pulmonary infection including those caused by *Pseudomonas aeruginosa* [158]
US20140302147	Distearoylphosphatidylcholine (DSPC) plus calcium chloride	1–5 μm	Glycopyrrolate, indacatrol, mometasone	Possible treatment of diseases such as COPD, asthma, idiopathic pulmonary fibrosis, and others [159]

6. Future directions

Analysis of modern achievements of various lung diseases by inhalation of nanomedicines clearly shows advantages of direct local delivery of nanopharmaceutics specifically to the diseased cells in the lungs. Based on the analysis of the literature data and our own extensive experience in this field, we can conclude that the future of nanotechnology-based inhalation treatment of lung diseases belongs to the targeted multifunctional approach where therapeutic agents are delivered specifically to the diseased cells in the lungs together with suppressors of their resistance to the therapy. In addition, most probably such advanced multifunctional treatment will include the delivery of several drugs with different mechanisms of action, enhance the efficacy of treatment of lung diseases and limit adverse side effects on healthy tissues.

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