CHAPTER

10

Nanomedicine-Based Inhalation Treatments for Lung Cancer

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OUTLINE

1. AN OVERVIEW OF PULMONARY DRUG DELIVERY IN LUNG CANCER THERAPY

1.1 Main Advantages

Compared to systemic delivery, aerosol chemotherapy could potentially lead to three main benefits for lung cancer therapy [1]. First, the use of the inhalation route for lung diseases represents a clear pharmacokinetic advantage. Inhalation allows administration of high doses of chemotherapy or targeted therapy directly to the lung tumor site and reduction of systemic distribution and toxicities. Together these advantages significantly enhance the therapeutic ratio [2]. Second, due to this sharp reduction in

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systemic toxicities, the use of inhalation could reduce treatment interruptions responsible for tumor cell repopulation [1]. And third, these inhalation procedures could increase drug penetration into the lung tumor by maintaining a significantly increased concentration gradient of the anticancer drug in the lung tumor site [1].

Moreover, pulmonary drug delivery allows the drug to target the solid lung tumors also through the local bloodstream. Once deposited in the respiratory tract, the anticancer drug can be absorbed into the local circulation. The rate of absorption depends on different factors, such as the deposition region in the lung, the physicochemical properties of the drug, and/or the formulation [3–5]. Through this local circulation, drug could also reach the lung tumors. Depending on their localization in the lung, the tumors are fed by either the bronchial vascularization (if they are located in the conducting zone) or by the pulmonary circulation (if located in the respiratory zone) [6]. Moreover, inhaled anticancer drugs could reach the lymphatic system, e.g., in surgically resected lymph nodes [7]. The lymphatic system, through hemithorax and regional lymph nodes, plays a main role in tumors spreading all over the body; this is particularly true in lung cancer. Among other mechanisms, lymphatic drainage is responsible for clearing small foreign particles (up to approximately 500 nm in diameter) from the alveoli. This clearance represents an additional pharmacological opportunity for inhaled nanomedicine in lung cancer [8,9].

1.2 Techniques, Devices, and Drug Formulations to Achieve Pulmonary Drug Delivery

Approaches to achieving pulmonary drug delivery can be classified into four main types according to the inhaler device, i.e., nebulizers, pressurized metered dose inhalers (pMDIs), Soft-Mist inhalers, and dry powder inhalers (DPIs).

Because pMDI and Soft-Mist devices can only deliver drug doses in the range of micrograms, they are not adapted to chemotherapy. However, some preclinical studies describe the use of a pMDI for delivering doxorubicin-based dendrimers [10] and siRNA [11].

Nebulizers present the advantage of requiring no specific inhalation technique. They can therefore be used by patients unable to carry out active inhalation (e.g., bedridden patients). Moreover, they require only simple drug formulations such as solutions and suspensions. Intravenous (i.v.) formulations can therefore be easily aerosolized by means of nebulizers. All these partly explain why nebulizers, and especially air jet nebulizers, are the only type of device that has been used in clinical trials so far [1,2,12]. However, air jet nebulizers display many crucial disadvantages for this application. With nebulization, the administration procedure to delivering therapeutic doses is long. For example, it took up to 6 h in the case of cisplatin liposomes delivered using an air jet nebulizer [13]. It is also often inefficient in terms of lung deposition. A low fraction of the nominal dose is deposited, usually 10% −15% [13,14], although up to 43% was deposited in the lungs in a trial with gemcitabine by means of a vibrating mesh nebulizer [15]. This second drawback leads to contamination of the device and the environment, which is difficult to manage. Moreover, liquid formulations used for nebulization require water-soluble drugs, which is unusual in anticancer drugs, or micronized drug suspensions. They also require a safe reconstitution procedure for lyophilized powder as liquid formulations present lower drug stability during long-term storage than a dry form.

DPIs offer many benefits compared to nebulizers, in particular for lung cancer chemotherapy [16]. DPI formulations are in a solid state, which is more stable for long-term storage and better adapted to drugs with poor water-solubility, such as conventional anticancer chemotherapeutics. Moreover, DPIs deliver high doses and are activated and driven by the patient's inspiratory flow for a short administration

time. They are easily transportable and less expensive, require less maintenance, and can be manufactured as disposable inhalers to limit device and environmental contamination compared with nebulizers. There have been recent developments of DPI formulations for lung cancer therapy, confirming the great interest in this approach [16−21].

The aerodynamic diameter (d_{ae}) is the most appropriate measure of aerosol particle size. This is because it relates to the dynamic behavior of a particle in an airflow, which depends on its geometric size, shape, and density. Aerodynamic diameter allows the main mechanisms of aerosol deposition to be described, gravitational settling and inertial impaction. Particles need to present aerodynamic diameters of between 1 and 5 µm to reach the lower respiratory tract, and between 1 and 3 µm for the respiratory zone (Table 10.1). Moreover, DPI formulations must display good flowability and good dispersion properties [22,23].

Safety issues are a major concern in developing new drug-delivery systems for the inhalation route [24]. Excipients in an inhaled formulation have to be well-tolerated by the respiratory tract. Reported local toxicities resulting from pulmonary administration of polymeric delivery systems are mainly inflammation and cytotoxicity, depending on the polymer constituent and the particle properties [25]. Because of a lack of information concerning the local toxicity induced by excipients for pulmonary use, stringent determination of the local tolerance profile for each new candidate excipient (i.e., for pulmonary application) is highly recommended [23].

1.3 Main Issues Encountered

As mentioned previously, despite clear advantages characterizing aerosol chemotherapy, no inhaled anticancer chemotherapy has come on to the market so far. This can be explained by four main observations.

Firstly, 10%−30% of conventional chemotherapies used in lung cancer induce lung toxicities [26]. Scientists and clinicians have concluded that higher concentrations of these drugs in the lungs would lead to higher pulmonary toxicities. Therefore, development of these drugs for an inhalation purpose has been severely limited [2,6]. Selection of a drug candidate that does not induce significant lung toxicity is crucial.

Secondly, the residence time of the anticancer drug close to the lung tumor could be too short to generate effective antitumor activity [12]. Once inhaled and deposited, the particles are cleared from the lungs more or less rapidly,

TABLE 10.1 Mechanisms of Aerosol Deposition

Site	D_{ae} (µm)	Mechanism	Comment
Large airways	• 5−9 (slow inhalation) • 3−6 (fast inhalation)	Impaction	Most deposition in segmental airways
Smaller airways	1−5	Sedimentation	Improved with slow and deep breaths
Respiratory bronchioles	1−3	Sedimentation	Improved with slow and deep breaths
Alveoli	<0.5	Brownian diffusion	Poor deposition—most exhaled

D_{ae}, aerodynamic diameter.

Adapted from 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.

depending on their physicochemical properties, the deposited region in the respiratory tract, the respiratory disease, etc. (Fig. 10.1) [27]. The particles are either cleared towards the upper airways by the mucociliary clearance from the conducting zone (80%−90% of inhaled material being excreted from the upper and central lung within 24 h [27]), cleared by alveolar macrophages from the respiratory zone (optimal phagocytosis for particles of 1.5−3 μm [4]), or dissolved. Once dissolved, the drug is able to exert its pharmacological action, degraded by enzymatic metabolism or absorbed in blood or the lymphatic circulation (Fig 10.1) [5,27]. Many formulation strategies have been described to overcome one (or more) of these lung clearance mechanisms (Table 10.2).

Thirdly, nebulizers have not allowed sufficient drug doses (dose limiting toxicity [DLT] in phase I) to be attained during clinical trials. This is because of their poor efficiency in terms of lung deposition and their long administration time. In phase I, no systemic limiting toxicities were reached for cisplatin at the highest delivered dose (60 mg/m^2). This dose was delivered in a total nebulization time of more than 6 h within two cycles of three consecutive inhalation days (cycle interval of 2 weeks) [13].

Finally, an additional issue relates to the limitation of environmental contamination and the necessary protection of medical staff during the preparation of this inhaled chemotherapy and its administration to patients [2]. Infrastructure, administration procedures, and devices have to
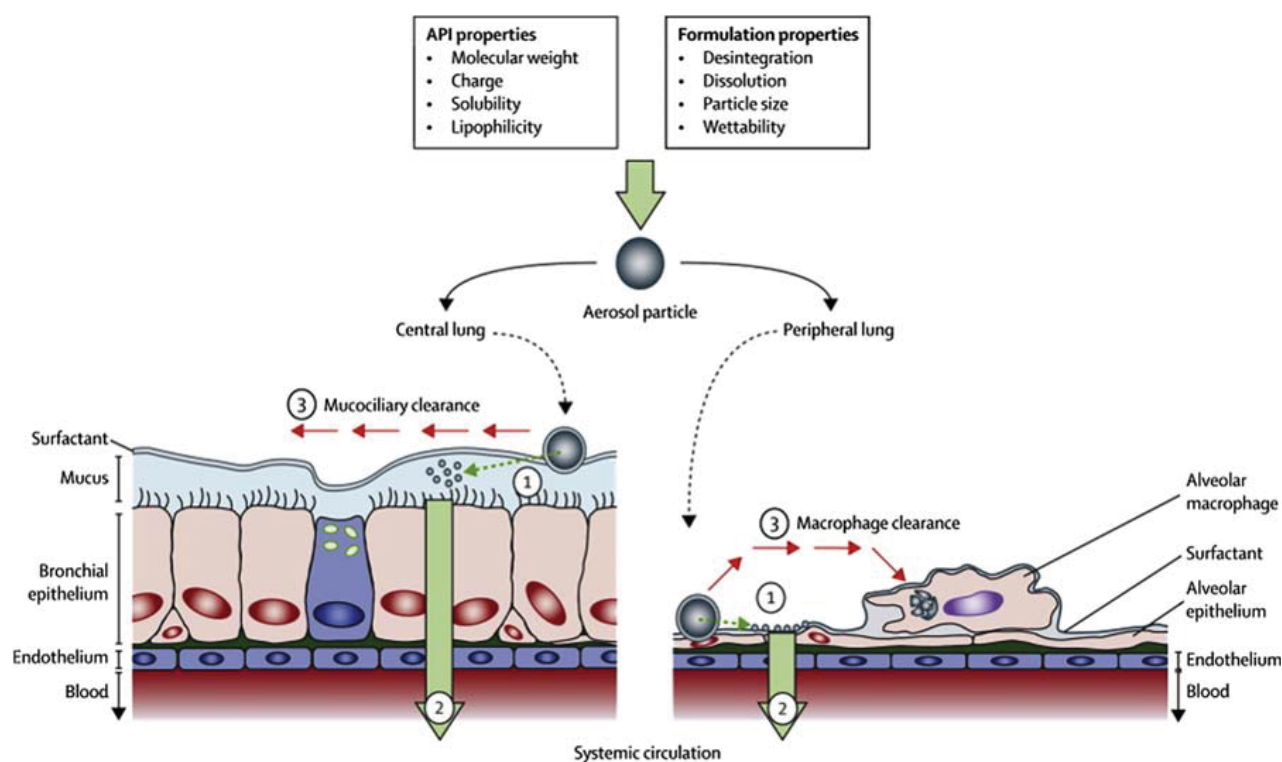

FIGURE 10.1   What happens to an aerosolized drug particle after deposition in the lungs? (1) First contact with the lung lining fluids: release of the drug compound from the deposited aerosol particle comprises different processes, governed by the amount and composition of the locally available lining fluid and by intrinsic properties of the drug and the carrier particle. (2) Absorption of the drug across the pulmonary epithelium: this process is mainly controlled by its physicochemical properties. (3) Clearance of the undissolved particle by mucociliary escalator or phagocytosis by macrophages. *From C.A Ruge, J. Kirch, C-M. Lehr. Pulmonary drug delivery: from generating aerosols to overcoming biological barriers—therapeutic possibilities and technological challenges, Lancet Respir. Med. 1 (2013) 402−413.* https://doi.org/10.1016/S2213-2600(13)70072-9.

TABLE 10.2 Example of Formulation Strategies Developed to Overcome Lung Clearance Mechanisms

Lung Clearance Mechanism to be Overcome	Strategy	Formulation Characteristic or Composition	References
Mucociliary clearance	Aerodynamic targeting—deposition in the alveoli	D_{ae} of 1.8—2.8 μm	[72]
	Mucoadhesion	Mucoadhesive agent-based formulations (e.g., chitosan, hyaluronan, HPMC)	[4,73]
Drug absorption	Micro- and/or nano-encapsulation of the drug	Micro- and nanoparticles (lipid, polymer-based)	[49,58,74,75]
Macrophage clearance	Modification of particle size[a]	Large porous particles, Trojan particles, nanoparticles	[51,58,59]
	Modification of particle shape	Varying particle geometric shapes (e.g., spheres, rectangular disks, elliptical disks)	[76]
	Stealth characteristics, surface modification	PEGylation	[41,77]
Physicochemical, enzymatic degradation	Encapsulation, complexation, degradation inhibitors	Liposomes, cyclodextrins, protease inhibitors	[41,78]

D_{ae}, aerodynamic diameter.

[a] Assuming optimal phagocytosis by macrophages for particles of 0.5—5 μm, and in particular the range of 1.5—3 μm.

be adapted for inhaled anticancer drug therapies. However, realistic and effective measures have been described in the literature in recent decades, such as a closed cabinet equipped with an air extractor and with both activated charcoal and HEPA filters [13,15,28—31].

2. INHALED NANOMEDICINE—AN ONGOING CONCEPT IN LUNG CANCER THERAPY

2.1 Advantages

Nanotechnology applications in medicine, defined as nanomedicine, have led to a number of applications for cancer imaging or treatment [32]. Nanopharmaceuticals are defined as "pharmaceuticals engineered on the nanoscale, i.e. pharmaceuticals where the nanomaterials plays the crucial therapeutic role or adds additional functionality to the active compound." [33] Like nanopharmaceuticals, nanocarriers consist of a colloidal nanoscale drug-delivery system composed of drugs, and polymeric and/or lipid material that improves the biokinetics and biodistribution of these drugs. The high and increasing number of nanocarriers developed for anticancer therapy can easily be explained by the clear benefits observed in preclinical and clinical development [34—36].

Nanomedicine presents many advantages in cancer therapy. This is especially true for pulmonary anticancer drug delivery. The advantages include the potential to (1) increase local drug bioavailability, (2) overcome biological barriers (e.g., mucus, cell membranes), (3) enhance or reduce the solubility of drug, (4) avoid or reduce phagocytosis by alveolar macrophages, (5) prolong pulmonary residence time, (6) protect

drug from degradation, (7) accumulate drug preferentially into tumors, (8) enhance drug internalization by cells, and (9) selectively and specifically recognize cancer cells [24,37,38].

2.2 Specific Requirements for Delivering Nanomedicine by Inhalation

Individualized nanoparticles present a too low d_{ae} to be deposited efficiently in the respiratory tract. As mentioned earlier, optimal d_{ae} for a particle to be deposited deep in the lungs is in the range of 0.5–5 μm (Table 10.1). As nanoparticles present an d_{ae} far below this range (because of their small size and/or density), they will (1) deposit mostly by diffusion in the alveolar region (i.e., random deposition related to their Brownian motion) or (2) be eliminated during exhalation [39]. Moreover, their enormous surface area is responsible for high free energy, leading to poor flowability and a high aggregation/agglomeration tendency [40]. Depending on the inhalation device, specific requirements are needed to achieve lung delivery of nanomedicine.

With a nebulizer, the nanoparticles are dispersed in liquid droplets aerosolized with appropriate d_{ae}. However, the nebulization can be responsible for destabilization of different types of nanocarriers, such as liposomes [41] and lipid nanocapsules [42]. Hureaux et al. observed that only vibrating mesh nebulizers are able to produce adequate aerosols containing lipid nanocapsules with good stability and performance (compared to jet, ultrasonic, and vibrating mesh nebulizers) [42].

With a DPI, the nanoparticles are contained in a formulation that must be designed to improve the DPI performance and reduce interparticle attraction forces. Examples of these dry powder formulations are nano-in-microparticles (or nano-embedded microparticles) or reversible nanoparticle agglomerates [17]. In nano-in-microparticles, the nanoparticles are embedded in

a microscale hydrophilic matrix able to release the nanoparticles in lung fluids [21,43,44],.

Many methods of producing nanomedicine-based DPI formulations have been described [23,40,45]. Among them, spray-drying has been recognized as a successful process for generating powders from solutions, dispersions, or suspensions in a single step. It converts a liquid feed (solution, coarse suspension, colloidal dispersion) to a dried particulate form. The main advantages of using this method to prepare DPI formulations include the ability to manipulate and control (1) mean particle size, (2) particle size distribution, shape, and density, and (3) macroscopic powder properties such as bulk density, flowability, and dispersibility. A typical spray-dryer produces particles of size ranging from 0.5 to 30 μm.

However, spray-drying nanoparticle dispersions is challenging. Destabilization of nanoparticle dispersions is usually observed during the spray-drying process, especially for materials characterized by low phase transition and/or melting temperatures such as lipids. This is due to the elevated temperature and large shear forces involved, leading to a dry powder that is unable to redisperse the initial nanoparticles [46,47]. Proposed measures to reduce the influence of temperature and shear forces during the spray-drying process have been to add hydrophilic excipients in solution into nanoparticle dispersions as well as to employ a hydroalcoholic solution instead of water as the dispersant [46,47]. Freitas and Müller explain that hydrophilic excipients, carbohydrates in their case, prevented the lipid nanocarriers from aggregating and protected them against heat by forming a crust after evaporation of the water during the drying step [46]. This crust has a spacer function and, once in contact with aqueous media, is able to dissolve quickly to re-disperse the initial nanocarriers. Typical hydrophilic excipients are lactose [48], mannitol [43,47], cyclodextrin [49], trehalose [50], and dextran [44]. Tsapis et al. also described the use

of additional soluble excipients (e.g., sugars, lipids, polymers, and proteins) in spray-dried nanoparticle-containing compositions to form large porous nanoparticle aggregates, so-called Trojan particles [51]. These relatively large particles (geometric sizes larger than 5 μm) are characterized by low densities leading to good aerosolization properties. In physiologic conditions, Trojan particles dissolve to release nanoparticles. As mentioned before, documentation on the safety profile of inhaled excipients is quite limited [23]. Therefore, the use of endogenous components, GRAS, and authorized excipients must be privileged in DPI formulations.

2.3 Clinical Development

The first type of nanocarrier developed for the delivery of chemotherapy by inhalation was liposome in the 1990s. So far, liposomes are the only type of nanocarrier that has been evaluated in clinical trials for this application (Table 10.3). Liposome vesicles are composed of endogenous lipids (e.g., phospholipids, cholesterol). These are biocompatible, biodegradable, and well-tolerated by the respiratory tract [41].

The first chemotherapeutic drug evaluated in clinical trials as an inhaled nanomedicine for lung cancer treatment was the topoisomerase I inhibitor 9-nitro-20(S)-camptothecin (9-NC), in 2004 [31]. A phase I study evaluating 9-NC-loaded dilauroyl phosphatidylcholine (DLPC) liposomes was performed in 24 patients with advanced pulmonary malignancies (both primary lung tumors and lung metastases). The inhaled doses necessary to obtain similar 9-NC plasma levels were lower than those administered orally (the usual route of administration for 9-NC). The systemic drug absorption observed was rapid and sustained. The authors determined the recommended dose by inhalation, i.e., $0.5\,mg/m^2/day$ (13.3 μg/kg/day), taken on five consecutive days per week for 8 weeks. This contrasts with a dose of $2\,mg/m^2/day$ for oral administration. This inhaled dose required a daily exposure of 60 min using a nebulizer. The DLT, at 26.6 μg/kg/day, was a chemical pharyngitis. It should be noted that bronchodilators and steroids allowed coughs and bronchial irritation to be alleviated clinically. Interestingly, hematological toxicity, i.e., the main toxicity for 9-NC, was not so often observed (grade 2 toxic effects included anemia and neutropenia for four and two patients, respectively). Two phase II evaluation trials were conducted: one in primary lung cancer, and one in metastatic endometrial cancer. The studies have been completed but no data have been published so far (ClinicalTrials.gov).

The most advanced development of inhaled nanomedicine is with liposomal cisplatin. Pt derivatives, including cisplatin, occupy a central position in adjuvant chemotherapy for lung cancer, especially for nonsmall-cell lung cancer (NSCLC). Cisplatin is one of the most potent and frequently used anticancer drugs despite severe toxicities following its systemic administration. These toxicities include cumulative nephrotoxicity (the main DLT), peripheral neuropathy, myelosuppression, and ototoxicity [13]. Cisplatin is therefore a potential candidate in aerosol chemotherapy for the reduction of systemic toxicities through use of this route of administration. A liposomal cisplatin formulation has been developed, consisting of cisplatin encapsulated in dipalmitoylphosphatidylcholine (DPPC)/cholesterol liposomes, referred to as sustained-release lipid inhalation targeting (SLIT) cisplatin. Preclinical studies have shown that the administration of SLIT cisplatin to the lungs induced limited systemic exposure in rats (including a decrease in ratios of lung/kidney levels compared to i.v. cisplatin) and was well-tolerated locally by beagle dogs (no histopathologic changes in the lungs, kidneys, or bone marrow) [13]. Moreover, SLIT cisplatin has shown significant antitumor activity in a murine Lewis lung tumor metastasis model, in contrast to the i.v. administration of cisplatin [13]. A phase I trial evaluated 17 patients

TABLE 10.3 Clinical Trials of Anticancer Nanomedicine Administered by Inhalation

Drug Formulation	Phase	Device Drug Concentration Time of Administration	Patients (n)	Posology	Deposition and/or Concentration in the Lungs	Local Adverse Effects and DLT	Systemic Exposure and Toxicities	Antitumor Activity	References
9-NC DLPC liposomes	I	Air jet nebulizer 2 mg (100 mg excipients) in 5 mL; 15 min/neb.; 2×/d	NSCLC and lung metastases (25); No response to treatments; 3–6 patients/cohort	Feasibility at 0.25 µg/m²/d for 5 consecutive d/w; 1, 2, 4, or 6 w (+2 w of rest); Dose escalation from 0.25 to 1 µg/m²/d; 5 d; 8 w (+2 w for rest)	4.2- to 10.6-fold higher drug concentrations in BAL than in serum	DLT (1 µg/m²/d): Chemical pharyngitis; Grade 2: Cough, bronchial irritation, skin rash around the face mask (0.5 µg/m²/d)	C_{max} (at 0.5 µg/m²/d) = 77 ± 39 ng/mL (vs. ~111 n/mL at 2 mg/m²/d per os); Less toxicities than with per os; Grade 2: Nausea/vomiting, anemia, neutropenia	Partial remission (3) and stable disease (3)	[31]
IL-2 DMPC liposomes	I	Twin jet nebulizer 20 min/neb.; 3×/d	Locally advanced or metastatic sarcomas or other refractory solid tumors (9) 3 patients per dose	1.5, 3.0 or 6.0 × 10⁶ IU 3×/d for 28 d	–	Respiratory parameters nonsignificantly modified	No significant toxicity	Complete remission (1 melanoma), stable disease (2 sarcoma and 1 renal carcinoma)	[79]
Cisplatin DPPC/Chol liposomes	I	Air jet nebulizer 1 mg/mL (23.5 mg excipients)/mL Up to 20 min/neb.; 3 consecutive neb./session; up to 3 sessions/d (2–3 h between 2 sessions)	Advanced NSCLC (16) and SCLC (1); No response to treatments	Dose escalation from 1.5 to 60 mg/m²; From 1 to 4 consecutive d/w for 1–3 w/cycle	Deposition in the lungs of 10%–15%	DLT not reached (>60 mg/m²); Grade 3: Bronchitis, decreased FEV₁, dyspnea	Low plasmatic concentrations; Grade 4: Thrombosis; Grade 3: Fatigue	Stable (12/18), progressive disease (4/18)	[13]
	Ib/IIa		Osteosarcoma with lung metastases only (≥1 cm) (19) treated with platinum-based regimens	24 or 36 mg/m²/2 w (=1 cycle)		Grade 2: Hoarseness	Grade 3: Nausea/vomiting	Complete (3 with tumors ≤2 cm + metastasectomy), partial response (1), stable (7) and progressive disease (8)	[52]

with primary lung cancer [13]. The most important observation was that the DLT of cisplatin was not reached. This was mainly explained by the poor performance of the jet nebulizer used to deliver the formulation. Indeed, nebulization required a long administration time for the formulation (the highest daily dose was administered through three sessions of 1 h with a break of 2—3 h between each session) because of the low deposition efficiency of the drug in the lungs. The dose reaching the target area was determined to be only 10% —15% of the delivered dose. The main toxicities observed were related to the respiratory tract, such as local irritation of the mucosa, bronchitis, or shortness of breath. For one patient, these local toxicities led to discontinuation of the trial. In all the patients, no signs of alveolar damage (computed tomography of the thorax) were observed. Despite the probability of more severe local toxicities resulting from the administration of higher doses, the authors concluded that the evaluated strategy was feasible and safe. The main factor limiting progress to phase II trials is the technical incapacity of the described nebulization procedure (related to the nebulizer chosen and to the concentration of cisplatin in the formulation). A further study was performed in recurrent osteosarcoma patients who only had pulmonary metastases (phase Ib/IIa trials) [52]. Issues encountered in osteosarcoma treatment are most often related to the inability to control metastases in the lungs. In this case, inhaled chemotherapy could also constitute a clear advantage.

As observed in the previous trial, the main relevant toxicities were associated with the respiratory tract (observed in 13/19 patients). The limited systemic drug exposure involved in using inhalation was also confirmed in this trial. In addition, only two patients presented serious adverse events due to the treatment, namely grade 3 vomiting and grade 1 dyspnea and chest pain.

2.4 Promising Strategies in Preclinical Development

Large numbers of preclinical studies on designing tumor-targeting nanocarriers delivered by the pulmonary route have been described in the two last decades (Table 10.4). These studies are largely discussed in two reviews [53,54]. By far the greatest numbers of these nanocarriers have been developed as liquid nanosuspensions, i.e., adapted to nebulizers.

Many studies have evaluated the accumulation of nanocarriers and/or the drug (i.e., contained in nanocarriers) in the lungs after pulmonary delivery (principally in mice or rats) [55—57]. Compared to i.v. injection, pulmonary delivery has led to lower systemic exposure of the encapsulated anticancer drug and, possibly by extension, related systemic toxicities [55]. Moreover, the use of drug-loaded nanocarriers locally has led to higher and longer drug retention in the lungs (up to 7 days [57]) than with the free drug [55]. For dendrimers, the time of retention within the lungs has been found to be size-dependent, with the largest nanoparticles (diameter of 20 nm) showing the highest accumulation and the longest retention in the lungs as a result of low absorption in the systemic circulation (Fig. 10.2) [56]. Nanocarriers have also been able to reach the lymphatic system after inhalation [9]. However, as discussed earlier, absorption in the systemic circulation is not the only mechanism of clearance in the lung and therefore not the only mechanism to bypass to prolong retention in the lung (Fig. 10.1 and Table 10.2). Phagocytosis by alveolar macrophages is also related to the inhaled particle size, with an optimal uptake when particles are characterized by a diameter in the range of 0.5—5 μm [27], in particular 1.5—3 μm [4]. Therefore, both smaller and larger particles [51,58,59] have been proposed to circumvent the uptake by macrophages (Table 10.2).

Pulmonary delivery has been proved to be therapeutically more effective than i.v. injection

TABLE 10.4 Examples of Preclinical Studies Describing Inhaled Nanomedicine for Lung Tumors

Nanocarrier	Formulation	Lung Delivery (Device if Described)	Evaluation and Models	Key Observations and Outcomes	References
Liposomes	Neutral and cationic[a] liposomes loaded with doxorubicin, antisense oligonucleotides or siRNA (all at once)	Liquid	• Human A549 adenocarcinoma cell line • Lung tumor orthotopic murine model (A549 cell line)	• Efficient intracellular delivery of the three agents in vitro • Endotracheal instillation led to higher peak concentrations and longer retention of fluorescent-labeled liposomes in the lungs compared with i.v. (AUC up to 7.5-fold higher)	[80]
	Paclitaxel-loaded liposomes	Liquid (AeroMist jet nebuliser)	• Healthy mice • Renca renal carcinoma pulmonary metastases murine model	• Increased exposure of the lungs to paclitaxel by using aerosol delivery compared to i.v. injection (AUC 26-fold higher) • Significant anticancer activity in vivo (versus nontreated)	[81]
Polymeric micelles	EpCAM-coated PEG–PLA nanoparticle[b] loaded with paclitaxel palmitate	Liquid (Microsprayer Aeroliser)	c-Raf transgenic lung cancer model	• Mild inflammation observed • Vehicles and loaded nanoparticles well-tolerated compared with a solution of the drug or the antibody • Significant antitumor activity in vivo (versus nontreated)	[63]
	Paclitaxel-loaded DSPE-PEG[c] micelles	Liquid	Healthy mice and rats	• Increased exposure of the lungs to paclitaxel by using aerosol delivery compared to i.v. injection and by using the micelles compared to Taxol locally (AUC 45-fold and threefold higher, respectively) • Limited paclitaxel systemic exposure • Vehicle well-tolerated locally (no increase of lung toxicity markers ALP and NAG)	[55]
	Paclitaxel-loaded F-PEG-HMD[d] micelles	Liquid (Microsprayer Aeroliser) and DPI (DP-4M Insufflator and Axahaler)	• Murine M109-HiFR lung carcinoma and human HeLa ovarian cancer cell lines • Healthy mice • Lung tumor orthotopic murine model (M109-HiFR cell line)	• Cell uptake of fluorescent micelles in two folate receptor-expressing cell lines • Penetration and distribution of fluorescent micelles in tumors in vivo • No inflammation nor cytotoxicity observed following endotracheal administration of the vehicle (excipient-based dry powder)	[21]

Lipid nanoparticles	LHRH receptor-targeted NLC[e] loaded with siRNAs (MRP1 and BCL2 proteins) and doxorubicin or paclitaxel	Liquid (nebulizer)	Lung tumor orthotopic murine model (A549 cell line)	• Intracellular delivery of NLC payloads in lung tumor cells in vivo and in vitro • Compared to nontargeted NLC, labeled targeted NLC preferentially accumulate in lung tumors in vivo (avoiding healthy lung tissues) • Significant enhancement of antitumor activity (compared to both i.v. and inhaled free drug)—quasi elimination of tumor burdens	[61]
	Paclitaxel-loaded SLN[f]	Liquid	• Murine mammary adenocarcinoma MXT-B2 cell line • MXT-B2 lung metastases murine model	• Significantly higher cytotoxic efficacy than Taxol in vitro (20-fold increase) • Loaded SLN was more effective in suppressing the pulmonary metastases than i.v. Taxol, decrease in both the number (up to 10-fold) and the volume • Complete remission in 75% of the animals • Labeled SLN reached the lymphatic system after inhalation	[9,60]
	F-PEG-HTCC[g]-coated, paclitaxel-loaded SLN	Liquid (Microsprayer Aeroliser)	• Murine M109-HiFR lung carcinoma and human HeLa ovarian cancer cell lines • Healthy BALB/c mice • Lung tumor orthotopic murine model (M109-HiFR cell line)	• Cell uptake of fluorescent coated SLN in two folate receptor-expressing cell lines • Significantly higher antiproliferative activity than Taxol in vitro (sixfold increase) • Prolonged pulmonary exposure to paclitaxel to up to 6 h and low systemic exposure vs. aerosolized and i.v. Taxol • Penetration and distribution of fluorescent coated SLN in tumors in vivo	[70]
	Paclitaxel-loaded LNC[h]	Liquid (eFlow rapid mesh nebulizer (PARI, Germany) and Microsprayer Aeroliser)	• Characterization of preclinical batches and scale-up studies • Healthy rat	• Nebulized LNCs had similar characteristics (structure, drug payload, and cytotoxicity) as fresh LNC and good aerodynamic properties • Prolonged lung retention of radiolabeled tracer-loaded LNC (compared to the tracer without LNC) with a lung half-time of 8.8 ± 0.7 h • Transient 7-day alveolar inflammation (acute inflammation increased with paclitaxel-loaded LNC in comparison with blank LNC)	[82,83]
Polymer nanoparticles	siRNA (EGFP protein)-loaded dextran nanogel coated with folate-grafted pulmonary surfactant	Liquid	Human alveolar epithelial H1229 and A549 cell lines	• Enhancement of both cellular uptake and gene silencing potential • Effective knockdown at siRNA concentrations in the nanomolar range • Effective targeting of the folate receptors in vitro	[69]

TABLE 10.4 Examples of Preclinical Studies Describing Inhaled Nanomedicine for Lung Tumors—cont'd

Nanocarrier	Formulation	Lung Delivery (Device if Described)	Evaluation and Models	Key Observations and Outcomes	References
	Doxorubicin-loaded n-butylcyanoacrylate nanoparticles embedded in effervescent or noneffervescent matrix	DPI (DP-4M Insufflator)	• Healthy mice • Lung tumor orthotopic murine model (H460 cell line)	• Significant improvement of antitumor efficacy and survival rate (70% mice alive after 140 days vs. all mice dead within 50 days with i.v. doxorubicin) • No cardiac toxicity in contrast to inhaled free doxorubicin	[62]
	EGFR-targeted gelatin nanoparticles loaded with cisplatin	Liquid (nebulizer AP-100100)	Lung tumor orthotopic and subcutaneous murine models (A549 cell line)	• After inhalation, higher accumulation of targeted nanoparticle and of Pt (contained in nanoparticles) in lung tumors than nontargeted nanoparticles and free cisplatin, respectively • Preferential accumulation in tumor-grafted lungs than in healthy lungs (3.5-fold) • Significant antitumor activity in vivo after intratumor injection (vs. nontreated) (sixfold decrease in tumor volume)	[64,65]
Dendrimers	PEGylated poly-lysine dendrimers conjugated to doxorubicin	Liquid	Rat model of lung metastases of breast cancer (MAT-13762 IIIB tumors)	• Long drug retention in the lungs, with ~15% of the dose retained in the lung after 7 days • Higher reduction of lung tumor burden (vs. i.v. doxorubicin) • Less local toxicity (vs. intratracheal free doxorubicin)	[57]
Nanocrystals	Paclitaxel nanocrystal agglomerates embedded in cisplatin matrix	DPI	Pharmaceutical characterization	• Enhanced dissolution kinetics (threefold increase in solubilized paclitaxel within 1 h vs. paclitaxel commercial powder) • Desirable aerodynamic properties	[17]
Magnetic nanoparticles	Nano-in-microparticles (NIM) composed with SPION[i] and doxorubicin	DPI (DP-4M Insufflator)	• Pharmaceutical characterization • In vitro tracheal mimic study	Twofold increased deposition of NIM containing a fluorescent dye in regions under the influence of a strong magnetic gradient in vitro vs. a liquid suspension	[84]

[a] DOTAP-based liposomes.
[b] Epithelial cell adhesion molecule (EpCAM)-coated poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) nanoparticle.
[c] Phosphatidylethanolamine-poly(ethylene glycol) derivate.
[d] Folate-poly(ethylene glycol)—hydrophobically modified dextran.
[e] Luteinizing hormone-releasing hormone (LHRH) receptor-targeted nanostructured lipid carriers (NLCs).
[f] Solid lipid nanoparticle.
[g] Folate-poly(ethylene glycol)-(N-[2-hydroxy- 3-trimethylammonium)propyl] chitosan).
[h] Lipid nanocapsules.
[i] Fe_3O_4 superparamagnetic iron oxide nanoparticles.
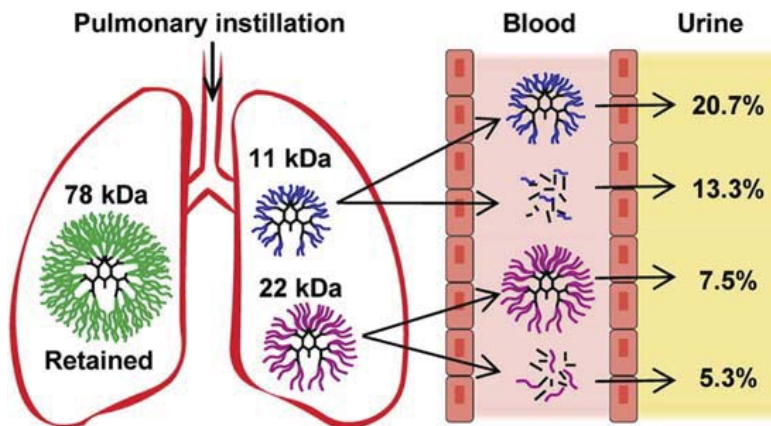Adapted from Rosière et al., Rev Mal Resp, in press.

FIGURE 10.2 Illustration of the observed correlation between dendrimer molecular weight (and therefore nanoparticle size) and absorption in the systemic circulation after pulmonary instillation. Diameters for the 11-, 22-, and 78-kDa dendrimers were 6, 12, and 20 nm, respectively. *From G.M. Ryan, L.M. Kaminskas, B.D. Kelly, D.J. Owen, M.P. McIntosh, C.J.H. Porter, Pulmonary administration of PEGylated polylysine dendrimers: absorption from the lung versus retention within the lung is highly size-dependent, Mol. Pharm. 10 (2013) 2986—2995. https://doi.org/10.1021/mp400091n.*

for various nanocarriers in many in vivo preclinical models [57,60—62]. When aerosolized, drug-loaded nanocarriers have also led to improved antitumor efficacy compared to free drug in solution [61]. In addition, the use of drug-loaded nanocarriers instead of solubilized free drug has led to lower toxicity, both local [63] and systemic [62,63].

Ligand-related targeting has also been explored in the context of an inhaled therapy. For this purpose, epidermal growth factor was grafted onto the surface of gelatine nanoparticles to target EGFR, overexpressed in NSCLC [64,65]. When delivered by inhalation, the presence of EGF induced a higher accumulation of nanoparticles (than with nontargeted nanoparticles) in A549 lung tumors of a murine model that overexpresses EGFR (Fig. 10.3). Moreover, the EGFR-targeted nanoparticles accumulated preferentially in the A549 tumor-grafted mouse lungs compared to in healthy mouse lungs 30 min and 24 h after aerosol administration, confirming the effective targeting of the tumor tissues in vivo. The antiepithelial cell adhesion molecule (EpCAM) antibody [63] and the modified synthetic analog of luteinizing hormone-releasing hormone (LHRH) [61] are other examples of targeting moieties that have been grafted onto the surface of nanocarriers delivered by the pulmonary route (Table 10.4). Promisingly, Taratula et al. demonstrated that, compared to non-LHRH-targeted labeled nanostructured lipid carriers (NLCs), LHRH-targeted labeled NLC preferentially accumulate in lung tumors in vivo, avoiding healthy lung tissue (Fig. 10.4). To our knowledge, this study was the only one that has demonstrated in vivo nanocarrier selectivity for lung tumors compared to healthy tissues following aerosol delivery. In terms of the therapeutic response, targeted NLC loaded with paclitaxel and siRNA (silencing proteins related to efflux and antiapoptotic defense mechanisms, i.e., MRP1 and BCL2 proteins) led to improved antitumor activity compared to i.v. conventional solvent-based paclitaxel formulation (i.e., ∼40-fold decrease in tumor volume), allowing complete regression in 50% of mice [61].

Another target in lung cancer is the overexpression of folate receptors (FRs), in particular the alpha form (FR-α), on lung cancer cell membrane compared with healthy tissues. This overexpression has been observed in more than 60% of NSCLC [66,67], principally in adenocarcinoma [68]. Engraftment of folate groups onto the nanoparticle surface has been investigated in pulmonary delivery (Table 10.4) as a way of improving the uptake of siRNA by high FR-expressing lung cancer cells, compared to low FR-expressing cancer cells [69]. The nanocarrier has led to very promising results in vitro. For example, better cellular uptake and gene silencing were both demonstrated for targeted
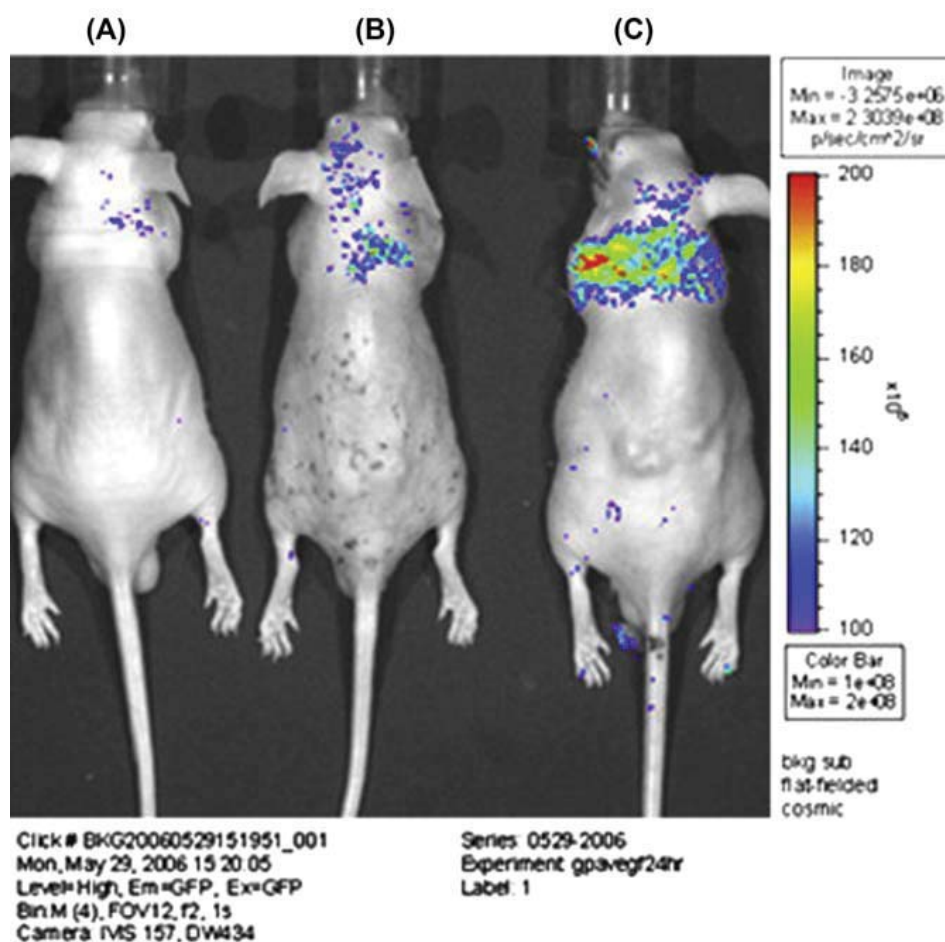
FIGURE 10.3   Proof of concept of the benefit of using an actively targeted nanocarrier by pulmonary delivery. (A) PBS-treated group, (B) nontargeted nanoparticle-treated group, and (C) EGFR-targeted nanoparticle-treated group. Green fluorescence spectra were obtained from live mice xenografted with the A549 human lung adenocarcinoma cells 1 day after inhalation delivery. *From C-L. Tseng, S.Y-H. Wu, W-H. Wang, C-L. Peng, F-H. Lin, C-C. Lin, T-H. Young, M-J. Shieh, Targeting efficiency and biodistribution of biotinylated-EGF-conjugated gelatin nanoparticles administered via aerosol delivery in nude mice with lung cancer, Biomaterials 29 (2008) 3014—3022.* https://doi.org/10.1016/j.biomaterials.2008.03.033.

nanocarriers compared to nontargeted nanocarriers in H1229 (an FR-expressing human alveolar cell line). The use of folate-grafted nanocarriers, micelles [21], and solid lipid nanoparticles (SLNs) [70] allowed the antiproliferative activity of paclitaxel to be increased in vitro (up to sixfold increase) and penetration into folate receptor lung cancer cells and tumors to be improved. Due to their sustained-release properties ($\sim$10% paclitaxel released each 24 h in vitro) and their physicochemical characteristics (i.e.,

size, surface modification), folate-grafted SLN prolonged pulmonary exposure to paclitaxel to up to 6 h following pulmonary delivery in healthy mice [70]. The nanocarriers in the composition of DPI formulations had good aerodynamic properties (broad deposition in the lungs and fine particle fractions up to 50%) and were able to re-disperse the initial nanocarrier in physiologic buffers [21,44]. The nanocarrier-based powders (without paclitaxel) were well-tolerated locally by healthy mice after inhalation.
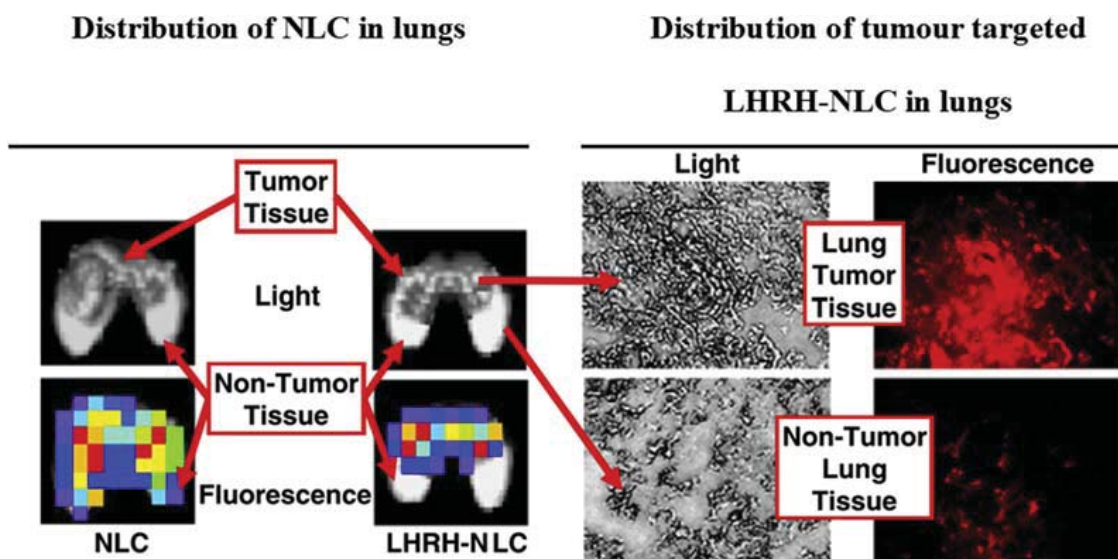
FIGURE 10.4 Accumulation of nanostructured lipid carrier (NLC) in mouse lungs bearing A549 human lung cancer. Distribution of fluorescently labeled nontargeted and LHRH-tumor-targeted NLC in mouse lungs (left image). Distribution of fluorescently labeled LHRH-tumor-targeted NLC in mouse lungs (tumor and nontumor tissues; red [gray in print version] = NLC) (right image). *Adapted from 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. 171 (2013) 349–357.* https://doi.org/10.1016/j.jconrel.2013.04.018.

There was no significant modification in cell composition, protein, and proinflammatory cytokine concentration and LDH activity in BALF. The main limitation of these DPI formulations was their paclitaxel loading, which was less than 0.5% w/w. These were not sufficient to deliver effective paclitaxel doses within a reasonable time to patients. Therefore, nanocrystal-based DPI formulations were developed to increase the paclitaxel drug loading to up to 2% [43]. These formulations led to 10-fold and 25-fold increases in the fine particle dose compared to SLN- [44] and micelle-based [21] dry powders, respectively.

3. CURRENT LIMITATIONS AND FUTURE CHALLENGES

While offering very promising results, inhaled anticancer nanomedicine, and inhaled nanomedicine in general, presents major drawbacks to be overcome in the future [24]. For example, the aforesaid studies describe nanocarriers characterized by poor drug payload. Drug loading of nanocarriers is usually in the range of 1%–10% (w/w). Consequently, depending on the drug candidate, the aim of delivering sufficient anticancer drug doses to patients by means of these nanocarriers might be unrealistic. While this drawback is certain for i.v. perfusions [71], pulmonary delivery might be less affected by this drawback. This possibility is due to (1) the usual decrease in inhaled drug doses and (2) the relatively low volume of distribution in the respiratory tract compared to the systemic circulation. It must be considered that this poor loading also leads to pulmonary delivery of high doses of the excipients composing the nanocarriers and dry powders (i.e., for DPI formulations). Delivery of these high doses might cause local adverse effects. In addition, most of the aforesaid nanocarriers are composed of excipients for which no data relating to toxicity/tolerance

following aerosol delivery are available. Future toxicity studies should therefore be more consistent in the evaluation of these new nanomedicine-based therapies. Safety is considered to be one of the most obvious challenges [24].

Another consistent challenge for these therapies is related to the method of producing the nanocarriers [24]. The described methods are often too complex to produce large batches, as they consist of multiple steps. Indeed, although these methods are relevant for lab-scale production, only a few are easy to scale up to produce acceptable clinical batches. An effective method will result in nanocarriers with highly reproducible characteristics (i.e., size, shape, drug payload, stability, drug release, etc.).

It should also be noted that almost all these studies have been published within the last decade. Developing this treatment approach is novel and therefore very challenging. However, these challenges must be taken up in the future due to the very promising results obtained in preclinical studies, especially in aerosol delivery of active tumor-targeted nanomedicine.

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