Current Topics

Recent Progress in Drug Delivery System for Cancer Therapy

Review

Present Situation and Future Progress of Inhaled Lung Cancer Therapy: Necessity of Inhaled Formulations with Drug Delivery Functions

Tomoyuki Okuda* and Hirokazu Okamoto

Faculty of Pharmacy, Meijo University; 150 Yagotoyama, Tempaku-ku, Nagoya 468–8503, Japan.
Received January 27, 2020

Inhaled lung cancer therapy is promising because of direct and noninvasive drug delivery to the lungs with low potential for severe systemic toxicity. Thus chemotherapeutic drugs have been administered clinically by nebulization of solution or suspension formulations, which demonstrated their limited pulmonary absorption and relatively mild systemic toxicity. In all these clinical trials, however, there was no obviously superior anticancer efficacy in lung cancer patients even at the maximum doses of drugs limited by pulmonary toxicity. Therefore methods that deliver both higher anticancer efficacy and lower pulmonary toxicity are strongly desired. In addition to the worldwide availability of pressured metered dose inhalers (pMDIs) and dry powder inhalers (DPIs) to treat local respiratory diseases, recent innovations in medicines and technologies are encouraging next steps toward effective inhaled lung cancer therapy with new therapeutic or drug delivery concepts. These include the discovery of target cells/molecules and drug candidates for novel cancer therapy, the development of high-performance inhalation devices for effective pulmonary drug delivery, and the establishment of manufacturing technologies for functional nanoparticles/microparticles. This review highlights the present situation and future progress of inhaled drugs for lung cancer therapy, including an overview of available inhalation devices, pharmacokinetics, and outcomes in clinical trials so far and some novel formulation strategies based on drug delivery systems to achieve enhanced anticancer efficacy and attenuated pulmonary toxicity.

Key words aerosol inhalation system; lung cancer therapy; drug delivery function; inhaled formulation; inhalation device; pulmonary pharmacokinetics

1. Introduction

Lung cancer is one of the world's most common and serious malignancies. The GLOBOCAN database for 185 countries and 36 cancer types (produced by the International Agency for Research, IARC) estimates that lung cancer globally caused 2.09 million new cases (11.6% of the total cases) and 1.76 million deaths (18.4% of the total cancer deaths) in 2018, both of which were the largest numbers among all cancer types.[1] Histopathologically, lung cancer is categorized into two broad classes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC is extremely malignant due to its high proliferative and metastatic potential, but has relatively good sensitivity to radiotherapy and chemotherapy. On the other hand, NSCLC with lower sensitivity to these therapies is more common.

Most chemotherapeutic drugs for SCLC and NSCLC are systemically administered *via* oral and intravenous routes to prevent metastases after surgical resection at early stage or to inhibit growth of unresectable tumors at more advanced stage. However, satisfaction with these drugs' performance is often very poor; severe adverse effects such as bone marrow suppression limit dosing, consequently leading to insufficient anticancer effects. Recently, the advent of molecular-targeted therapy with therapeutic monoclonal antibodies and signal transduction activators/inhibitors has greatly contributed to improved outcomes for patients with various types of cancer in which the target molecules are highly expressed. In lung cancer therapy, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib were demonstrated to achieve higher objective response rates and progression-free survival than chemotherapy in previously untreated patients with EGFR mutations.[2] Recently, necitumumab and nivolumab, therapeutic monoclonal antibodies specific to EGFR and programmed death-1 (PD-1), respectively, have been approved for clinical use in the treatment of lung cancer.[3] On the other hand, some severe adverse effects have been observed for molecular-targeted drugs. For example, EGFR TKIs commonly induce skin rash, and interstitial pneumonia in rare but fatal cases, whereas therapeutic monoclonal antibodies frequently cause hypersensitivity reactions including infusion-related and cytokine-released effects.[4,5] Moreover, acquired resistance to chemotherapeutic and molecular-targeted drugs is a well-known unwanted development in many cases.[2,6]

Thus innovative medical strategies and technologies are strongly demanded for further improvement of chemotherapeutic and molecular-targeted drugs against lung cancer—and one of the key factors is effective drug delivery to target sites.

* To whom correspondence should be addressed. e-mail: tokuda@meijo-u.ac.jp

2. Aerosol Inhalation Systems for Pulmonary Delivery

The lungs are attractive organs as both action and absorption sites of drugs because of various histological characteristics including direct and noninvasive access *via* the respiratory tract, low enzyme activity, large surface area comparable to small intestine, and thin cell layer with high membrane permeability.[7] Therefore pulmonary delivery *via* the respiratory tract is promising to promote efficacy of chemotherapeutic and molecular-targeted drugs against lung cancer with minimal systemic adverse effects. In preclinical studies in small animals, pulmonary drug delivery has been often performed through intranasal or intratracheal instillation of drug solution or suspension,[8] although this method is not readily applicable in human due to perceived high invasiveness. For noninvasive and effective pulmonary drug delivery in human, aerosol inhalation systems are considered practical. All these systems comprise specially designed formulations and inhalation devices. Three major types of the devices are commonly used: nebulizers, pressurized metered dose inhalers (pMDIs), and dry powder inhalers (DPIs).[9]

All the above devices generate liquid or solid pharmaceutical aerosols from the formulations, followed by their transfer *via* inspiratory airflow to the respiratory tract. After transfer, aerosols are deposited on the respiratory epithelium under various mechanisms including inertial impaction, sedimentation, diffusion, interception, and electrostatic interaction.[10] The deposition patterns of aerosols in the respiratory tract are predominantly determined by their aerodynamic particle size calculated with the following equation:

$$d_a = d_g \left(\frac{\rho_p}{\rho_0 \chi} \right)^{0.5}$$

where d_a, d_g, ρ_p, ρ_0, and χ are aerodynamic particle size, geometric particle size, particle density, unit density, and dynamic shape factor, respectively. In general, larger-sized aerosols ($d_a > 10\,\mu$m) are deposited on an upper position of the respiratory tract, whereas smaller-sized aerosols ($d_a < 0.5\,\mu$m) may be emitted out of the body through expiratory airflow. Therefore for effective pulmonary delivery of pharmaceutical aerosols, their aerodynamic particle size is adjusted mainly within the range of 1–5 μm.[10] In a lower position of the respiratory tract, where temperature and relative humidity increase up to approximately 37°C and 100%, respectively, deposition of hygroscopic aerosols is considered promoted due to increases in their particle size and density through moisture absorption.

2.1. Nebulizers Among commercial inhalation devices, nebulizers, which generate single-micron-sized liquid aerosols from solution or suspension formulations, have the longest history of development (since the mid-1800 s).[11] Currently, three major types of nebulizers are available on the market: jet, mesh, and ultrasonic nebulizers, which generate liquid aerosols by compressed air, ultrasonic vibration, and ultrasonic cavitation, respectively.[12,13] Many nebulizers are inconvenient to carry due to their relatively large size, although small-sized mesh nebulizers with portability (*e.g.*, MicroAir U100, Omron Healthcare Co., Ltd.) have been developed recently. Even now, nebulizers are predominantly chosen in the first clinical trials of inhalation therapy with drug candidates because the trial can be easily started with only drug solutions or suspensions. Indeed, only nebulizers have been applied to clinical trials for inhaled lung cancer therapy so far, as described below. However, they have many practical issues including the necessity of electric power supply for operation, poor pulmonary delivery efficiency, time-consuming administration, and low storage stability of the formulations due to their liquid forms. Moreover, large portions of aerosols generated and emitted from nebulizers are not inhaled but released to the environment, possibly causing exposure of anticancer drugs with severe adverse effects to medical workers or neighbors.

2.2. pMDIs pMDIs are mainly composed of metering valves, actuators, and canisters containing solution or suspension formulations with liquefied gas propellants.[14,15] After pressing the canister, a constant volume of the formulation (25–100 μL) is aerosolized *via* the metering valve, followed by instant emission from the actuator. As the propellants, chlorofluorocarbons (CFCs) with many suitable properties including no toxicity, no flammability, high drug compatibility, appropriate boiling points, and appropriate densities were often used at the past, but they have since been largely replaced with hydrofluoroalkanes (HFAs) because CFCs damage the stratospheric ozone layer.[14,16] However, HFAs have much greater greenhouse effects than carbon dioxide, implying that further alternative propellants without environmental effects are required.[14,16] For pMDIs, many drugs are formulated as suspensions through micronization due to their low solubility in the propellants. In contrast, solution formulations for pMDIs are prepared mainly by adding alcohols and surfactants as solubilizers, which can attain not only higher dose uniformity but also smaller particle size of emitted aerosols than suspension formulations.[16]

pMDIs have been widely accepted as effective and practical devices in inhalation therapy for asthma and chronic obstructive pulmonary disease (COPD) because of many advantages including small aerosol size, portability, convenience, and relatively low cost.[14] However, their disadvantages are also well recognized including propellant-induced throat irritation (the so-called cold Freon effect), large oropharyngeal deposition caused by high spray velocity, and a difficult administration skill to coordinate inhalation with actuation of the devices.[14] Spacer attachment to the devices, which can take some distance between the aerosol emission point and a patient's mouth, is effective to overcome these disadvantages. Breath-actuated pMDIs that have no need of coordination are currently available on the market. In general, slow deep inhalation is recommended for effective pulmonary drug delivery in pMDIs to minimize aerosol deposition on the oropharynx by inertial impaction.

To the best of our knowledge, there is little information about preclinical and clinical studies with pMDIs for inhaled lung cancer therapy. One of the reasons may be because specialized and complex techniques and systems are necessary to fill the formulations into the canisters.[17]

2.3. DPIs DPIs are the most commonly used inhalation devices in adult patients because of many practical advantages including ease of use, portability, relatively high pulmonary delivery efficiency, and high storage stability of dry powder formulations due to their solid forms.[18] Inhalation automatically actuates powder dispersion in the devices, followed by emission as solid aerosols therefrom *via* inspiratory airflow. Unlike pMDIs, therefore, DPIs do not need propellants and co-

ordination of inhalation with actuation, whereas they demand relatively high inspiratory flow rates in patients (>30 L/min) for effective powder dispersion.[19] Currently, various types of DPIs with different handling are available commercially, which sometimes causes poor patient adherence by incorrect handling. Moreover, these devices have different airflow resistance, generally corresponding to their powder dispersion efficiency.[20] In general, devices with higher airflow resistance tend to have higher powder dispersion efficiency, whereas inspiratory flow rates through the devices achievable in patients become lower. Lower inspiratory flow rate avoids aerosol deposition on the oropharynx caused by inertial impaction, consequently leading to better pulmonary delivery, although devices with extremely high resistance may be uncomfortable in patients.[20] Although preferences for resistance were not consistent in some studies,[20] Levy *et al.*[21] have recently commented that the peak inspiratory flow requirement of 30 L/min in the high-resistance devices does not usually pose any practical limitations for patients with asthma and COPD.

In the conventional development of DPI formulations, bulk drug powder is micronized by milling to attain aerodynamic particle size suitable to pulmonary delivery.[18] However, micronized drug particles have poor flowability and dispersibility due to their high cohesiveness. To improve these particle properties, micronized drug particles are commonly formulated by carrier- and agglomerate-based designs.[22] In carrier-based design, drug particles are physically attached on coarse carrier particles such as sieved lactose, whereas in agglomerate-based designs the drug particles are loosely agglomerated with or without excipient particles to form relatively large particles. After inhalation of these formulations, detachment and deagglomeration of the micronized drug particles in the airstream are necessary for their pulmonary delivery—even though satisfactory efficiency has not yet been achieved in commercial DPI products. In some commercial DPI products, furthermore, the delivered doses to the lungs greatly depend on inspiratory flow rates through the devices in patients,[20] which can cause considerable inter- and intra-patient change of their therapeutic effects. Thus the development of novel dry powder particles for inhalation with high pulmonary delivery efficiency independent of inspiratory flow rates and device performance has been actively progressed with various excipient and powderization techniques, as described below.

Recently, some papers about preclinical studies with DPIs for inhaled lung cancer therapy have been published as mentioned in Section 5.4, although there is little information about their clinical trials, as with pMDIs. DPIs can minimize release of aerosols to the environment, consequently avoiding their exposure to medical workers or neighbors. On the other hand, exposure to industrial workers during the production and loading of highly dispersible dry powders with anticancer drugs remains a matter of concern.

2.4. Novel Inhalation Devices Soft mist inhalers (SMIs) are novel multidose, propellant-free, hand-held, liquid inhalation devices for solution or suspension formulations, in which constant doses of slow-moving aerosols are generated by impact of two liquid jets after pressing the dose-release button. Slow aerosol emission from SMIs enables the user easily to coordinate inhalation with actuation of the devices and to avoid aerosol impact to the oropharynx. Pulmonary delivery efficiency in SMIs has been reported much higher than that achievable with pMDIs and DPIs.[23]

An evaporation/condensation inhaler named Staccato® system is a novel single-use breath-actuated inhalation device that has been adopted in the development of inhaled loxapine (Adasuve®) for a rapid action through pulmonary absorption. A thin drug film in the product is thermally evaporated through inhalation, followed by instant formation of condensed aerosols with 1–3 μm diameter in the airstream.[24]

3. Fate of Drugs after Pulmonary Delivery

Biodistribution of inhaled drugs is greatly affected by the histological characteristics of the respiratory system and the physicochemical characteristics of the drugs. For successful inhaled lung cancer therapy, effective delivery to primary and metastatic cancer cells in the lungs is usually needed, whereas some inhaled drugs can achieve therapeutic effects by delivery to other target cells in the lungs, which may be more easily accessible than cancer cells (Fig. 1). For example, alveolar macrophages are the target cells for inhaled immunostimulators, where immune responses to lung cancer are promoted.[25] In inhaled gene therapy against lung cancer, the target cells for nucleic acids encoding anticancer cytokines or antibodies are both normal and cancer cells in the lungs, where these anticancer proteins are generated and secreted by effective transfection. Furthermore, lymph nodes surrounding the lungs are the target site where inhaled drugs can inhibit metastasis to and from the lungs. On the other hand, it is essential to minimize pulmonary absorption of inhaled drugs so as to limit their potential for causing systemic adverse effects. Hence the development of prodrugs may be practical as inhaled anticancer drugs for their specific delivery/actions to lung cancer with minimized local toxicity caused by oropharyngeal deposition.[26]

3.1. Histological Characteristics of Respiratory System In human lungs, the alveolar epithelium has a thickness of 0.1–0.2 μm, which is much thinner than that of the upper bronchial epithelium (50–60 μm).[7] In addition, the alveolar region has a large surface area (>100 m^2), low enzymatic activity, and extensive vasculature.[7] These histological characteristics in the alveolar region greatly contribute to high drug absorption efficiency after pulmonary delivery. In the intercellular space of the respiratory epithelium, on the other hand, tight junctions are formed to restrict paracellular permeation of hydrophilic and macromolecular drugs. Besides, there are various types of drug transporters in the respiratory epithelium, such as P-glycoprotein (P-gp), multidrug resistance proteins (MRPs), organic cation transporters (OCTs), organic anion transporters (OATs) and peptide transporters (PEPT1/PEPT2), although their concrete relations with drug distribution after pulmonary delivery remain unclear.[27] Some anticancer drugs are widely known as substrates for P-gp and MRPs, and their biodistribution can be affected by these transporters.

The respiratory system has several barrier functions against foreign substances including inhaled drugs. The bronchial epithelium is covered with mucus secreted by goblet cells, which can restrict dissolution and diffusion of drugs. Furthermore, drugs on the bronchial epithelium can be carried to the larynx by mucociliary clearance, followed by their transfer to the gastrointestinal tract by swallowing. Mucociliary clearance is performed at the fastest rate in the central airway and at a slower rate with increasing airway generations.[28] In con-

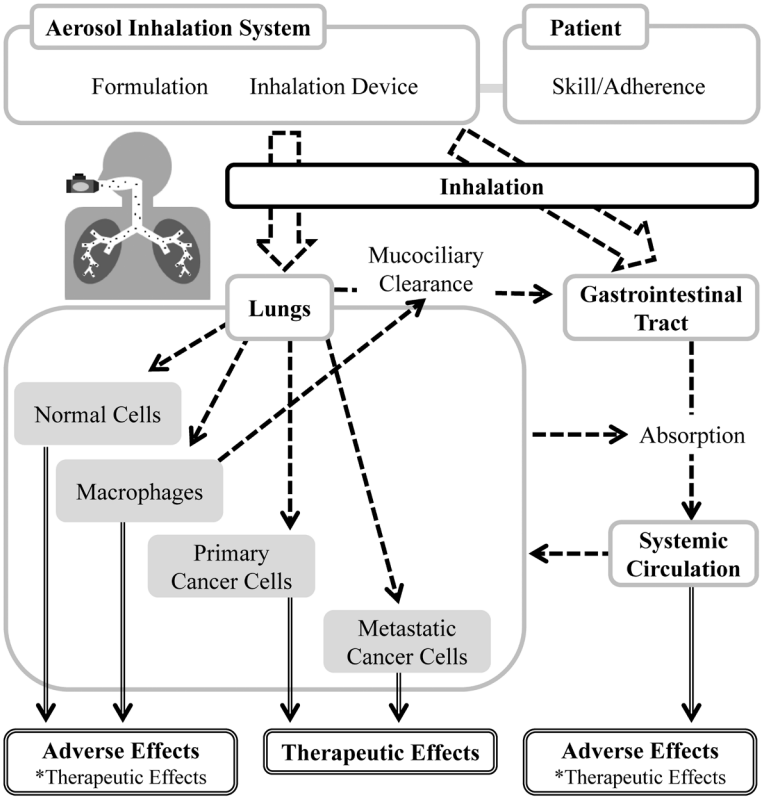

Fig. 1. Pharmacokinetics and Pharmacodynamics of Inhaled Drugs for Lung Cancer Therapy

*Therapeutic effects can be achieved only by induction of anticancer immune responses or expression/secretion of anticancer proteins (*e.g.*, anticancer cytokines).

trast, the alveolar epithelium is covered with lung surfactant secreted by alveolar type II cells, which can promote dissolution and diffusion of drugs.[29] Moreover, drugs on the alveolar epithelium can be taken up by alveolar macrophages through phagocytosis, leading to lysosomal degradation or transfer toward the upper respiratory tract, followed by mucociliary clearance.

3.2. Physicochemical Characteristics of Drugs The physicochemical characteristics of drugs are the most important factors determining their pharmacokinetics. To predict oral bioavailabilities of drugs, for example, in 1997 Lipinski[30] proposed the Rule of Five on the basis of four physicochemical parameters: molecular weight, number of hydrogen-bond donors (NHD), number of hydrogen-bond acceptors (NHA), and octanol-water partition coefficient ($\log P$). In this proposed scheme, drugs with molecular weight >500, NHD >5, NHA >10, or $\log P>5$ will be estimated to have poor absorption or permeation. However, Choy and Prausnitz[31] indicated that even drugs outside the Rule of Five limits can be clinically developed as inhaled formulations.

Hypothetically, pulmonary bioavailability should be higher for hydrophobic than hydrophilic drugs because of effective permeation through passive diffusion. In various drugs with the $\log P$ ranging between -6 and 6, however, their pulmonary bioavailability was not correlated with their hydrophobicity.[32] In contrast, amphotericin B and all-*trans* retinoic acid, which have extremely high hydrophobicity, show prolonged retention in the lungs and insignificant translocation to the systemic circulation after pulmonary delivery,[33,34] which may be due to their strong hydrophobic interaction with epithelial membranes in the lungs.

The molecular weight and size of drugs are also the important factors related with their pulmonary bioavailability. Although higher molecular weight drugs generally have lower pulmonary bioavailability, the absorption rates of macromolecules in the lungs are higher than those in the other tissues including the small intestine. As inhaled macromolecular products, a nebulizer formulation of deoxyribonuclease (Pulmozyme®; 37 kDa) has been clinically used to improve pulmonary function in patients with cystic fibrosis. Furthermore, DPI formulations of insulin (5.8 kDa) with rapid actions *via* pulmonary absorption (Exubera® and Afrezza®) were approved by the U.S. Food and Drug Administration (FDA). Regarding the detailed biodistribution of macromolecules with different molecular weights in the lungs, two studies investigated pulmonary administration of polyethylene glycol (PEG), a clinically approved hydrophilic polymer, and PEG-modified poly-L-lysine dendrimers, biodegradable dendritic polymers, into rats.[35,36] In the former study PEG <2 kDa disappeared from alveolar macrophages and lung tissue within 48 h, whereas PEG >5 kDa remained in these sites for up to 7 d.[35] In the latter study dendrimers <22 kDa showed relatively high pulmonary bioavailability (20–30%) with limited retention and partial degradation in the lungs, whereas 78-kDa dendrimers exhibited extremely low pulmonary bioavailability (approximately 2%) with prolonged retention and limited degradation in the lungs.[36]

Apart from macromolecules, nanoparticles/microparticles have also attracted interest for biodistribution studies through pulmonary delivery from some perspectives such as drug delivery carriers and particulate pollutants related to environmental health hazard. Choi *et al.*[37] comprehensively

investigated the biodistribution of various nanoparticles with different chemical composition, size, and surface charge after pulmonary administration into rats. They observed neutral and anionic nanoparticles with less than approximately 34 nm hydrodynamic diameter quickly translocated from the lungs to mediastinal lymph nodes, irrespective of chemical composition and shape, followed by their transfer to the bloodstream. Kreyling *et al.*[38] studied the biodistribution of iridium nanoparticles (Ir NPs) 20 and 80 nm in diameter after pulmonary administration into rats, and noted both Ir NPs were retained predominantly in the lungs; however, small fractions absorbed into the systemic circulation: 20-nm Ir NP fractions translocated to the liver, spleen, kidneys, heart, brain, and blood were about one order of magnitude higher than those of 80-nm Ir NP. It should be noted that both nanoparticles and microparticles exhibit more prolonged pulmonary retention in humans than other species including rodents.[39]

Regarding electric charge, positively charged drugs can electrostatically interact with negatively charged mucus and epithelial membranes, leading to their delayed mucus penetration and prolonged pulmonary retention. Indeed, Choi *et al.*[37] reported that cationic nanoparticles with approximately 29 nm hydrodynamic diameter were retained in the lungs with limited translocation to mediastinal lymph nodes and bloodstream, unlike neutral and anionic nanoparticles with similar hydrodynamic diameters. Moreover, both positively and negatively charged drugs can be eliminated through phagocytosis by alveolar macrophages.[25,40] Besides, the activity of phagocytosis in alveolar macrophages greatly depends on geometric particle size, being the greatest for particles with 1–3 μm and the least for those with less than 0.2 μm or more than 10 μm diameter.[25]

4. Clinical Trials of Inhaled Lung Cancer Therapy

The first clinical trial for inhaled lung cancer therapy was published in 1968.[41] As mentioned above, nebulizers have been predominantly adopted until now in all the clinical trials for inhaled lung cancer therapy. The drugs evaluated included not only chemotherapeutics but also therapeutic cytokines to induce immune responses to lung cancer, as described below and shown in Table 1. In some of these clinical trials, patients not only with primary cancer (SCLC and NSCLC) but also with metastatic cancer in the lungs were eligible subjects. For inhalation of chemotherapeutic drugs in the trials, specialized barrier systems (*e.g.*, full barrier protection clothing and a high-efficiency particulate air (HEPA)-filtered airborne scavenging tent) were constructed to protect patients and medical workers from unnecessary exposure to cytotoxic aerosols. Most of the clinical trials demonstrated limited systemic translocation and few systemic adverse effects of the inhaled drugs, although it remains unclear whether these drugs can exhibit higher anticancer efficacy against lung cancer than systemically administered drugs. In some trials, the inhaled drugs were additionally administered into patients receiving systemic chemotherapy, which complicated accurate assessment of their intrinsic anticancer efficacy. The major dose-limiting toxicity (DLT) of the inhaled drugs was due to their local actions on the respiratory tract rather than systemic adverse events.

4.1. 5-Fluorouracil 5-Fluorouracil (5-FU) is a chemotherapeutic drug with antimetabolic activity that has been widely used clinically as oral and intravenous formulations for treatment against several types of cancer such as stomach, colon, and breast cancer. The major adverse effects of systemically administered 5-FU include anorexia, diarrhea, nausea, vomiting, stomatitis, and hair loss, whereas the severe unwanted effects include myelosuppression. Tatsumura *et al.*[42] tested inhaled 5-FU with an ultrasonic nebulizer in lung cancer patients. Biodistribution analysis of inhaled 5-FU in lung and blood samples revealed that the concentrations of 5-FU in tumor tissue and lymph nodes surrounding the lungs were higher than those in normal lung tissue, whereas 5-FU was not detected in the blood. There were no notable adverse effects in the trial.

4.2. Cisplatin and Carboplatin Cisplatin (CDDP), a platinum complex, is one of the most commonly used chemotherapeutic drugs. It is intravenously injected into patients with various types of cancer such as bladder, prostate, ovary, stomach, and lung cancer; however, its clinical use is limited due to severe adverse effects including neurotoxicity and nephrotoxicity. Wittgen *et al.*[43] performed a phase I trial of inhaled CDDP as a liposomal formulation with a jet nebulizer. This formulation, containing dipalmitoyl phosphatidylcholine (DPPC) and cholesterol as liposome components, was intended to exhibit sustained release of encapsulated CDDP in the lungs and bypass acute kidney injury caused by rapid accumulation of free CDDP in the kidneys after pulmonary absorption. Inhaled liposomal CDDP was well tolerated and did not show neurotoxicity, nephrotoxicity, and ototoxicity. Major adverse effects were pulmonary injury including pulmonary function loss, dyspnea, and hoarseness, except for fatigue, nausea, and vomiting, although its DLT was not found even at a maximum dose of 60 mg/m^2. Pharmacokinetic data for inhaled liposomal CDDP showed low blood platinum concentrations. Another clinical trial of inhaled liposomal CDDP, which was performed in patients with lung metastases of osteosarcoma, also confirmed low blood platinum concentrations and no CDDP-specific adverse effects.[44] In the trial, the platinum concentration in tumor tissue was not significantly different from that in surrounding lung tissue.

Carboplatin (CBDCA) is a CDDP analog with fewer adverse effects. In a clinical trial of CBDCA reported by Zarogoulidis *et al.*,[45] solo treatment with its inhaled formulation (160–230 mg/d on days 1–3) and combination treatment with its inhaled formulation (160–230 mg on day 1) and intravenous formulation (320–460 mg on day 1) were compared *versus* solo treatment with its intravenous formulation (550–700 mg on day 1). A jet nebulizer was used for pulmonary delivery through inhalation. As for adverse effects of the inhaled formulation, a statistically significant loss of pulmonary function was found in the solo treatment but not in the combination treatment. Interestingly, combination treatment with the inhaled and intravenous formulations achieved prolonged survival with a statistically significant difference from solo treatment with the intravenous formulation (275 *vs.* 211 d), although solo treatment with the inhaled formulation did not (250 d).

4.3. Doxorubicin Doxorubicin (DOX) is an anthracycline antibiotic with a wide spectrum of anticancer activity. Its intravenous formulations have been clinically used against various types of cancer including lung, stomach, liver, colon, and breast cancer, although it can cause severe adverse effects such as cardiotoxicity. A liposomal formulation of DOX for

Table 1. Clinical Trials of Inhaled Lung Cancer Therapy

Drug	Dose per day	Cancer type	Evaluation	Response rate [total no. patients]	Ref.
5-Fluorouracil	250 mg	NSCLC	Biodistribution/Toxicity/Efficacy	CR: 20%, PR: 40%, SD + PD: 40% [10]	42)
Cisplatin (liposomal)	1.5–60 mg/m^2	NSCLC/SCLC	PK/Toxicity/Pulmonary function/Efficacy	SD: 71%, PD: 24% [17]	43)
Cisplatin (liposomal)	24 or 36 mg/m^2	MC (osteosarcoma)	Biodistribution/Toxicity/Pulmonary function/Efficacy	CR: 16%, PR: 5%, SD: 37%, PD: 42% [19]	44)
Carboplatin	160–230 mg	NSCLC	PK/Toxicity/Pulmonary function/Efficacy	CR: 5%, PR: 20%, SD: 25%, PD: 50% [20]	45)
Carboplatin (+ i.v. carboplatin)	160–230 mg	NSCLC	PK/Toxicity/Pulmonary function/Efficacy	CR: 10%, PR: 30%, SD: 15%, PD: 45% [20]	45)
Doxorubicin	0.4–9.4 mg/m^2	NSCLC/MC	PK/Toxicity/Pulmonary function/Efficacy	PR: 2%, SD: 15% [53]	46)
Doxorubicin (+ i.v. cisplatin/ docetaxel)	6.0 or 7.5 mg/m^2	NSCLC	Toxicity/Pulmonary function/Efficacy	CR: 4%, PR: 25%, SD: 54% [24]	47)
Gemcitabine	1–4 mg/kg	NSCLC	PK/Toxicity/Efficacy	PR: 9%, SD: 36%, PD: 36% [11]	48)
9-Nitrocamptothecin (liposomal)	6.7–26.6 μg/kg	PC/MC	PK/Toxicity/Pulmonary function/Efficacy	PR: 8%, SD: 12% [25]	49)
Interferon-α	3 or 18 MU	NSCLC	Toxicity/Pulmonary function/Efficacy	PD: 83% [6]	51)
Interferon-α	18–216 $\times$ 10^6 IU	NSCLC/Breast cancer	PK/Toxicity	—	52)
Interferon-γ	0.1–5.4 mg	NSCLC/SCLC	PK/Toxicity/BALF cell analysis/ Oxygen radical production	—	53)
Interferon-γ	250–1000 μg	— (healthy subjects)	PK/Toxicity/Pulmonary function/ Interferon-γ-induced gene expression	—	54)
Interleukin-2	1–6 $\times$ 10^6 IU	NSCLC/MC (RCC)	PK/Toxicity/Pulmonary function/ BALF and blood cell analysis/Efficacy	CR: 6%, PR: 6%, SD: 38%, PD: 44% [16]	55)
Interleukin-2	1.8–3.6 $\times$ 10^7 IU	MC (melanoma)	Toxicity/Efficacy	CR: 19%, PR: 30%, SD: 19%, PD: 30% [27]	56)
GM-CSF	60–240 μg	MC	Toxicity/Pulmonary function/ Blood cell analysis/Efficacy	CR: 14%, PR: 14%, SD: 43%, PD: 29% [7]	57)
GM-CSF	500–2000 μg	MC (melanoma)	Toxicity/Pulmonary function/ Systemic anticancer immunity/Efficacy	PR: 3%, SD: 13% [40]	58)

i.v.: intravenous, GM-CSF: granulocyte macrophage colony-stimulating factor, NSCLC: non-small cell lung cancer, SCLC: small cell lung cancer, PC: primary cancer in the lungs, MC: metastatic cancer in the lungs, RCC: renal cell cancer, PK: pharmacokinetics, CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease.

intravenous administration (Doxil®) has also been approved for clinical use to achieve prolonged blood circulation and attenuated cardiotoxicity of DOX. A phase I study of inhaled DOX was performed with a jet nebulizer, in which a solution formulation of DOX (non-liposomal) was prepared in 20% ethanol/80% water.[46] The DLT of inhaled DOX includes pulmonary toxicity (respiratory distress and chemotoxic reaction) at a dose of 9.4 mg/m^2, although there was no significant systemic toxicity related to DOX. Pharmacokinetic data for inhaled DOX showed low blood DOX concentrations (a maximum concentration of approximately 40 ng/mL at a dose of 9.4 mg/m^2). In another clinical trial, inhaled DOX was combined with intravenously administered CDDP and docetaxel.[47] The trial clarified that inhaled DOX was safe even combined with systemic chemotherapy, although it did not demonstrate significantly improved response rate.

4.4. Gemcitabine Gemcitabine (GEM) is a water-soluble chemotherapeutic drug belonging to the nucleoside analog family, which has been clinically used as intravenous formulations for treatment against lung, pancreatic, and breast cancer. It has relatively mild toxicity after intravenous administration compared with other chemotherapeutic drugs, but its major adverse effects include myelosuppression and influenza-like symptoms. A clinical trial of inhaled GEM for lung cancer

therapy was performed with a mesh nebulizer.[48] The DLT of inhaled GEM was bronchospasm at a dose of 3 mg/kg; other adverse effects also observed included fatigue, vomiting, dyspnea, and cough. Scintigraphy using a ^{99m}Tc derivative as tracer of GEM aerosols confirmed that approximately 42% of the initial GEM dose in the nebulizer was delivered to the lungs of patients. Pharmacokinetic data for inhaled GEM showed low blood GEM concentrations.

4.5. 9-Nitrocamptothecin 9-Nitrocamptothecin (9-NC) is a topoisomerase I inhibitor and lipophilic camptothecin analog for oral administration. The major adverse effects of orally administered 9-NC are hematologic (*e.g.*, anemia and neutropenia) and gastrointestinal (*e.g.*, nausea, vomiting, and anorexia). In a clinical trial of inhaled 9-NC for lung cancer therapy, a liposomal formulation containing dilauroyl phosphatidylcholine (DLPC) as a liposome component was atomized with a jet nebulizer.[49] Inhaled liposomal 9-NC showed no hematological toxicity, and its DLT was chemical pharyngitis at a dose of 26.6 μg/kg. Pharmacokinetic data of inhaled liposomal 9-NC showed that 9-NC was absorbed from the lungs to reach a maximum blood concentration (76.7 ng/mL at a dose of 13.3 μg/kg [equivalent to 0.5 mg/m^2]) similar to that observed with orally administered 9-NC (111 ng/mL at a dose of 2 mg/m^2). At the end of treatment, on the other hand, the concentration of 9-NC in bronchoalveolar lavage fluid (BALF) was much higher than that in the blood.

4.6. Interferon Interferon (IFN) is a well-known cytokine with antiviral, anticancer, and immunomodulatory functions. So far, various classes of IFN have been identified including IFN-α (19 kDa), IFN-β (23 kDa for β-1a and 19 kDa for β-1b), and IFN-γ (17 kDa), which have been clinically used against chronic hepatitis C, relapsing-remitting multiple sclerosis, and chronic granulomatous disease, respectively.[50] The most common adverse effects of systemically administered IFN are influenza-like symptoms including fever, headache, and malaise, whereas depression, neutropenia, and interstitial pneumonia have also been observed. IFN-α, IFN-β, and IFN-γ have been tested by nebulization in clinical trials against lung cancer.[50] In a clinical trial reported by Kinnula *et al.*,[51] inhaled natural IFN-α was not effective against lung cancer, and its DLT was bronchial hyperreactivity and/or bronchoconstriction. Furthermore, the same group found that inhaled recombinant IFN-α had different pharmacokinetics from inhaled natural recombinant IFN-α and caused milder systemic adverse effects.[52] On the other hand, the same group[53] and Jaffe *et al.*[54] reported that inhaled recombinant IFN-γ showed activation of alveolar macrophages without significant pulmonary absorption and systemic adverse effects in clinical trials.

4.7. Interleukin-2 Interleukine-2 (IL-2; 16 kDa) is an activated lymphocyte-secreted cytokine with various immunostimulatory functions. The major adverse effects of systemically administered IL-2 include influenza-like symptoms, fatigue, and hepatic toxicity. In particular, high-dose IL-2 can cause capillary leak syndrome, a life-threatening respiratory failure. A phase I trial of inhaled IL-2 was performed with a jet nebulizer in patients with NSCLC and lung metastases of renal cell cancer (RCC).[55] The predominant dose-dependent adverse effect of inhaled IL-2 was nonproductive cough, and there were no severe adverse effects including capillary leak syndrome. Pharmacokinetic data for inhaled IL-2 showed low blood IL-2 concentrations. Moreover, inhaled IL-2 dose-dependently increased the number of activated lymphocytes in BALF, although there were no significant responses of lymphocytes in peripheral blood. In another clinical trial of inhaled IL-2, which was performed in patients with lung metastases of melanoma, some therapeutic responses were observed without severe adverse effects, whereas there were no therapeutic responses in extra-lung metastases.[56]

4.8. Granulocyte Macrophage Colony-Stimulating Factor Granulocyte macrophage colony-stimulating factor (GM-CSF; 14–35 kDa, depending on degree of glycosylation), also called colony-stimulating factor 2, is a cytokine that stimulates growth and differentiation of hematopoietic progenitor cells and increases the functional activities of neutrophils, monocytes, macrophages, and dendritic cells. In clinical applications, GM-CSF has been subcutaneously or intravenously injected into patients receiving systemic chemotherapy to promote neutrophil recovery. Besides, GM-CSF is promising as an immunostimulatory adjuvant to induce anticancer immunity. A clinical study of inhaled GM-CSF was performed with a jet nebulizer in patients with lung metastases of leiomyosarcoma, RCC, Ewing's sarcoma, osteosarcoma, and melanoma.[57] In the trial, there were no adverse effects, and pulmonary function changes were minor. In another clinical trial of inhaled GM-CSF in patients with lung metastases of melanoma, significant systemic anticancer immunity was not achieved even at a maximum dose of 2000 μg.[58]

5. Inhaled Formulations with Drug Delivery Functions for Lung Cancer Therapy

As mentioned above, most clinical trials of inhaled lung cancer therapy to date have demonstrated attenuated systemic adverse effects but not remarkable therapeutic success. One reason for these disappointing results may be insufficient delivery of drugs to target sites after inhalation. Further to improve efficiency, therefore, novel inhaled drugs (or formulations) with drug delivery functions including sustained release, prolonged retention, and targeting in the lungs have been actively developed in preclinical studies so far (Table 2).

To add these drug delivery functions to inhaled drugs, there are two major technical strategies: chemical conjugation with polymers and encapsulation into nanoparticles/microparticles.[59,60] Chemical conjugation with polymers has been adapted in some commercial products for systemic administration including PEG-conjugated proteins, although application of this technique is limited due to some disadvantages including necessity of having active functional groups in the drugs to conjugate with polymers and lowered activity of the drugs by conjugation. Encapsulation into nanoparticles/microparticles can be applied to a wider range of drugs, whereas controlled release of encapsulated drugs (*i.e.*, stable encapsulation) must be achieved in the body to exhibit drug delivery functions of nanoparticles/microparticles. In the systemic circulation, many biocomponents such as blood cells, serum proteins, and lipids can bind to nanoparticles/microparticles through electrostatic and hydrophobic interaction, causing rapid release of encapsulated drugs after intravenous administration. In contrast, there are relatively fewer biocomponents in the lungs, where inhaled nanoparticles/microparticles may escape from these interactions more reliably to exert their drug delivery functions including controlled release. Indeed, Garbuzenko *et al.*[61] demonstrated that the pulmonary pharmacokinetics of inhaled

Table 2. Inhaled Formulations with Drug Delivery Functions for Lung Cancer Therapy

Drug	Delivery system	Drug loading method to delivery system	Targeting moiety	Dosage form	Ref.
Paclitaxel	Poly-L-glutamic acid	Covalent binding	—	SS	69)
Paclitaxel	PEG	Covalent binding	—	SS	70,71)
Cisplatin	Hyaluronic acid	Metal complex formation	—	SS	72,73)
Doxorubicin	PEG-PLL	Covalent binding	—	SS	74)
Doxorubicin	Liposomes	Encapsulation	—	SS	77)
9-Nitrocamptothecin	Liposomes	Encapsulation	—	SS	78)
Paclitaxel	Liposomes	Encapsulation	—	SS	79)
Paclitaxel	Solid lipid nanoparticles	Encapsulation	—	SS	84)
Paclitaxel	PEG-lipid micelles	Encapsulation	—	SS	85)
Paclitaxel	PLGA microparticles	Encapsulation	—	SS	86)
p53 pDNA	PLL/Protamine	Electrostatic complex formation	—	SS	96)
Anti-WT1 siRNA	Polyethylene imine	Electrostatic complex formation	—	SS	97)
FKN pDNA	704	Electrostatic complex formation	—	SS	98)
AT2R pDNA	Modified TAT peptide	Electrostatic complex formation	—	SS	99)
CpG oligonucleotide	Polyketal microparticles	Encapsulation	—	SS	100)
Doxorubicin	Liposomes	Encapsulation	Transferrin	SS	102)
Cisplatin	Gelatin nanoparticles	Metal complex formation	EGF	SS	104)
Doxorubicil/Paclitaxel/ Anti-MRP1 siRNA/ Anti-BCL2 siRNA	Lipid nanoparticles	Encapsulation/Electrostatic complex formation	LHRH	SS	105)
Anti-VEGF siRNA	Gold nanoparticles	Covalent binding	M2pep	SS	106)
Anti-EGFR mAb	Antibody	—	Antibody	SS	107)
Anti-VEGF mAb	Antibody	—	Antibody	SS	108)
Cisplatin	Solid lipid microparticles	Encapsulation	—	DP	121,122)
Paclitaxel	Solid lipid microparticles	Encapsulation	—	DP	123,124)
Doxoribicin	PLGA microparticles	Encapsulation	—	DP	125)
Doxorubicil/Paclitaxel	PLGA microparticles	Encapsulation	—	DP	126)
Gemcitabine	Liposomes	Encapsulation	—	DP	127)
Paclitaxel	PEG-HMD micelles	Encapsulation	Folate	DP	103)
Doxorubicin	Polybutyl cyanoacrylate nanoparticles	Encapsulation	—	DP	128)
Interferon-β pDNA	Chitosan	Electrostatic complex formation	—	DP	132)
Anti-VEGF siRNA	Chitosan	Electrostatic complex formation	—	DP	136)

p53: tumor suppressor, pDNA: plasmid DNA, WT1: Wilms' tumor gene 1 (tumor antigen), siRNA: small interfering RNA, FKN: fractalkine, AT2R: angiotensin II type 2 receptor, CpG: unmethylated cytosine-guanine motif, MRP1: multidrug resistance-associated protein 1 (multidrug efflux pump), BCL2: b-cell lymphoma 2 (apoptosis suppressor), VEGF: vascular endothelial growth factor, EGFR: epidermal growth factor receptor, mAb: monoclonal antibody, PEG: polyethylene glycol, PLL: poly-L-lysine, PLGA: poly-lactic-*co*-glycolic acid, 704: tetrafunctional amphiphilic block copolymer, TAT: transactivator of transcription, HMD: hydrophobically modified dextran, EGF: epidermal growth factor, LHRH: luteinizing hormone-releasing hormone, M2pep: tumor associated macrophages-targeting peptide, SS: solution or suspension, DP: dry powder.

polymers or nanoparticles is considerably different to intravenously administered equivalents. Interestingly, the authors also clarified that lipid-based nanocarriers show higher and more prolonged pulmonary accumulation after inhalation than non-lipid-based vehicles. Moreover, surface modifications of nanoparticles/microparticles with PEG, cell penetrating peptides, and targeting moieties (*e.g.*, ligand and antibodies) can provide additional drug delivery functions for further prolonged pulmonary retention, intracellular uptake, and active targeting, respectively.

As a strategy for cancer targeting, chemical conjugation with polymers to reach more than 40 kDa in total and encapsulation into nanoparticles with 10–500 nm diameter allow effective drug delivery to tumor tissue after systemic administration by passive targeting based on the enhanced permeability and retention (EPR) effect.[62,63] Furthermore, various markers including EGFR and vascular endothelial growth factor (VEGF) have been found in lung cancer, and targeting moieties for these markers have been applied to the modification of drugs for active targeting in lung cancer therapy.[64] Recently, M2-like tumor-associated macrophages (TAMs), which populate the tumor microenvironment and promote tumor angiogenesis, survival, and growth through immunosuppressive effects on the adaptive immune responses in early tumorigenesis, have attracted much attention as an alternative target site for cancer therapy.[65] However, the delivery efficiency of inhaled drugs to cancer cells and other targets in the lungs by passive and active targeting remains to be fully validated.

5.1. Chemical Conjugation with Polymers For small molecule drugs, chemical conjugation with polymers is one of the simplest techniques to obtain successfully prolonged retention ability in the administration site or systemic circulation. PEG and dextran are most commonly used as polymers conjugated to attain these drug delivery functions, because they have high hydrophilicity and good tolerability. These polymers can also provide further functions including improved water solubility for hydrophobic drugs and attenuated immunogenicity/enzymatic degradation for biomacromolecular drugs. As with the functions mentioned above, however, the therapeutic activity of drugs conjugated with polymers is affected by various conjugation conditions including conjugation sites in the chemical structure of drugs, conjugation modes between drugs and polymers (*e.g.*, direct ester or amide linkages and spacer application), molar ratios of drugs to polymers, and

total molecular weight. For example, the potencies of many macromolecular drugs are slightly or greatly lost by conjugation with PEG.[66] As for polymer–drug conjugates *via* ester linkages, the drugs may be rapidly released from the conjugates through enzymatic hydrolysis in the blood circulation after systemic administration,[67] whereas the conjugates may be relatively stable in the lungs after inhalation because of less potential for enzymatic degradation. For the application of polymer–drug conjugates to cancer therapy, pH-sensitive hydrazone linkages and cathepsin-sensitive peptide spacers may be effective to achieve selective drug release from conjugates in the tumor microenvironment.[68]

As a polymer–drug conjugate for lung cancer therapy, Zou *et al.*[69] investigated the usefulness of paclitaxel (PTX, a lipophilic chemotherapeutic drug) conjugated with 42-kDa poly-L-glutamic acid (PGA) through a direct ester linkage. The PGA–PTX conjugate (Opaxio™) improved water solubility of PTX without any solubilizers and reached phase III clinical trials for ovarian cancer therapy. After pulmonary administration into mice at a therapeutically equivalent dose, the PGA–PTX conjugate showed lower toxicity than a commercial PTX formulation (Taxol®) with ethanol and Cremophor EL as solubilizers. Moreover, Luo *et al.*[70] developed innovative PEG–PTX conjugates with 6- and 20-kDa linear PEG by click chemistry with azide linker triazole rings and ester linkages, which had the sustained release ability of PTX in BALF and serum through enzymatic hydrolysis of ester linkages. Pulmonary administration of these PEG–PTX conjugates showed higher maximum tolerated doses in normal mice and higher anticancer efficacy in lung metastasis-bearing mice than both intravenous and pulmonary administration of Taxol®.[71] In particular, the conjugate with 20-kDa PEG had less toxicity and equivalent anticancer efficacy at a lower dose as PTX than that with 6-kDa PEG (20 and 50 mg/kg as PTX for the conjugates with 20- and 6-kDa PEG, respectively), which might be attributed to the larger molecular weight of PEG. In other reports, CDDP was conjugated with 35-kDa hyaluronic acid (HA, a natural polysaccharide) through metal complex formation.[72,73] In a biodistribution study in rats, pulmonary administration of the HA–CDDP conjugate (HylaPlat™) demonstrated higher platinum accumulation in lymph nodes surrounding the lungs as well as the whole lungs, limiting platinum translocation to other tissues, than its intravenous administration or intravenous and pulmonary administration of free CDDP.[72] Furthermore, the HA–CDDP conjugate showed higher anticancer efficacy after pulmonary administration into lung metastasis-bearing mice at the same dose as free CDDP.[73] In addition, a novel 56-kDa conjugate of DOX with a biodegradable PEGylated poly-L-lysine dendrimer was synthesized with a 4-(hydrazinosulfonyl) benzoic acid linker.[74] Pulmonary administration of the dendrimer–DOX conjugate showed prolonged retention and sustained absorption of DOX in the lungs of normal rats, leading to higher anticancer efficacy in lung metastasis-bearing rats than its intravenous administration as well as intravenous administration of free DOX.

5.2. Encapsulation into Nanoparticles/Microparticles Lipids and biodegradable/biocompatible polymers are the major components of clinically used nanoparticles/microparticles for drug encapsulation because of their low toxicity. Lipid-based nanoparticles/microparticles include liposomes, lipid nanospheres/microspheres, and solid lipid nanoparticles/microparticles; polymeric micelles and polymeric nanospheres/microspheres are also widely used. In the aim of encapsulation, all nanoparticles/microparticles can be applied to hydrophobic drugs through hydrophobic interaction, whereas only liposomes or polymeric nanospheres/microspheres prepared by a water-in-oil-in-water (w/o/w) emulsion method can encapsulate hydrophilic drugs. Nanoparticles/microparticles and encapsulated drugs have been elaborately designed under various techniques to achieve controlled drug release (or stable drug encapsulation) in the body. Some of the techniques are based on remote loading (for liposomes), promoted hydrophobic interaction (for lipid nanospheres/microspheres), limited inner diffusion (for solid lipid nanoparticles/microparticles), induced structural interaction (for polymeric micelles), and limited polymer degradation (for polymeric nanospheres/microspheres).

Liposomes are the most advanced nanoparticles for clinical application as inhaled formulations. Besides inhaled chemotherapeutic drugs mentioned in Section 4 (CDDP and 9-NC), clinical trials of inhaled antibiotics are progressing by nebulization of liposomal formulations (Arikace® for amikacin and Lipoquin® or Pulmaquin® for ciprofloxacin).[75,76] Among various lipids used as liposome components, DPPC is considered one of the most suitable drug delivery materials for inhalation therapy, because it is a major element (accounting for 40% [w/w]) of lung surfactant and exerts excellent safety. In many preclinical studies in small animals, pulmonary delivery of various liposomal chemotherapeutic drugs including DOX, 9-NC, and PTX demonstrated promising outcomes for lung cancer therapy including prolonged pulmonary retention with limited systemic translocation, attenuated systemic adverse effects, and potent anticancer efficacy.[77–79] However, the physical stability of liposomes during nebulization is a matter of concern in their application to inhalation therapy. High shear forces exerted by nebulizers for aerosol generation from liposomal suspensions can destabilize the structure of liposomes, leading to losses of their drug delivery functions. Some reports demonstrated that jet and mesh nebulizers are quite acceptable for delivering liposomes, whereas ultrasonic nebulizers are the least suitable.[80,81] Moreover, the stability of liposomes during nebulization may be improved by various techniques including the adoption of large mesh apertures for mesh nebulizers and the addition of cholesterol or phospholipids with high phase transition temperatures as liposome components.[82,83] Besides liposomes, solid lipid nanoparticles, PEG-lipid micelles, and poly-lactic-*co*-glycolic acid (PLGA) microspheres have been applied for encapsulation of PTX, demonstrating prolonged pulmonary retention and high anticancer efficacy in mice after pulmonary administration.[84–86] However, there is little information of their superiority over liposomes in drug delivery functions and stability during nebulization.

For realizing gene therapy, various cationic nanocarriers (*e.g.*, cationic polymers and liposomes), which electrostatically interact with negatively charged therapeutic nucleic acids to form nanocomplexes, have been developed to attain various functions including nuclease resistance, enhanced cellular uptake *via* endocytosis, and effective intracellular translocation *via* endosomal escape.[87] As an alternative strategy for successful gene therapy, development as inhaled formulations is promising because of some advantages including direct

and noninvasive delivery to the lungs (target tissue) and low nuclease activity in the lungs. In preclinical studies in small animals, interestingly, pulmonary delivery of naked nucleic acids (*e.g.*, plasmid DNAs (pDNAs) and small interfering RNAs (siRNAs)) achieved effective gene transfection and therapeutic actions in the lungs even without any cationic nanocarriers.[88–92] As with liposomes shown above, however, naked nucleic acids can be destabilized during nebulization. Furthermore, naked oligonucleotides (ODNs) with relatively small molecular weight including siRNAs may be transferred to the systemic circulation after pulmonary administration.[93–95] Thus cationic nanocarriers remain useful in the development of inhaled formulations for gene therapy, because nanocomplex formation protects nucleic acids from various stresses induced during nebulization or the production process of the formulations and prolongs their pulmonary retention with limited transfer to the systemic circulation after inhalation. So far, various therapeutic nucleic acids such as a pDNA encoding p53 (a tumor suppressor) and a siRNA specific to WT1 (a tumor antigen) have been applied with cationic nanocarriers including polyethylene imine (PEI, a cationic polymer) to form nanocomplexes for lung cancer therapy, showing effective gene transfection and anticancer actions in orthotopic lung tumor- and lung metastasis-bearing mice after pulmonary administration.[96–99] For immunotherapy against lung cancer, Sato *et al.*[100] recently developed novel pH-sensitive biodegradable polyketal microparticles loaded with a CpG ODN. After pulmonary administration into normal and orthotopic lung tumor-bearing mice, the CpG ODN-loaded microparticles showed higher accumulation and more prolonged retention in tumor nests (primarily macrophages and dendritic cells within the tumors), higher activation of immune cells in the lungs, and higher anticancer efficacy than naked CpG ODN.

5.3. Modification with Targeting Moieties Active drug targeting to marker-expressing cells in the lungs might not be sufficiently achieved by systemic administration, because these cells are usually present even in other organs. In contrast, inhalation can directly deliver drugs to marker-expressing cells in the lungs and minimize their transfer to other organs. Thus far, mannose- and transferrin-modified liposomes, folate-modified polymeric micelles, and EGF-modified gelatin nanoparticles have been developed for active drug targeting to alveolar macrophages and lung cancer cells after pulmonary administration, and demonstrated more effective drug delivery to the target cells and higher efficacy than their non-modified counterparts as well as good tolerability in small animals.[101–104] Besides, Taratula *et al.*[105] constructed multifunctional lipid nanoparticles modified with PEG and a luteinizing hormone-releasing hormone (LHRH) analog (a targeting moiety for lung cancer) for codelivery of anticancer drugs (DOX and PTX) and siRNAs specific to MRP1 (a multidrug efflux pump) and BCL2 (an apoptosis suppressor). After pulmonary administration into orthotopic lung tumor-bearing mice, the nanoparticles predominantly accumulated in tumor tissue, avoiding normal lung tissue, and showed higher anticancer efficacy than comparator nanoparticles without any siRNAs. For immunotherapy against lung cancer, Conde *et al.*[106] developed novel gold nanoparticles (AuNPs) functionalized with a TAM-targeting peptide (M2pep) and a siRNA specific to VEGF. After pulmonary administration into orthotopic lung tumor-bearing mice, the bi-functionalized AuNPs were internalized into TAMs and lung cancer cells to inhibit VEGF expression in the lungs and reduced recruitment of TAMs. Interestingly, the bi-functionalized AuNPs showed higher anticancer efficacy than those without M2pep, which were internalized into lung cancer cells but not TAMs to inhibit VEGF expression in the lungs without reducing recruitment of TAMs.

Inhaled antibodies are considered to have two drug delivery functions: one is active targeting to antigens (target proteins) in the lungs and the other is prolonged pulmonary retention attained from their large molecular weight. In two preclinical studies in mice with intrabronchially implanted cancer cells and with K-ras-induced lung adenocarcinoma, aerosolized anticancer full-length monoclonal antibodies showed therapeutic actions with limited absorption to the systemic circulation.[107,108] In addition to full-length antibodies, various engineered antibody fragments including antigen-binding fragments (Fabs), single-chain variable fragments (scFvs), and single-domain antibodies (sdAbs or Nanobodies®) are also promising because of some attractive properties such as enhanced tissue and tumor penetration, binding to cryptic epitopes, and avoidance of crystallizable fragment (Fc) receptor-dependent toxicity.[109,110]

5.4. Development as DPI Formulations For pulmonary administration of inhaled drugs with drug delivery functions in preclinical and clinical studies, solution or suspension formulations have been predominantly used with nebulizers and other aerosolization apparatuses. However, the low storage stability of these formulations due to their liquid forms, which can cause degradation of drugs and irreversible aggregation of nanoparticles/microparticles, is a matter of concern in clinical application. Among alternative aerosol inhalation systems, DPI formulations are promising because expected to show higher storage stability due to their solid forms. In particular, innovative particle design of dry powders based on excipient and powderization techniques has attracted much attention, enabling the production of powders with high aerosol performance for effective pulmonary delivery through inhalation. Such powderization techniques include freeze drying (FD), spray drying (SD), spray freeze drying (SFD), and supercritical carbon dioxide ($scCO_2$) precipitation.[111]

Highly porous particle structure of dry powders for inhalation may be advantageous because its low particle density enables achievement of aerodynamic particle size suitable for inhalation therapy (1–5 μm, as mentioned in Section 2) with larger geometric particle size, successfully improving the flowability and dispersibility of the powders.[18] In recent commercial DPI products, a tobramycin powder (TOBI® Podhaler™) and an insulin powder (Afrezza®) have highly porous structure, as constructed by PulmoSphere™ and Technosphere™ technologies, respectively.[112,113] PulmoSphere™ technology is based on SD of component solutions with distearoyl phosphatidylcholine (DSPC, a hydrophobic shell-forming reagent) and perfluorooctyl bromide (a pore-forming reagent), whereas Technosphere™ technology is based on FD of component solutions with fumaryl diketopiperazine (FDKP, an excipient). In contrast, SFD can produce highly porous dry powders from component solutions, regardless of types of drugs and excipients.[114]

For successful development of macromolecule- and nanoparticle-embedded DPI formulations, effective reconstitution of macromolecules and nanoparticles after dissolution of

the powders is essential to maintain their original physicochemical and biological (or functional) properties. However, macromolecule/nanoparticle reconstitution can be disturbed by various thermal and physical stresses induced during each production process of the powders including agitation, atomization, heating, freezing, and drying. Thus optimization of functional excipients and powderizaion conditions is necessary to obtain powders with high macromolecule/nanoparticle reconstitution efficiency. For example, some sugars (*e.g.*, sucrose (Suc), lactose (Lac), and trehalose (Tre)), polyols (*e.g.*, mannitol (Man)), and amino acids are useful as excipients to stabilize the structure of macromolecules and nanoparticles in powders, possibly through water replacement and amorphous matrix creation (or vitrification).[115] However, amorphousization in powders generally lowers their physical stability, which can promote moisture absorption and reduce aerosol performance. In contrast, the addition of leucine (Leu, a hydrophobic amino acid) as a component of the powders has been demonstrated greatly to improve their aerosol performance and anti-hygroscopicity.[116–118] In two reports about comparison between nanoparticle-embedded SD and SFD powders with Man as an excipient, D'Addio *et al.*[119] found that SFD powder showed higher nanoparticle reconstitution efficiency than SD powder, whereas Yu *et al.*[120] demonstrated an opposite trend in the case of other nanoparticles. For clinical application of macromolecule- and nanoparticle-embedded DPI formulations, furthermore, their high-dose administration as powders may be demanded, because large amounts of excipients are necessary to stabilize the structure of macromolecules and nanoparticles, consequently leading to low occupation ratio of the main drugs (*i.e.*, drugs conjugated with polymers or encapsulated into nanoparticles) in the powders.

As DPI formulations for lung cancer therapy, Levet *et al.*[121,122] developed dry CDDP powders with sustained release ability by SD of CDDP microcrystalline suspensions. The addition of tristearin and PEG derivatives as excipients greatly improved the aerosol performance of the powders.[121] In particular, powder with PEG2000-distearoyl phosphoethanolamine (DSPE) showed the most prolonged retention and sustained absorption of CDDP in the lungs after pulmonary administration into mice.[122] Furthermore, Meenach *et al.*[123,124] successfully produced dry PTX powders with sustained release ability and high aerosol performance by SD of component solutions with several lipids and PEG-lipids as excipients. Moreover, Kim *et al.*[125] and Feng *et al.*[126] prepared highly porous PLGA microspheres loaded with DOX and PTX by a w/o/w emulsion method with ammonium bicarbonate as a pore-forming reagent, followed by FD for their powderization. The dried DOX-loaded microspheres showed prolonged retention of DOX in the lungs over 14 d after pulmonary administration into mice.[125] In addition, the dried DOX-PTX-coloaded microspheres exhibited synergistically higher anticancer efficacy in lung metastasis-bearing mice after pulmonary administration compared with the dried DOX-loaded or PTX-loaded microspheres.[126]

As for nanoparticle-embedded DPI formulations for lung cancer therapy, Gandhi *et al.*[127] successfully produced GEM-loaded liposomal dry powders with high aerosol performance by FD of liposomal suspensions with Suc, Lac, and Tre as cryoprotectants and Leu as a dispersion enhancer. The reconstituted liposomes from the powders had almost the same particles size as the original particles (approximately 300 nm) and high entrapment efficiency of GEM (>90%). After pulmonary administration into rats, the liposomal powders prolonged retention of GEM in BALF and lung tissue with attenuated pulmonary toxicity. Furthermore, Rosière *et al.*[103] developed inhaled dry powders embedded with PTX-loaded folate-PEG-hydrophobically modified dextran micelles by SD of their suspensions with Man and Leu as an excipient and a dispersion enhancer, respectively. The polymeric micellar powders showed high aerosol performance, although their reconstituted polymeric micelles had larger particle size than the originals. Moreover, Roa *et al.*[128] produced inhaled dry powders embedded with DOX-loaded polybutyl cyanoacrylate nanoparticles by SFD of their suspensions with Lac as an excipient. Citric acid, sodium carbonate, and ammonium hydroxide were further added in the suspension to produce effervescent nanoparticle-embedded powder. Interestingly, the effervescent nanoparticle-embedded powder had higher nanoparticle reconstitution efficiency and exhibited higher anticancer efficacy in lung metastasis-bearing mice after pulmonary administration than the non-effervescent powder. The authors speculated that the higher anticancer efficacy of the effervescent nanoparticle-embedded powder might be because it actively released nanoparticles in the lungs, leading to their prevented aggregation and enhanced dispersion. For inhaled gene therapy, our group developed various dry powders embedded with nanocomplexes formed between nucleic acids (pDNAs and siRNAs) and cationic polymers (PEI, chitosan, and biodegradable polycations) by $scCO_2$ precipitation or SFD of their suspensions with Man as an excipient.[129–136] The nanocomplex-embedded powders had high nanocomplex reconstitution efficiency with maintained integrity of nucleic acids. Furthermore, they exhibited higher gene transfection efficiency in both normal and tumor tissue within the lungs after pulmonary administration into normal and lung metastasis-bearing mice than the original nanocomplex suspensions.[129,130,132–135] The reason may be due to local, highly concentrated exposure to the nanocomplexes reconstituted after direct attachment of the powders on the respiratory epithelium. Moreover, the anticancer nanocomplex-embedded powders with a pDNA encoding IFN-β and a siRNA specific to VEGF showed potent anticancer efficacy in lung metastasis-bearing mice after pulmonary administration.[132,136]

6. Conclusion and Perspectives

Over the last five decades, preclinical and clinical studies for inhaled lung cancer therapy with chemotherapeutic drugs have been performed, demonstrating their limited pulmonary absorption and relatively mild systemic toxicity. To the best of our knowledge, however, there are still no inhaled chemotherapeutic drugs that have progressed to phase III clinical trials due to their insignificant anticancer efficacy even at the maximum doses (predominantly limited by pulmonary toxicity). Thus both higher anticancer efficacy and lower pulmonary toxicity are strongly desired in inhaled formulations for lung cancer therapy, which may be achieved by adding drug delivery functions including sustained release, prolonged retention, and targeting in the lungs. On the other hand, recent preclinical and clinical achievements with novel anticancer drugs including molecular-targeted drugs, immune modulators, and gene medicines strongly indicate that these may be successful-

ly applied as inhaled lung cancer therapies in future. However, the addition of drug delivery functions should be essential even in these new applications. Moreover, combinations of various anticancer drugs by codelivery through inhalation or by separate delivery through inhalation and systemic administration may achieve comprehensive, additive, and synergistic anticancer actions against lung cancer.

On the basis of drug delivery strategies including conjugation with polymers, encapsulation into nanoparticles/microparticles, and modification with targeting moieties, the development of innovative inhaled drugs or formulations has been actively progressed in recent preclinical studies to achieve superiority in pharmacokinetics and anticancer efficacy. From the viewpoint of widely acceptable final products, furthermore, the interest of researchers is steadily shifting from nebulizer systems to DPI systems, although information about the development of DPI formulations for lung cancer therapy has been extremely limited so far. On the other hand, it can be speculated that inspiratory flow rates through DPI devices achievable in lung cancer patients are generally lower than those in healthy persons, because patients may have reduced pulmonary function *via* not only cancer progression but also treatment including lung resection surgery, thoracic radiotherapy, and chemotherapy. Therefore the development of formulations and devices for DPI systems that can guarantee high pulmonary drug delivery efficiency even at low inspiratory flow rates in lung cancer patients is crucial for the clinical application of inhaled lung cancer therapy.

In summary, it is no doubt that inhaled lung cancer therapy will be certainly progressed in the future by comprehensively integrating innovative drugs and technologies, although there are still many issues to be discussed, including the validity of small animal models for human primary and metastatic cancer in the lungs, pharmacokinetic/pharmacodynamic relation of inhaled drugs, delivery efficiency of inhaled drugs to primary and metastatic cancer cells in the lungs, acceptability of inhalation devices in lung cancer patients, and so on. For successful introduction of inhaled lung cancer therapy in clinic, not only demonstrating its superiority over conventional systemic therapy in clinical trials is extremely important, but also its cost performance and patient satisfaction/preference should be closely discussed: the importance of these viewpoints can be understood from the failure of inhaled insulin products (Exubera® and Afrezza®) in the market.[137]

Conflict of Interest The authors declare no conflict of interest.

References

1) Bray F., Ferlay J., Soerjomataram I., Siegel R. L., Torre L. A., Jemal A., *CA Cancer J. Clin.*, **68**, 394–424 (2018).
2) Herbst R. S., Morgensztern D., Boshoff C., *Nature* (London), **553**, 446–454 (2018).
3) Hirsch F. R., Scagliotti G. V., Mulshine J. L., Kwon R., Curran W. J. Jr., Wu Y. L., Paz-Ares L., *Lancet*, **389**, 299–311 (2017).
4) Silvestri G. A., Rivera M. P., *Chest*, **128**, 3975–3984 (2005).
5) Hansel T. T., Kropshofer H., Singer T., Mitchell J. A., George A. J., *Nat. Rev. Drug Discov.*, **9**, 325–338 (2010).
6) Scagliotti G. V., Novello S., Selvaggi G., *Ann. Oncol.*, **10** (Suppl. 5), S83–S86 (1999).
7) Patton J. S., Byron P. R., *Nat. Rev. Drug Discov.*, **6**, 67–74 (2007).
8) Fernandes C. A., Vanbever R., *Expert Opin. Drug Deliv.*, **6**, 1231–1245 (2009).
9) Dolovich M. B., Dhand R., *Lancet*, **377**, 1032–1045 (2011).
10) Carvalho T. C., Peters J. I., Williams R. O. III, *Int. J. Pharm.*, **406**, 1–10 (2011).
11) Anderson P. J., *Respir. Care*, **50**, 1139–1150 (2005).
12) O'Callaghan C., Barry P. W., *Thorax*, **52** (Suppl. 2), S31–S44 (1997).
13) Ari A., *Eurasian J. Pulmonol.*, **16**, 1–7 (2014).
14) Newman S. P., *Respir. Care*, **50**, 1177–1190 (2005).
15) Stein S. W., Sheth P., Hodson P. D., Myrdal P. B., *AAPS PharmSciTech*, **15**, 326–338 (2014).
16) Myrdal P. B., Sheth P., Stein S. W., *AAPS PharmSciTech*, **15**, 434–455 (2014).
17) Vallorz E., Sheth P., Myrdal P., *AAPS PharmSciTech*, **20**, 177 (2019).
18) Telko M. J., Hickey A. J., *Respir. Care*, **50**, 1209–1227 (2005).
19) Haidl P., Heindl S., Siemon K., Bernacka M., Cloes R. M., *Respir. Med.*, **118**, 65–75 (2016).
20) Frijlink H. W., De Boer A. H., *Expert Opin. Drug Deliv.*, **1**, 67–86 (2004).
21) Levy M. L., Carroll W., Izquierdo Alonso J. L., Keller C., Lavorini F., Lehtimäki L., *Adv. Ther.*, **36**, 2547–2557 (2019).
22) Islam N., Cleary M. J., *Med. Eng. Phys.*, **34**, 409–427 (2012).
23) Dalby R. N., Eicher J., Zierenberg B., *Med. Devices*, **4**, 145–155 (2011).
24) Dinh K. V., Myers D. J., Noymer P. D., Cassella J. V., *J. Aerosol Med. Pulm. Drug Deliv.*, **23**, 253–260 (2010).
25) Lee W. H., Loo C. Y., Traini D., Young P. M., *Expert Opin. Drug Deliv.*, **12**, 1009–1026 (2015).
26) Baptist A. P., Reddy R. C., *J. Clin. Pharm. Ther.*, **34**, 1–12 (2009).
27) Gumbleton M., Al-Jayyoussi G., Crandon-Lewis A., Francombe D., Kreitmeyr K., Morris C. J., Smith M. W., *Adv. Drug Deliv. Rev.*, **63**, 110–118 (2011).
28) Kreyling W., Scheuch G., "Particle-Lung Interactions," ed. by Gehr P., Heyder J., Marcel Dekker, New York, 2000, pp. 323–376.
29) Hidalgo A., Cruz A., Pérez-Gil J., *Eur. J. Pharm. Biopharm.*, **95** (Pt A), 117–127 (2015).
30) Lipinski C. A., Lombardo F., Dominy B. W., Feeney P. J., *Adv. Drug Deliv. Rev.*, **23**, 3–25 (1997).
31) Choy Y. B., Prausnitz M. R., *Pharm. Res.*, **28**, 943–948 (2011).
32) Patton J. S., Fishburn C. S., Weers J. G., *Proc. Am. Thorac. Soc.*, **1**, 338–344 (2004).
33) Beyer J., Schwartz S., Barzen G., Risse G., Dullenkopf K., Weyer C., Siegert W., *Infection*, **22**, 143–148 (1994).
34) Brooks A. D., Tong W., Benedetti F., Kaneda Y., Miller V., Warrell R. P. Jr., *Cancer Chemother. Pharmacol.*, **46**, 313–318 (2000).
35) Gursahani H., Riggs-Sauthier J., Pfeiffer J., Lechuga-Ballesteros D., Fishburn C. S., *J. Pharm. Sci.*, **98**, 2847–2856 (2009).
36) Ryan G. M., Kaminskas L. M., Kelly B. D., Owen D. J., McIntosh M. P., Porter C. J., *Mol. Pharm.*, **10**, 2986–2995 (2013).
37) Choi H. S., Ashitate Y., Lee J. H., Kim S. H., Matsui A., Insin N., Bawendi M. G., Semmler-Behnke M., Frangioni J. V., Tsuda A., *Nat. Biotechnol.*, **28**, 1300–1303 (2010).
38) Kreyling W. G., Semmler-Behnke M., Seitz J., Scymczak W., Wenk A., Mayer P., Takenaka S., Oberdörster G., *Inhal. Toxicol.*, **21** (Suppl. 1), 55–60 (2009).
39) Geiser M., Kreyling W. G., *Part. Fibre Toxicol.*, **7**, 2 (2010).
40) Pei Y., Yeo Y., *J. Control. Release*, **240**, 202–211 (2016).
41) Shevchenko I. T., Resnik G. E., *Neoplasma*, **15**, 419–426 (1968).
42) Tatsumura T., Koyama S., Tsujimoto M., Kitagawa M., Kagamimori S., *Br. J. Cancer*, **68**, 1146–1149 (1993).
43) Wittgen B. P., Kunst P. W., van der Born K., van Wijk A. W., Perkins W., Pilkiewicz F. G., Perez-Soler R., Nicholson S., Peters G. J., Postmus P. E., *Clin. Cancer Res.*, **13**, 2414–2421 (2007).
44) Chou A. J., Gupta R., Bell M. D., Riewe K. O., Meyers P. A., Gorlick R., *Pediatr. Blood Cancer*, **60**, 580–586 (2013).

45) Zarogoulidis P., Eleftheriadou E., Sapardanis I., Zarogoulidou V., Lithoxopoulou H., Kontakiotis T., Karamanos N., Zachariadis G., Mabroudi M., Zisimopoulos A., Zarogoulidis K., *Invest. New Drugs*, **30**, 1628–1640 (2012).
46) Otterson G. A., Villalona-Calero M. A., Sharma S., Kris M. G., Imondi A., Gerber M., White D. A., Ratain M. J., Schiller J. H., Sandler A., Kraut M., Mani S., Murren J. R., *Clin. Cancer Res.*, **13**, 1246–1252 (2007).
47) Otterson G. A., Villalona-Calero M. A., Hicks W., Pan X., Ellerton J. A., Gettinger S. N., Murren J. R., *Clin. Cancer Res.*, **16**, 2466–2473 (2010).
48) Lemarie E., Vecellio L., Hureaux J., Prunier C., Valat C., Grimbert D., Boidron-Celle M., Giraudeau B., le Pape A., Pichon E., Diot P., el Houfia A., Gagnadoux F., *J. Aerosol Med. Pulm. Drug Deliv.*, **24**, 261–270 (2011).
49) Verschraegen C. F., Gilbert B. E., Loyer E., Huaringa A., Walsh G., Newman R. A., Knight V., *Clin. Cancer Res.*, **10**, 2319–2326 (2004).
50) Thipphawong J., *Adv. Drug Deliv. Rev.*, **58**, 1089–1105 (2006).
51) Kinnula V., Cantell K., Mattson K., *Eur. J. Cancer*, **26**, 740–741 (1990).
52) Maasilta P., Halme M., Mattson K., Cantell K., *Lancet*, **337**, 371 (1991).
53) Halme M., Maasilta P., Repo H., Ristola M., Taskinen E., Mattson K., Cantell K., *Int. J. Radiat. Oncol. Biol. Phys.*, **31**, 93–101 (1995).
54) Jaffe H. A., Buhl R., Mastrangeli A., Holroyd K. J., Saltini C., Czerski D., Jaffe H. S., Kramer S., Sherwin S., Crystal R. G., *J. Clin. Invest.*, **88**, 297–302 (1991).
55) Lorenz J., Wilhelm K., Kessler M., Peschel C., Schwulera U., Lissner R., Struff W. G., Huland E., Huber C., Aulitzky W. E., *Clin. Cancer Res.*, **2**, 1115–1122 (1996).
56) Enk A. H., Nashan D., Rübben A., Knop J., *Cancer*, **88**, 2042–2046 (2000).
57) Anderson P. M., Markovic S. N., Sloan J. A., Clawson M. L., Wylam M., Arndt C. A., Smithson W. A., Burch P., Gornet M., Rahman E., *Clin. Cancer Res.*, **5**, 2316–2323 (1999).
58) Markovic S. N., Suman V. J., Nevala W. K., Geeraerts L., Creagan E. T., Erickson L. A., Rowland K. M. Jr., Morton R. F., Horvath W. L., Pittelkow M. R., *Am. J. Clin. Oncol.*, **31**, 573–579 (2008).
59) Marasini N., Haque S., Kaminskas L. M., *Curr. Opin. Colloid In.*, **31**, 18–29 (2017).
60) Abdelaziz H. M., Gaber M., Abd-Elwakil M. M., Mabrouk M. T., Elgohary M. M., Kamel N. M., Kabary D. M., Freag M. S., Samaha M. W., Mortada S. M., Elkhodairy K. A., Fang J. Y., Elzoghby A. O., *J. Control. Release*, **269**, 374–392 (2018).
61) Garbuzenko O. B., Mainelis G., Taratula O., Minko T., *Cancer Biol. Med.*, **11**, 44–55 (2014).
62) Maeda H., Nakamura H., Fang J., *Adv. Drug Deliv. Rev.*, **65**, 71–79 (2013).
63) Torchilin V., *Adv. Drug Deliv. Rev.*, **63**, 131–135 (2011).
64) Dabbagh A., Abu Kasim N. H., Yeong C. H., Wong T. W., Abdul Rahman N., *J. Aerosol Med. Pulm. Drug Deliv.*, **31**, 139–154 (2018).
65) Ovais M., Guo M., Chen C., *Adv. Mater.*, **31**, 1808303 (2019).
66) Fishburn C. S., *J. Pharm. Sci.*, **97**, 4167–4183 (2008).
67) Greish K., Fang J., Inutsuka T., Nagamitsu A., Maeda H., *Clin. Pharmacokinet.*, **42**, 1089–1105 (2003).
68) Cheng W., Gu L., Ren W., Liu Y., *Mater. Sci. Eng. C*, **45**, 600–608 (2014).
69) Zou Y., Fu H., Ghosh S., Farquhar D., Klostergaard J., *Clin. Cancer Res.*, **10**, 7382–7391 (2004).
70) Luo T., Magnusson J., Préat V., Frédérick R., Alexander C., Bosquillon C., Vanbever R., *Pharm. Res.*, **33**, 1671–1681 (2016).
71) Luo T., Loira-Pastoriza C., Patil H. P., Ucakar B., Muccioli G. G., Bosquillon C., Vanbever R., *J. Control. Release*, **239**, 62–71 (2016).
72) Xie Y., Aillon K. L., Cai S., Christian J. M., Davies N. M., Berkland C. J., Forrest M. L., *Int. J. Pharm.*, **392**, 156–163 (2010).
73) Ishiguro S., Cai S., Uppalapati D., Turner K., Zhang T., Forrest W. C., Forrest M. L., Tamura M., *Pharm. Res.*, **33**, 2517–2529 (2016).
74) Kaminskas L. M., McLeod V. M., Ryan G. M., Kelly B. D., Haynes J. M., Williamson M., Thienthong N., Owen D. J., Porter C. J., *J. Control. Release*, **183**, 18–26 (2014).
75) Ehsan Z., Wetzel J. D., Clancy J. P., *Expert Opin. Investig. Drugs*, **23**, 743–749 (2014).
76) Cipolla D., Blanchard J., Gonda I., *Pharmaceutics*, **8**, E6 (2016).
77) Garbuzenko O. B., Saad M., Betigeri S., Zhang M., Vetcher A. A., Soldatenkov V. A., Reimer D. C., Pozharov V. P., Minko T., *Pharm. Res.*, **26**, 382–394 (2009).
78) Koshkina N. V., Kleinerman E. S., Waidrep C., Jia S. F., Worth L. L., Gilbert B. E., Knight V., *Clin. Cancer Res.*, **6**, 2876–2880 (2000).
79) Koshkina N. V., Waldrep J. C., Roberts L. E., Golunski E., Melton S., Knight V., *Clin. Cancer Res.*, **7**, 3258–3262 (2001).
80) Elhissi A. M., Taylor K. M., *J. Drug Del. Sci. Tech.*, **15**, 261–265 (2005).
81) Lehofer B., Bloder F., Jain P. P., Marsh L. M., Leitinger G., Olschewski H., Leber R., Olschewski A., Prassl R., *Eur. J. Pharm. Biopharm.*, **88**, 1076–1085 (2014).
82) Elhissi A. M., Faizi M., Naji W. F., Gill H. S., Taylor K. M., *Int. J. Pharm.*, **334**, 62–70 (2007).
83) Niven R. W., Schreier H., *Pharm. Res.*, **7**, 1127–1133 (1990).
84) Videira M., Almeida A. J., Fabra A., *Nanomedicine*, **8**, 1208–1215 (2012).
85) Gill K. K., Nazzal S., Kaddoumi A., *Eur. J. Pharm. Biopharm.*, **79**, 276–284 (2011).
86) Sato H., Wang Y. M., Adachi I., Horikoshi I., *Biol. Pharm. Bull.*, **19**, 1596–1601 (1996).
87) Tros de Ilarduya C., Sun Y., Düzgüneş N., *Eur. J. Pharm. Sci.*, **40**, 159–170 (2010).
88) Bragonzi A., Dina G., Villa A., Calori G., Biffi A., Bordignon C., Assael B. M., Conese M., *Gene Ther.*, **7**, 1753–1760 (2000).
89) Ito T., Okuda T., Takashima Y., Okamoto H., *Mol. Pharm.*, **16**, 489–497 (2019).
90) Bitko V., Musiyenko A., Shulyayeva O., Barik S., *Nat. Med.*, **11**, 50–55 (2005).
91) D'Alessandro-Gabazza C. N., Kobayashi T., Boveda-Ruiz D., *et al.*, *Am. J. Respir. Cell Mol. Biol.*, **46**, 397–406 (2012).
92) Ito T., Okuda T., Takayama R., Okamoto H., *J. Pharm. Sci.*, **108**, 2661–2667 (2019).
93) Moschos S. A., Frick M., Taylor B., *et al.*, *Mol. Ther.*, **19**, 2163–2168 (2011).
94) Lipka J., Semmler-Behnke M., Wenk A., Burkhardt J., Aigner A., Kreyling W., *Int. J. Pharm.*, **500**, 227–235 (2016).
95) Okuda T., Toyoda Y., Murakami T., Okamoto H., *Int. J. Pharm.*, **565**, 294–305 (2019).
96) Zou Y., Tornos C., Qiu X., Lia M., Perez-Soler R., *Clin. Cancer Res.*, **13**, 4900–4908 (2007).
97) Zamora-Avila D. E., Zapata-Benavides P., Franco-Molina M. A., Saavedra-Alonso S., Trejo-Avila L. M., Reséndez-Pérez D., Méndez-Vázquez J. L., Isaias-Badillo J., Rodríguez-Padilla C., *Cancer Gene Ther.*, **16**, 892–899 (2009).
98) Richard-Fiardo P., Cambien B., Pradelli E., Beilvert F., Pitard B., Schmid-Antomarchi H., Schmid-Alliana A., *Cancer Gene Ther.*, **18**, 761–772 (2011).
99) Kawabata A., Baoum A., Ohta N., Jacquez S., Seo G. M., Berkland C., Tamura M., *Cancer Res.*, **72**, 2057–2067 (2012).
100) Sato T., Shimosato T., Ueda A., Ishigatsubo Y., Klinman D. M., *Mol. Cancer Ther.*, **14**, 2198–2205 (2015).
101) Wijagkanalan W., Higuchi Y., Kawakami S., Teshima M., Sasaki H., Hashida M., *Mol. Pharmacol.*, **74**, 1183–1192 (2008).

102) Gaspar M. M., Radomska A., Gobbo O. L., Bakowsky U., Radomski M. W., Ehrhardt C., *J. Aerosol Med. Pulm. Drug Deliv.*, **25**, 310–318 (2012).
103) Rosière R., Van Woensel M., Mathieu V., Langer I., Mathivet T., Vermeersch M., Amighi K., Wauthoz N., *Int. J. Pharm.*, **501**, 148–159 (2016).
104) Tseng C. L., Su W. Y., Yen K. C., Yang K. C., Lin F. H., *Biomaterials*, **30**, 3476–3485 (2009).
105) Taratula O., Kuzmov A., Shah M., Garbuzenko O. B., Minko T., *J. Control. Release*, **171**, 349–357 (2013).
106) Conde J., Bao C., Tan Y., Cui D., Edelman E. R., Azevedo H. S., Byrne H. J., Artzi N., Tian F., *Adv. Funct. Mater.*, **25**, 4183–4194 (2015).
107) Maillet A., Guilleminault L., Lemarié E., Lerondel S., Azzopardi N., Montharu J., Congy-Jolivet N., Reverdiau P., Legrain B., Parent C., Douvin D. H., Hureaux J., Courty Y., De Monte M., Diot P., Paintaud G., Le Pape A., Watier H., Heuzé-Vourc'h N., *Pharm. Res.*, **28**, 2147–2156 (2011).
108) Hervé V., Rabbe N., Guilleminault L., Paul F., Schlick L., Azzopardi N., Duruisseaux M., Fouquenet D., Montharu J., Redini F., Paintaud G., Lemarié E., Cadranel J., Wislez M., Heuzé-Vourc'h N., *MAbs*, **6**, 1638–1648 (2014).
109) Nelson A. L., Reichert J. M., *Nat. Biotechnol.*, **27**, 331–337 (2009).
110) Muyldermans S., *Annu. Rev. Biochem.*, **82**, 775–797 (2013).
111) Emami F., Vatanara A., Park E. J., Na D. H., *Pharmaceutics*, **10**, E131 (2018).
112) Geller D. E., Weers J., Heuerding S., *J. Aerosol. Med. Pulm. Drug Deliv.*, **24**, 175–182 (2011).
113) Almeida A. J., Grenha A., "Mucosal Delivery of Biopharmaceuticals: Biology, Challenges and Strategies," ed. by Neves J.D., Sarmento B., Springer, New York, 2014, pp. 483–498.
114) Wanning S., Süverkrüp R., Lamprecht A., *Int. J. Pharm.*, **488**, 136–153 (2015).
115) Chen L., Okuda T., Lu X. Y., Chan H. K., *Adv. Drug Deliv. Rev.*, **100**, 102–115 (2016).
116) Otake H., Okuda T., Okamoto H., *Chem. Pharm. Bull.*, **64**, 239–245 (2016).
117) Li L., Sun S., Parumasivam T., Denman J. A., Gengenbach T., Tang P., Mao S., Chan H. K., *Eur. J. Pharm. Biopharm.*, **102**, 132–141 (2016).
118) Yu J., Chan H. K., Gengenbach T., Denman J. A., *Eur. J. Pharm. Biopharm.*, **119**, 224–234 (2017).
119) D'Addio S. M., Chan J. G., Kwok P. C., Benson B. R., Prud'homme R. K., Chan H. K., *Pharm. Res.*, **30**, 2891–2901 (2013).
120) Yu H., Teo J., Chew J. W., Hadinoto K., *Int. J. Pharm.*, **499**, 38–46 (2016).
121) Levet V., Rosière R., Merlos R., Fusaro L., Berger G., Amighi K., Wauthoz N., *Int. J. Pharm.*, **515**, 209–220 (2016).
122) Levet V., Merlos R., Rosière R., Amighi K., Wauthoz N., *Int. J. Pharm.*, **517**, 359–372 (2017).
123) Meenach S. A., Anderson K. W., Hilt J. Z., McGarry R. C., Mansour H. M., *AAPS PharmSciTech*, **15**, 1574–1587 (2014).
124) Meenach S. A., Anderson K. W., Zach Hilt J., McGarry R. C., Mansour H. M., *Eur. J. Pharm. Sci.*, **49**, 699–711 (2013).
125) Kim I., Byeon H. J., Kim T. H., Lee E. S., Oh K. T., Shin B. S., Lee K. C., Youn Y. S., *Biomaterials*, **33**, 5574–5583 (2012).
126) Feng T., Tian H., Xu C., Lin L., Xie Z., Lam M. H., Liang H., Chen X., *Eur. J. Pharm. Biopharm.*, **88**, 1086–1093 (2014).
127) Gandhi M., Pandya T., Gandhi R., Patel S., Mashru R., Misra A., Tandel H., *Int. J. Pharm.*, **496**, 886–895 (2015).
128) Roa W. H., Azarmi S., Al-Hallak M. H., Finlay W. H., Magliocco A. M., Löbenberg R., *J. Control. Release*, **150**, 49–55 (2011).
129) Okamoto H., Nishida S., Todo H., Sakakura Y., Iida K., Danjo K., *J. Pharm. Sci.*, **92**, 371–380 (2003).
130) Okamoto H., Sakakura Y., Shiraki K., Oka K., Nishida S., Todo H., Iida K., Danjo K., *Int. J. Pharm.*, **290**, 73–81 (2005).
131) Mohri K., Okuda T., Mori A., Danjo K., Okamoto H., *J. Control. Release*, **144**, 221–226 (2010).
132) Okamoto H., Shiraki K., Yasuda R., Danjo K., Watanabe Y., *J. Control. Release*, **150**, 187–195 (2011).
133) Okuda T., Kito D., Oiwa A., Fukushima M., Hira D., Okamoto H., *Biol. Pharm. Bull.*, **36**, 1183–1191 (2013).
134) Okuda T., Suzuki Y., Kobayashi Y., Ishii T., Uchida S., Itaka K., Kataoka K., Okamoto H., *Pharmaceutics*, **7**, 233–254 (2015).
135) Okuda T., Morishita M., Mizutani K., Shibayama A., Okazaki M., Okamoto H., *J. Control. Release*, **279**, 99–113 (2018).
136) Miwata K., Okamoto H., Nakashima T., Ihara D., Horimasu Y., Masuda T., Miyamoto S., Iwamoto H., Fujitaka K., Hamada H., Shibata A., Ito T., Okuda T., Hattori N., *Mol. Ther. Nucleic Acids*, **12**, 698–706 (2018).
137) Oleck J., Kassam S., Goldman J. D., *Diabetes Spectr.*, **29**, 180–184 (2016).