*Review*

# Airway local endoscopic pharmacological treatment; current applications and future concepts

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**Abstract**

**Introduction**: Local treatment of the airways and lung parenchyma has been used in clinical practice for several years for a variety of diseases. **Methods**: A variety of endoscopic tools for local treatment exist, especially for treating malignancies. Using these endoscopic tools, one can administer drugs specifically designed for the airways. **Discussion**: This article presents all locally administered treatment options and provides useful insights for future local endoscopically applied treatments.

**Keywords**: Bronchoscopy; Hemostasis; Drug; Stents; Sirolimus; Everolimus; Zotarolimus; Cisplatin; Taxanes; Antibiotics; Interferon; Anti-VEGF; EBUS

## 1. Introduction

Local endoscopic treatments have been in use for several years. A variety of endoscopic tools for local treatment exist such as argon plasma coagulation (APC), laser, cryoprobes and electrocatheters. One can use these tools through an endoscope in order to open a channel through a tumor or granulomatous tissue. Such invasive procedures are usually followed by a stent placement (silicon/metallic) with a balloon dilation system [1].

Specifically designed needles, used for applying glue in post-operative fistulas within the airways, exist. Moreover, specially designed catheters which produce spray for applying interferon and anti-vascular endothelial factor (VEGF) in the case of endobronchial HPV infections can also be found [2,3]. Other types of catheters can spray biopolymer powder used for hemostasis with the help of a portable air-compressor. The same air-compressor can be used in order to generate aerosol formulations of different drugs such as interferon and anti-VEGF.

Stents have been used as a mechanical means of ensuring patency of the airways [4–8]. In the case of benign diseases, such as tracheomalacia, silicon stents have been used for some time, and recently drug eluting silicon stents containing everolimus, sirolimus and zotarolimus have been used for the prevention of granulomatous tissue formation. In the case of malignancies, metallic auto-expandable stents coated with paclitaxel or cisplatin have been used.

There is extensive research activity going on towards creating and introducing tissue friendly biogradable stents. Polymers and co-polymers are currently considered the best available option [9].

Efforts for improving stents include the use of electrospinning as a method to cover all types of stents. The main issue with drug eluting stents has been the time limitations in drug release. By using electrospinning, one can place large drug doses on the relatively small surface of stents.

The various interactions between the surface of stents and the mucosa have also been of interest in the recent years. Nowadays, stents can be custom made because it is feasible to use the information acquired from a CT of the thorax to create suitable stents using a 3D printer [6].

In the past year physicians have also had the opportunity to use a local ablation microwave system de-

veloped by Bronchus®. Until recently, radiologists had been using radiofrequency, microwave and thermosphere systems for lung cancer ablation under the guidance of computed tomography. The endoscopic microwave ablation system can identify vessels and lesions using the ARCHEMEDES®navigation system [10,11]. Biopsy needles, such the 19G Olympus®, have been used to apply chemotherapeutic agents in large lung cancer lesions and lymphnodes [12].

The future success of local treatments using endoscopic tools depends on the technological advancements, particularly in molecular biology and radiology techniques. Positron emission tomography (PET-CT) can identify active lesions and it can be used for treatment re-evaluation. Rapid on-site evaluation systems such as the Cellvisio® can be used to identify local disease and treatment efficiency. Other novel radiology techniques such as the Cios-Spin can assist in the navigation and application of local interventional treatments [13]. This article aims to present the existing knowledge supporting choosing local treatments applied through the use of endoscopic tools for treating lung diseases.

## 2. Local parameters of the airways for drug absorption

### 2.1 Clearance mechanisms

Aerosol particles are deposited in the airways are removed by mucociliary clearance mechanisms. The airway surface consists of epithelial goblet cells and submucosal glands secreting mucus. These two cell types produce a two-layer mucus blanket over the ciliated epithelium: (a) the low-viscosity sol layer and (b) a high-viscosity gel layer. All insoluble particles are trapped in the gel layer and with the help of the beating cilia are moved towards the pharynx or the gastrointestinal tract or even removed through coughing. The activity of the mucus varies depending on the airway area and the clearance is also synergistically determined by the number of ciliated cells and their beat frequency which differs in different parts of the airways. For normal mucociliary clearance there must be no underlying lung disease. Furthermore, the chemical composition of the sol layer should be optimal along with the rheology of the mucus. Mucociliary clearance is dysfunctional in lung diseases such as immotile cilia syndrome, chronic obstructive pulmonary disease (COPD), cystic fibrosis, bronchiectasis, and asthma [14]. It has been observed that lipophilic molecules pass easily through the airway epithelium via passive transport, while the hydrophilic molecules cross via extracellular pathways and exocytosis [15]. Particles deposited on the bronchial epithelium are absorbed into the systemic bronchial circulation and then into the lymphatic system. Aerosol particles that are deposited in the alveolar region may either be phagocytosed and cleared by alveolar macrophages, or they are absorbed into the pulmonary circulation. Alveolar macrophages situated in the alveoli are phagocytic cells and they are the first defense layer against inhaled microorganisms and particles [16]. Macrophages phagocytose insoluble particles that are deposited in the alveoli, and are either cleared by the lymphatic system or moved with the help of beating cilia covered in alveolar fluid in the upper airways to the gastrointestinal tract [17]. However; depending on the particle's chemical structure, this process can take weeks to months to complete [18]. There are cases where particles can be enzymatically degraded intracellularly with the help of local enzymes [19]. The bacterial biofilm of the mucus also plays a crucial role in the rate of drug absorption of the bronchial epithelium and alveoli [20]. An extensive investigation of these factors has been done in cystic fibrosis patients and therefore all factors have been more or less elucidated [21]. Pretreatment with aerosolized corticosteroids or saline in other cases provides enhanced local drug absorption, in dosages of 1 mg/2 mL × 3 budesonide or saline 2mL × 3 per day.

### 2.2 Bronchial-systematic circulation

The lungs receive the largest part of the cardiac output through the pulmonary arteries and are considered to be the highest perfused organs in the body. The alveoli are the parts of the lungs that are the most highly supplied. Only 1% of the cardiac output circulates to the trachea and bronchi. In bronchiectasis the bronchial blood flow is augmented from 1% to as much as 30% of the cardiac output [22]. It has been observed that inhaled drugs that are deposited in tracheobronchial regions can be locally absorbed by the local vessels [23].

### 2.3 Tumor size

It has been observed in previous studies that tumor size affects the distribution and deposition of an inhaled compound and therefore the maximum diameter of the tumors had to be up to 5 cm upon diagnosis, otherwise patients were excluded from trials [24–29]. Anticancer drugs have been observed to penetrate normal tissues by both diffusion and convection and finally reabsorption into the lymphatic circulation. However; a major issue is the unstructured neo-angiogenesis and lack of functional lymphatics in several tumors [30,31]. This tumor microenvironment increases the levels of interstitial fluid pressure in tumors [32–34], reduces convection and inhibits distribution of macromolecules [35,36]. The penetration of a drug particle depends on its: (a) charge, (b) shape, (c) molecular weight, and (d) aqueous solubility. Water-soluble drugs are distributed in the extracellular matrix and diffuse efficiently around and between the cells. Lipid-soluble drugs, on the other hand, penetrate lipid membranes, and so can be transported through cells. One can use 22G, 21G and 19G needles for local drug administration. Indeed, 19G needles can deliver larger quantities, however; depending on the stiffness of the tissue, the drug penetration will differ. Needles with pores on their sides exist to facilitate absorption

**Table 1. Diseases of lung for which currently local treatment has used.**

| |
|---|
| -Cystic fibrosis |
| -Lung cancer |
| -COPD |
| -Asthma |
| -Pulmonary hypertension |
| -Diabetes |
| -Emphysema |
| -Benign tracheal stenosis |
| -Lung Abscess |
| -Pleura effusion |
| -Airways HPV |
| -Fistulas |

of more cells during biopsy. Such needles can also be used to deliver more drug in different parts of a lesion when they are used for drug delivery [12,37].

## 2.4 Physical properties of drug formulations

The most important physical properties that have to be considered when creating an aerosolized drug are: (a) viscosity ionic strength, (b) pH and (c) osmolarity. It has been observed that if pH and osmolarity are not in the normal range, then bronchoconstriction and coughing will occur due to mucosal irritation [38,39].

## 2.5 Lung disease

It has been observed when studying aerosolized drugs that lung disease plays a crucial role in local drug absorption. This knowledge comes from studies of large patient populations with diabetes and aerosolized insulin administration, either as liquid or powder form [24]. However; all these drugs are deposited to a large percentage of the surface of the airway epithelium. In the case of local intratumoral administration only the extracellular matrix (ECM) and the parameters contained in this environment play a key role. However, one should keep in mind that chronic obstructive pulmonary disease (COPD) or heart failure might be contraindications to performing an endoscopy for the administration of intratumoral therapy. In cases of underlying disease exacerbation, one should increase the dosage and administrations. Additional issues such as the oxygenation of a patient need to be resolved and aerosol pretreatment as previously described must be increased up to double. In the worst case scenario, one should switch to intravenous administration until the exacerbation is adequately managed. All this information can be found in a previously published article by Zaroglidis *et al.* [24].

# 3. Drug carriers

## 3.1 Liposomes

Liposomes are drug carriers that help secure sustained release, reduced toxicity and reduced irritation to the lung parenchyma. They can be easily manipulated and have stability [40]. The dose of the drug carried by the liposomes, its release rate and the deposition in the alveoli depends on: (a) lipid composition, (b) particle size, (c) charge, (d) drug/lipid ratio, and (e) method of delivery [41–43]. Liposomes are produced from phospholipids, and they may or may not be electrically charged [44,45]. Their structure consists of an aqueous part, which is entrapped by a synthetic lipid single layer or bilayer, with or without the addition of cholesterol. They can encapsulate either hydrophilic or lipophilic chemical entities [46]. However, drugs with intermediate solubility are poorly retained by liposomes, so they have to be manipulated to achieve a higher degree of retention [47]. Liposomes are prepared for inhalation either in liquid or dry powder form [48]. Unfortunately, it has been observed that during nebulization, an amount of the formulation is lost and hence the operating conditions need to be optimized in order to minimize loss [49–52]. The dry powder liposome formulations are produced by lyophilization, usually followed by milling or by spray-drying [53,54]. The sustained release capability of liposomes has been observed in examples of aerosolized treatments for lung diseases in several studies [24,55,56]. In order to enhance the sustained-release properties of liposomes, a polymer surface coating called polyethylene glycol (PEG) has been developed recently. PEG coating provides a "stealth" shield to the molecule to bypass the body's defense mechanisms [47,57,58].

## 3.2 Microparticles

Microparticles are produced from naturally occurring or synthetic polymers. Their size ranges between 0.1 and 500 $\mu$m. Their physical and chemical properties allow them to be more stable than liposomes, and so are capable of higher drug loading. Microparticles are an ideal carrier system for proteins and peptides [59,60]. The main factors affecting them are: (a) pH, (b) heat, (c) moisture, (d) solvents, (d) oxygen, and (e) mechanical stresses. The preparation for aerosolized delivery of microparticles can be undertaken using: (a) emulsion-solvent evaporation, (b) spray-drying, (c) phase separation, (d) emulsion-solvent diffusion and e) supercritical fluid technology [61–67]. The following parameters influence drug release: (a) porosity, (b) size, (c) solubility, (d) molecular weight, (e) nature of micromolecular drug, (f) concentration, g) tortuosity and h) uniformity of the polymer. A coating can be added to improve the time release characteristics, and also 1,2-dipalmitoylphosphatidylcholine is also added to poly(DL-lactide-co-glycolide) microspheres to decrease the uptake by macrophages [64]. When chitosan and hydroxypropylcellulose are added to the particles, their local absorption

over time is increased [67]. It was observed in previous studies that particles of 1–3 $\mu$m, tend to aggregate [68] and are cleared by alveolar macrophages [69]. Therefore it was necessary to develop large porous particles of a geometric diameter of 0.5 $\mu$m, an aerodiameter of 0.5 $\mu$m, and a low density of 0.1 mg/mL [65,70].

Further development of this molecules led to the development of "Trojan" particles [69], these have the ability to escape both phagocytic and mucociliary clearance of the respiratory system. They are prepared from nanoparticles, of low density (0.1 mg/mL). These particles can be aerosolized from dry powder [61]. A single cancer cell can ingest one or multiple microparticles and these particles can be arranged in such a way so as to reduce the space occupied inside the cell [71,72].

### 3.3 Carbohydrates

There are currently three carbohydrates used as used drug carriers: (a) lactose (a-lactose monohydrate), (b) glucose, and (c) mannitol. These carriers act also as stability enhancers. In the last decade several carriers were assessed and it was observed that mannitol is the best candidate for dry powder inhaler formulations [73]. Current techniques that are used to produce a inhalable formulation are: (a) supercritical fluid technology, (b) lyophilizing followed by milling/jet milling or spray-drying, (c) freeze-drying, and (d) spray-freeze drying [74–78]. Lactose enhances the uptake of poly-D-lysine into airway cells, increasing intracellular localization of proteins and peptides [79]. Lactose can be mixed with fine lactose particles (about 5 $\mu$m in diameter) with coarse lactose to improve disaggregation, as well as the fine particle fraction [80]. Lactose can have a ternary component, such as L-leucine, to the formulation to increase dispersibility [45].

Cyclodextrins (cyclic oligosaccharides), have also been proven to be useful excipients in the respiratory distribution of small molecules [81]. In a recent study, the use of dimethyl-$\beta$-cyclodextrin resulted in increased bioavailability of carbohydrates by increasing concentrations of cyclodextrin [73].

### 3.4 Pegylation

Polyethylene glycol (PEG) added to proteins enables a sustained release at the site of deposition, due to a "stealth" effect, by bypassing the defense mechanisms of the respiratory tract (macrophages), and by decreasing degradation of the formulation in the lungs [47,82]. PEG has been shown to be a safe carrier for inhalable agents [83].

### 3.5 Biodegradable polymers

Polylactic acid has properties that favor sustained release, but it is not suitable for lung delivery due to its prolonged biological half-life. However, an oligomer of lactic acid, with a shorter half-life (6–8 days), can be used instead for drug delivery. Hydroxypropyl cellulose, is another option absorbed over approximately 24 hours that bypasses mucociliary clearance. However, not enough data exist about its the toxicity profile [45,84].

### 3.6 Bioadhesives

Bioadhesives have been used to prolong the attachment of the carrier-drug complex to the bronchial cells of the airways [85,86]. Several multivalent binding agents have been incorporated in drug-carrier systems, such as; lectins, heparin, octa-arginine, peptides, heparin sulfate, and antibodies [87,88].

## 4. Cell targeting for drug transportation

Cell targeting has been used in recent years as the best method to improve local absorption. Gene therapy is considered the best method for cell-selective targeting [89]. Additionally alveolar macrophages have been investigated as a vehicle by which to deliver a chemotherapeutic agent to the lymph nodes through the lymphatic circulation. It has been observed that liposomes and microspheres are engulfed by alveolar macrophages. Several receptors which are overexpressed in tumors, such as epidermal growth factor, have been exploited to target specific cells in cancer therapy [90,91]. Low-density lipoprotein has been previously used for receptor assimilation [92].

### 4.1 Intracellular targeting

Intracellular targeting is an additional strategy enabling a drug to reach the proper surface area [93,94]. Most chemotherapy regimens interact within the reproductive cell cycle, so this delivery strategy should be further pursued [95]. Three are the most important parameters concerning the cell microenvironment and drug-formulation interactions: (a) endosomal release, (b) intracellular trafficking, and (c) nuclear localization.

### 4.2 ATP-binding cassette transporters

ABC transporters are a large family (50 members) of transmembrane proteins, consisting of seven subfamilies. ABC transporters act as a defense mechanism in lung epithelial tissue [96]. P-glycoprotein, also known as multidrug resistant proteins, has been shown to play a role in multidrug resistance, especially in chemotherapy resistance [97]. P-glycoprotein and its properties have been extensively studied in the lung. It has been observed that its presence decreases oral drug absorption, prevents drug entry in the central nervous system, and it is responsible for many drug to drug interactions [98]. The transporter is found on the apical membrane of the bronchial and bronchiolar epithelium [99–102], in the endothelial cells of the bronchial capillaries [103], and in alveolar macrophages [100,101]. Unfortunately the expression of P-glycoprotein in smokers with lung disease versus people with normal lungs has not been adequately investigated. It has been observed that P-glycoprotein and immunohistochemical multidrug-

resistant protein analyses can predict a patient's response to chemotherapy [104]. However; in one study, the mRNA levels in the lung tissue of smokers, non-smokers, and ex-smokers were not found to be significantly different [99]. Several studies have previously demonstrated a direct or indirect connection between the P-glycoprotein transporter expression and underlying lung disease. In cystic fibrosis, for example, due to changes induced by the disease, it has been shown that the P-glycoprotein transporter is upregulated [105,106]. Furthermore, it has been observed in another study that toxins released from microorganisms infecting patients with cystic fibrosis also inhibit P-glycoprotein [107]. On the other hand, there are no significant data indicating modification of P-glycoprotein expression between different disease stages in chronic obstructive pulmonary disease patients [108,109] and no relevant data exist for asthma patients. It has been observed that corticosteroids administered by inhalation, oral, and intraperitoneal routes upregulate the P-glycoprotein transporter [110,111]. In a previous study, inhibition of P-glycoprotein was observed by lipid nanocapsules, which is a crucial mechanism of resistance for paclitaxel [112]. Nine multidrug resistance proteins (MRPs) exist. In normal lung tissue, MRP 1 and MRP 5 have been found to be highly expressed, MRP 6 and MRP 7 are moderately expressed, and MRPs 2, 3, 4, 8, and 9 are either low or undetectable [113,114]. MRP-1 and MRP-2 are found in the bronchial and bronchiolar epithelium [100,115,116]. MRP-1 is also found in alveolar macrophages [100,115]. MRP-1 expression levels are altered in patients with chronic obstructive pulmonary disease [108,115]. It has been shown that smoking downregulates these transporters, and these transporters play a protective role against cell damage [108,117]. Ipratropium, N-acetylcysteine, and budesonide stimulate MRP-1 efflux and activity [118]. Formoterol on its own does not have an effect on the transporter, however, the co-administration of inhaled corticosteroids reduces the transporter expressions [118]. In a previous study on the use of inhaled doxorubicin, MRP-1 and MRP-2 were overexpressed [119]. This information is important to keep in mind, because most lung cancer patients are also diagnosed with chronic obstructive disease and because most are or have been smokers, the transporter expression has been found to be increased after doxorubicin use.

### 4.3 Organic cation transporters

Organic cation transporters comprise of five types of carriers: (a) electrogenic OCT 1-3, (b) electroneutral OCT N1 and (c) OCT N2. OCT 1–3 can be found in the trachea, smooth muscles of the airway, and ciliated bronchial cells. OCT N1 is expressed in the tracheal epithelium and alveolar macrophages. OCT N2 is expressed in the alveolar epithelium and airway epithelium [120–123]. Previously published data from animal and in vitro cell lines studies implicate upregulation or downregulation of OCT transporters upon induced inflammation or drug interactions related to asthma and/or chronic obstructive pulmonary disease. Nevertheless, these data have not been clearly demonstrated in humans [122–125].

### 4.4 Peptide transporters

Peptide transporters belong to the proton-coupled oligopeptide transporter group. PEPT 1 and PEPT 2 are the two main transporters, which contribute to the high bioavailability of peptide-like molecules. These two peptides can affect the absorption and distribution of inhaled antibiotics and antiviral drugs [126]. PEPT 2 and PEPT 1 have been detected in the airway epithelium and bronchial epithelium [127,128]. PEPT 1 and PEPT 2 have been found in animal tissues and various cell lines [129,130]. Their mechanism of action, however, has not been fully investigated [131].

### 4.5 Organic anion transporters

There are six members identified: (a) OAT 1–4, (b) URAT 1, and (c) OAT 5, mostly found in the kidneys [132]. Gene microarrays have confirmed their absence in human and murine lungs, but OAT 2 is highly expressed at these sites [120]. Moreover; OAT 4 mRNA was highly expressed in the bronchial cell lines Calu-3 and 16HBE14o- [129].

### 4.6 Organic anion transporting polypeptides

There are 11 human organic anion transporting polypeptides (OATPs), which are divided into six families [133]. Their actual tissue distribution has not been fully investigated [133]. OATP 2B1, OATP 3A1, OATP 4C1, and OATP 4A1 expression has been observed in: (a) cell lines, (b) human lungs, and (c) animals [120,129,134].

## 5. Chemotherapy drugs

### 5.1 Platinum analogs

Several studies have been performed, both in humans and in animals, with inhaled platinum analogs and instillation day 1 and cisplatin (50 mg/m$^2$ daily) on days 1 and 2. The administration was performed with jet-nebulizers as a production system and under strict protection measures such as the high efficiency particulate air (HEPA) filter or a protective chamber [27,135–140]. Safety and efficacy assessment was performed with: radiographic examinations (x-rays, computed tomography scans), blood and urine analysis, ki-67 cell proliferation, pathological findings, bronchoalveolar lavage, high-performance liquid chromatography, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay and recording of Eastern Cooperative Oncology Group common toxicity criteria. The main adverse effects observed were dose-dependent: (a) cough, (b) fatigue, (c) nausea and (d) weight loss.

## 5.2 5-fluorouracil

5-FU was the first chemotherapy drug to be used as local chemotherapy treatment almost 30 years ago [141]. A high-efficiency particulate arrestance filter and a plexiglass chamber were used for the administration. Safety and efficacy were evaluated via blood samples, high performance liquid chromatography and imaging techniques [142–145]. The drug was administered intrabronchially and regression of the tumor was observed with endoscopy in all their treatment sessions. The drug has not been delivered intratumorally as in other studies 100 mg/m$^2$ on day [146]. It was observed that the aerosol drug was absorbed via the alveoli to the systematic circulation. This has not been reported in other studies [135]. This was an early study, conducted before intratumoral administration began [37], where the aerosol as a local therapy interacted with the surface of the tumor and was absorbed by the extracellular matrix of the tumor and alveoli [147]. The adverse effects observed were cough, weight loss, dizziness and bronchospasm, and were dose dependent. 5-FU has been administered either alone or coated with lipids or liposome nanoparticles [144,145].

## 5.3 Taxanes

Taxanes (paclitaxel and docetaxel) have been administered locally in the respiratory system alone or in combination with other drugs [148,149]. In one of the first published studies cyclosporin A was co-administered as a method to augment the efficiency of aerosolized taxanes paclitaxel (175 mg/m$^2$) [150]. The safety and efficacy was evaluated with blood samples, pathology findings, high performance liquid chromatography and V/Q scans. All previously published studies were performed in animals and the administration was performed in sealed cages. The drug formulation administered was either coated with liposomes or a lipid base formulation. Unfortunately, severe adverse effects, such as weight loss and neurological toxicity were observed.

## 5.4 Doxorubicin

This chemotherapy drug has been administered as local treatment. Safety and efficacy assessments were performed with respiratory capacity tests (spirometry, diffusion capacity and 6 minute walking test), high performance liquid chromatography, V/Q display, imaging techniques, pathology findings, blood samples, confocal laser scanning microscopy and colorimetric assay for cell proliferation. Adverse effects observed included (a) acute chest pain, (b) wheezing, alveolar hemorrhage, (c) weight loss, (d) cardiac toxicity, (e) hypoxia and >20% reduction of pulmonary function tests values were observed. In order to cope with the adverse effects intravenous corticosteroids were administered. Consequently, novel compounds were developed to engulf doxorubicin as loaded microparticles or liposomes to coat the drug. The previously observed side effects were subsequently reduced [26,119,151,152]. The administration of the aerosolized drug was performed in specially designed cages, chambers or HEPA with hood 60 mg/m$^2$ q14D.

## 5.5 Gemcitabine

Gemcitabine has less severe systemic side effects when administered intravenously [153]. In previous studies gemcitabine was administered as aerosol in specially designed chambers and cages 2362 mg/m$^2$/week for 3 weeks. The evaluation of the drug distribution, safety and efficacy were performed with: (a) V/Q scan, (b) high performance liquid chromatography, (c) bronchoalveolar lavage, (d) blood samples, (e) pathology findings and (f) cell proliferation. The most severe adverse effects observed were emesis, bronchospasm and excessive cough and were dose depended. Moreover; acute pulmonary toxicity was observed in the form of pulmonary edema, neovascularization, and connective tissue formation [154–156].

## 5.6 9-nitrocamptothecine

9-NC has been previously investigated in animals and humans as aerosol and via instillation. The safety and efficiency profile has been investigated with blood and urine samples, imaging techniques, Ki-67 proliferation, pathology samples, platelet-endothelial cell adhesion and performance liquid chromatography. The only adverse effects observed included; (a) cough, (b) pharyngitis and (c) bronchitis and these were dose-dependent. The 9-NC has been administered only as a liposome form in doses of 26.6 $\mu$g/kg/day [55,157].

## 5.7 Bevacisumab

Bevacisumab has not been administered as a local treatment for NSCLC, however, it is a strong anti-neovascularization drug used to treat NSCLC [158]. It has been used for hereditary hemorrhagic telangiectasia-associated epistaxis as anasal spray and submucosal injections [159,160]. It has also been used as local instillation treatment in bronchial HPV patients [3]. The concept for its usage in general is to block the local anarchic proliferation of the vessels. The adverse effects observed were mainly ageusia, anosmia, headache, hemorrhage, hypertension, septal perforation and nasal obstruction (15 mg/kg on day 1Assessment of safety and efficacy was performed via blood samples, epistaxis severity score and phone administered custom-made questionnaires.

## 5.8 Immunotherapy

In a recently published multicenter study immunotherapy was administered locally with the help of 19G needles under the guidance of endobronchial ultrasound with a convex probe. The patients were stage four NSCLC and the safety and efficiency of their treatment was evaluated with positron emission tomography (PET-CT) and next genera-

tion sequencing (NGS) [12]. Indeed, the combination of intratumoral chemotherapy and immunotherapy improved survival rates in patients with ≤50% PD-L1 expression and in total to all patients that received intratumoral treatment versus only intravenous treatment (Nivolumab 10 mg/mL was purchased from Bristol-Myers-Squibb with the dosage being administered at 3 mg/kg, and calculated according to the weight). The reason for this positive effect was probably due to the local transformation of the tumor matrix were initial local application of chemotherapy initiated a production of proteins that enhanced the synergistic local effect of immunotherapy administration. The same concept as i.v chemotherapy plus immunotherapy, however, the major local adverse effect was the formation of abscess. Again abscess formation has been observed in a previous case series due to high PD-L1 expression and it is common in large NSCLC lesions [161]. There are two more studies that support that local aerosol administration of immunotherapy has a favorable effect and could be used alternatively in NSCLC for selected patients [162,163].

## 6. Local application

Local drug application can be achieved through instillation with a simple or spraying catheter [2]. There are different catheters that one can use for the instillation of a liquid drug in a specific area via bronchoscopic guidance, Small compressors exist that can be used to produce compressed air and with the use of a catheter with specially designed tips one can either instill a drug or spray it with the help of the compressor (Fig. 1).
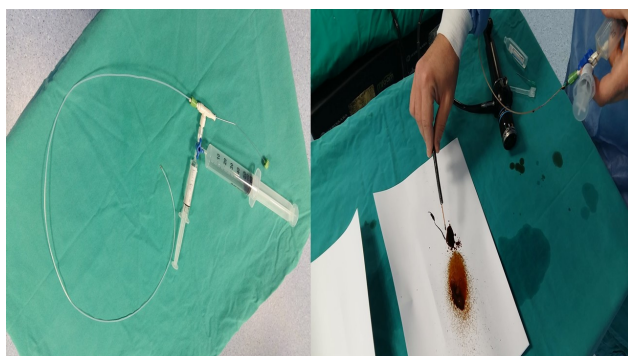


**Fig. 1. Left is the spraying catheter that produces aerosol with the help of positive pressure from a 60cc needle.** Right the procedure using iodine solution on a piece of paper to present the spraying effect. An air compressor can be used if available.

Through a simple catheter one can spray a drug in powder form (Fig. 2).

Biopsy needles exist that can be used to administer drugs locally within a tumor. These needles come in different sizes: 22G, 21G and 19G [12] (Figs. 3, 4, 5, 6).

Another needle design exists that facilitates the com-



**Fig. 2. Left an air compressor (yellow arrow), middle the drug in powder form, right green arrow sprayed powder inside a bronchus.**



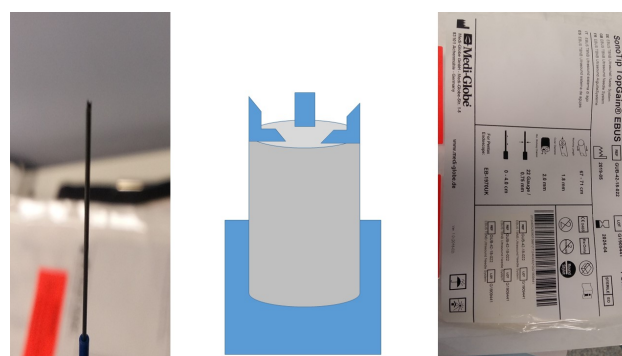**Fig. 3. 21G Olympus needle that can be used to apply drug solutions intratumorally.**



**Fig. 4. 22G Mediglobe Sonotip needle with a "CORONA" tip for enhanced cut-through effect and local drug application.**

bination of two drugs at the tip of the needle and the final combination can be applied in the designated exact location with the help of the bronchoscope (Fig. 7).

The "Blue fish balloon needle" is also available where a balloon catheter is expanded and at 12 o'clock a 34G needle is coming out to puncture the walls of a bronchus [164]. This needle is usually used to apply drugs through stents as local treatment for granulomatous tissue formation or lung cancer tissue (Figs. 8, 9).
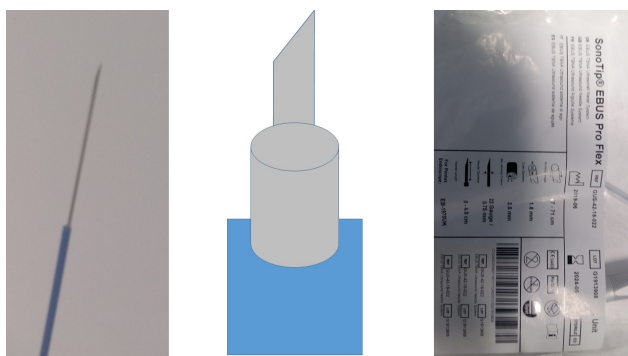
**Fig. 5. 22G Mediglobe simple needle that can be used to apply intratumorally drug solutions.**
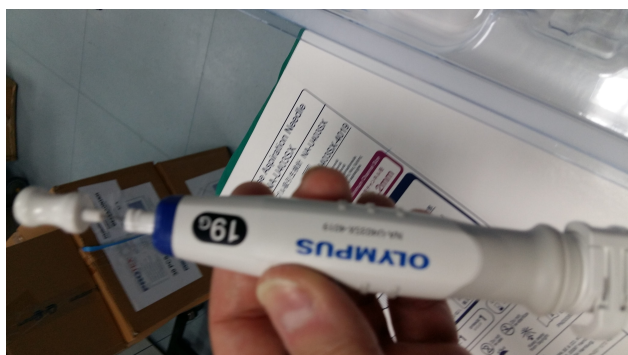


**Fig. 6. 19G Olympus needle that can be used to apply intratumorally drug solutions.**

## 7. Discussion

Several locally administered drugs have been used for the treatment of non-small cell lung cancer, delivered through inhalation, instillation or intratumoral administration. All these different forms of local administration come with different issues to resolve. In the case of aerosolized drugs administered through inhalation, underlying airway disease is an issue as previously demonstrated with inhaled insulin [24]. Another issue of course is the equipment that is used to produce small aerodynamic particles for inhalation. Upgrading the currently available equipment or coming up with novel equipment and techniques is necessary. In the case of central tumor obstruction, local ablation systems should be used prior to drug administration in order to secure airway patency. Various catheters can be used for different clinical situations, depending on the method of drug administration and drug chemical characteristics. Issues like spilling of possibly toxic to the surrounding tissues drugs need to be taken into consideration, because such occurrences might induce adverse effects such as pulmonary edema.

There is clearly need to use suitable equipment for targeted local administration and to create formulations that diffuse easily through the matrix of a lesion, to enhance fast, localized absorption. Autologous blood transplanta-



**Fig. 7. Needle with two separate channels in order to mix drugs at the tip.** It can be used for the application of coagulation drugs that need mixing.

tion is another method of local treatment and can be used in a variety of medical conditions [165,166]. The pros and cons of local application for each drug has been reported in the above sections. Local treatment has the major advantage of efficiency with less adverse effects. However; in order to achieve this we have to choose the optimal delivery system and the optimal drug for each environment. The treating physician has to choose according to a case by case scenario and choose the appropriate method. Another issue is the mode of ventilation and sedation for each patient. Again, this has to be chosen based on the underlying disease and method of application. The best combination of all these factors will have the best result (Table 1).
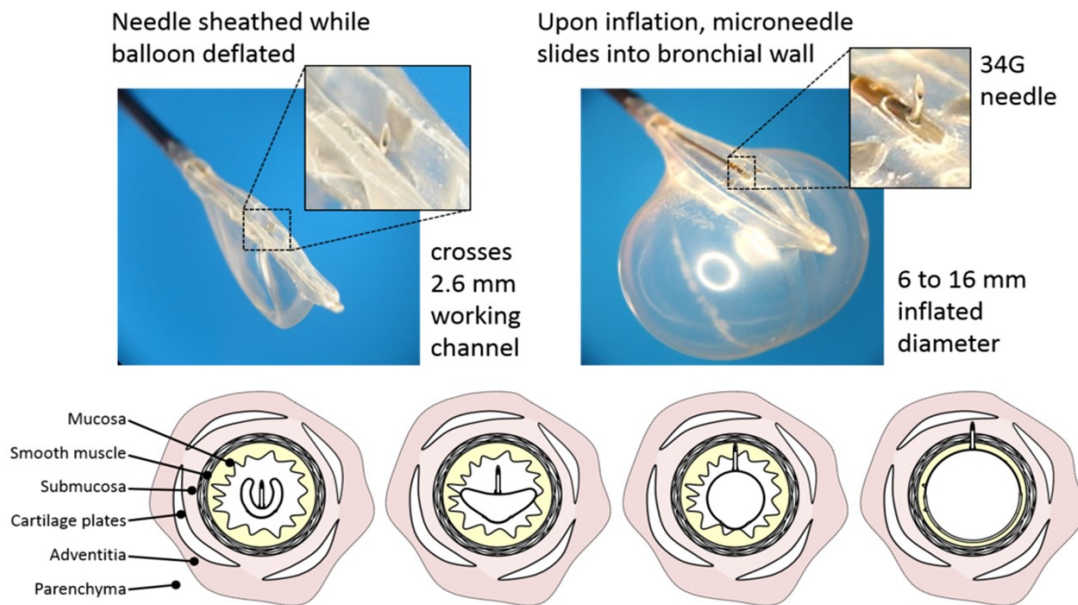
**Fig. 8. 34G "Blow Fish" needle that can apply drug in the bronchial epithelium through a silicon stent.**
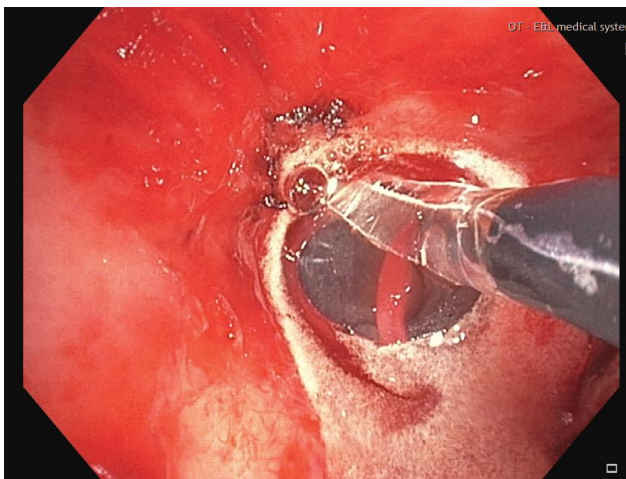


**Fig. 9. 34G "Blow Fish" needle in the trachea form the record of Professor Lutz Freitag, Department of Pulmonology, University Hospital Zurich, Rämistrasse 100, 8091, Zurich Switzerland.**

## 8. Conclusions

Local drug treatment of lung diseases is feasible, and suitable for managing many diseases because it reduces systemic adverse effects and increases local drug efficiency. It should be an option depending on the patients' current medical condition and history. However, novel delivery systems are needed and therefore clinicians should work closely with the pharmaceutical industry to produce the much-needed solutions.

## Author contributions

PZ, CK, KS, WHS, DM, KT, AL, CB, HH, CA, AI, CS wrote the manuscript and collected the data.

## Ethics approval and consent to participate

Not applicable.

## Conflict of interest

The authors declare no conflict of interest.

## References

[1] Criner GJ, Eberhardt R, Fernandez-Bussy S, Gompelmann D, Maldonado F, Patel N, *et al*. Interventional Bronchoscopy. American Journal of Respiratory and Critical Care Medicine. 2020; 202: 29–50.

[2] Zarogoulidis P, Tryfon S, Sapalidis K, Tsakiridis K, Baka S, Huang H, *et al*. Bronchial HPV; the good the bad and the unknown. Respiratory Medicine Case Reports. 2020; 30: 101053.

[3] Zarogoulidis P, Hatzibougias D, Tsakiridis K, Hohenforst-Schmidt W, Huang H, Bai C, *et al*. Interventional bronchoscopy for HPV 16 and 66 with the use of spraying interferon-alpha (2b) plus bevacizumab and anti-reflux agent. Respiratory Medicine Case Reports. 2021; 33: 101398.

[4] Zarogoulidis P, Darwiche K, Walter R, Li Q, Teschler H, Freitag L, *et al*. Research Spotlight: Sirolimus-coated stents for air-

way tracheal stenosis: a future 3D model concept with today's knowledge. Therapeutic Delivery. 2013; 4: 1093–1097.

[5] Zarogoulidis P, Sardeli C, Konstantinou F, Sapalidis K. Conventional Versus Therapeutic Stents for Airway Malignancies: Novel Local Therapies Underway. EBioMedicine. 2018; 33: 10–11.

[6] Freitag L, Gördes M, Zarogoulidis P, Darwiche K, Franzen D, Funke F, *et al*. Towards Individualized Tracheobronchial Stents: Technical, Practical and Legal Considerations. Respiration. 2017; 94: 442–456.

[7] Hohenforst-Schmidt W, Zarogoulidis P, Pitsiou G, Linsmeier B, Tsavlis D, Kioumis I, *et al*. Drug Eluting Stents for Malignant Airway Obstruction: a Critical Review of the Literature. Journal of Cancer. 2016; 7: 377–390.

[8] Huang H, Chen C, Bedi H, Bai C, Li Q, Hohenforst-Schmidt W, *et al*. Innovative use of a Montgomery cannula in the bronchoscopic management of tracheal stenosis and failed tracheostomy decannulation. Respiratory Medicine Case Reports. 2017; 22: 130–132.

[9] Zarogoulidis P, Darwiche K, Tsakiridis K, Teschler H, Yarmus L, Zarogoulidis K, *et al*. Learning from the Cardiologists and Developing Eluting Stents Targeting the Mtor Pathway for Pulmonary Application; a Future Concept for Tracheal Stenosis. Journal of Molecular and Genetic Medicine. 2013; 7: 65.

[10] Schwarz Y. Electromagnetic navigation. Clinics in Chest Medicine. 2010; 31: 65–73.

[11] Yuan H, Wang X, Sun J, Xie F, Zheng X, Tao G, *et al*. Flexible bronchoscopy-guided microwave ablation in peripheral porcine lung: a new minimally-invasive ablation. Translational Lung Cancer Research. 2019; 8: 787–796.

[12] Zarogoulidis P, Hohenforst-Schmidt W, Huang H, Zhou J, Wang Q, Wang X, *et al*. Intratumoral Treatment with Chemotherapy and Immunotherapy for NSCLC with EBUS-TBNA 19G. Journal of Cancer. 2021; 12: 2560–2569.

[13] Cho RJ, Senitko M, Wong J, Dincer EH, Khosravi H, Abraham GE. Feasibility of Using the O-Arm Imaging System during ENB-rEBUS–guided Peripheral Lung Biopsy. Journal of Bronchology & Interventional Pulmonology. 2021; 28: 248–254.

[14] Houtmeyers E, Gosselink R, Gayan-Ramirez G, Decramer M. Regulation of mucociliary clearance in health and disease. The European Respiratory Journal. 1999; 13: 1177–1188.

[15] Summers QA. Inhaled drugs and the lung. Clinical and Experimental Allergy. 1991; 21: 259–268.

[16] Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. American Journal of Respiratory Cell and Molecular Biology. 1992; 6: 235–243.

[17] Folkesson HG, Matthay MA, Weström BR, Kim KJ, Karlsson BW, Hastings RH. Alveolar epithelial clearance of protein. Journal of Applied Physiology. 1996; 80: 1431–1445.

[18] Martonen TB. Mathematical model for the selective deposition of inhaled pharmaceuticals. Journal of Pharmaceutical Sciences. 1993; 82: 1191–1199.

[19] Suarez S, Hickey AJ. Drug properties affecting aerosol behavior. Respiratory Care. 2000; 45: 652–666.

[20] Finbloom JA, Sousa F, Stevens MM, Desai TA. Engineering the drug carrier biointerface to overcome biological barriers to drug delivery. Advanced Drug Delivery Reviews. 2020; 167: 89–108.

[21] Almughem FA, Aldossary AM, Tawfik EA, Alomary MN, Alharbi WS, Alshahrani MY, *et al*. Cystic Fibrosis: Overview of the Current Development Trends and Innovative Therapeutic Strategies. Pharmaceutics. 2020; 12: 616.

[22] Labiris NR, Dolovich MB. Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications. British Journal of Clinical Pharmacology. 2003; 56: 588–599.

[23] Deffebach ME, Charan NB, Lakshminarayan S, Butler J. The bronchial circulation. Small, but a vital attribute of the lung. The American Review of Respiratory Disease. 1987; 135: 463–481.

[24] Zarogoulidis P, Papanas N, Kouliatsis G, Spyratos D, Zarogoulidis K, Maltezos E. Inhaled insulin: too soon to be forgotten? Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2011; 24: 213–223.

[25] Gagnadoux F, Hureaux J, Vecellio L, Urban T, Le Pape A, Valo I, *et al*. Aerosolized chemotherapy. Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2008; 21: 61–70.

[26] Otterson GA, Villalona-Calero MA, Sharma S, Kris MG, Imondi A, Gerber M, *et al*. Phase I study of inhaled Doxorubicin for patients with metastatic tumors to the lungs. Clinical Cancer Research. 2007; 13: 1246–1252.

[27] Wittgen BPH, Kunst PWA, van der Born K, van Wijk AW, Perkins W, Pilkiewicz FG, *et al*. Phase I study of aerosolized SLIT cisplatin in the treatment of patients with carcinoma of the lung. Clinical Cancer Research. 2007; 13: 2414–2421.

[28] Kleinstreuer C, Zhang Z, Li Z. Modeling airflow and particle transport/deposition in pulmonary airways. Respiratory Physiology & Neurobiology. 2008; 163: 128–138.

[29] Zhang Z, Kleinstreuer C, Kim CS. Airflow and nanoparticle deposition in a 16-generation tracheobronchial airway model. Annals of Biomedical Engineering. 2008; 36: 2095–2110.

[30] Leu AJ, Berk DA, Lymboussaki A, Alitalo K, Jain RK. Absence of functional lymphatics within a murine sarcoma: a molecular and functional evaluation. Cancer Research. 2000; 60: 4324–4327.

[31] Jain RK, Munn LL, Fukumura D. Dissecting tumour pathophysiology using intravital microscopy. Nature Reviews Cancer. 2002; 2: 266–276.

[32] Jain RK. Delivery of molecular and cellular medicine to solid tumors. Advanced Drug Delivery Reviews. 1997; 26: 71–90.

[33] Milosevic MF, Fyles AW, Wong R, Pintilie M, Kavanagh MC, Levin W, *et al*. Interstitial fluid pressure in cervical carcinoma: within tumor heterogeneity, and relation to oxygen tension. Cancer. 1998; 82: 2418–2426.

[34] Heldin C, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure - an obstacle in cancer therapy. Nature Reviews Cancer. 2004; 4: 806–813.

[35] Jain RK. Barriers to drug delivery in solid tumors. Scientific American. 1994; 271: 58–65.

[36] Jain RK. Transport of molecules in the tumor interstitium: a review. Cancer Research. 1987; 47: 3039–3051.

[37] Celikoglu F, Celikoglu SI, Goldberg EP. Intratumoural chemotherapy of lung cancer for diagnosis and treatment of draining lymph node metastasis. The Journal of Pharmacy and Pharmacology. 2010; 62: 287–295.

[38] Weber A, Morlin G, Cohen M, Williams-Warren J, Ramsey B, Smith A. Effect of nebulizer type and antibiotic concentration on device performance. Pediatric Pulmonology. 1997; 23: 249–260.

[39] Eschenbacher WL, Boushey HA, Sheppard D. Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone. The American Review of Respiratory Disease. 1984; 129: 211–215.

[40] Storm G, Crommelin DJ. Colloidal systems for tumor targeting. Hybridoma. 1997; 16: 119–125.

[41] Suarez S, Gonzalez-Rothi RJ, Schreier H, Hochhaus G. Effect of dose and release rate on pulmonary targeting of liposomal triamcinolone acetonide phosphate. Pharmaceutical Research. 1998; 15: 461–465.

[42] Fielding RM, Abra RM. Factors affecting the release rate of terbutaline from liposome formulations after intratracheal instillation in the guinea pig. Pharmaceutical Research. 1992; 9: 220–223.

[43] Cryan S. Carrier-based strategies for targeting protein and peptide drugs to the lungs. The AAPS Journal. 2005; 7: E20–E41.

[44] Gonzalez-Rothi RJ, Suarez S, Hochhaus G, Schreier H, Lukyanov A, Derendorf H, *et al.* Pulmonary targeting of liposomal triamcinolone acetonide phosphate. Pharmaceutical Research. 1996; 13: 1699–1703.

[45] Labiris NR, Dolovich MB. Pulmonary drug delivery. Part II: the role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications. British Journal of Clinical Pharmacology. 2003; 56: 600–612.

[46] Gonzalez-Rothi RJ, Straub L, Cacace JL, Schreier H. Liposomes and pulmonary alveolar macrophages: functional and morphologic interactions. Experimental Lung Research. 1991; 17: 687–705.

[47] Allen TM. Liposomal drug formulations. Rationale for development and what we can expect for the future. Drugs. 1998; 56: 747–756.

[48] Schreier H, Gagné L, Bock T, Erdos GW, Druzgala P, Conary JT, *et al.* Physicochemical properties and *in vitro* toxicity of cationic liposome cDNA complexes. Pharmaceutica Acta Helvetiae. 1997; 72: 215–223.

[49] Niven RW, Schreier H. Nebulization of liposomes. I. Effects of lipid composition. Pharmaceutical Research. 1990; 7: 1127–1133.

[50] Desai TR, Hancock REW, Finlay WH. A facile method of delivery of liposomes by nebulization. Journal of Controlled Release. 2002; 84: 69–78.

[51] Niven RW, Speer M, Schreier H. Nebulization of liposomes. II. the effects of size and modeling of solute release profiles. Pharmaceutical Research. 1991; 8: 217–221.

[52] Niven RW, Carvajal TM, Schreier H. Nebulization of liposomes. III. the effects of operating conditions and local environment. Pharmaceutical Research. 1992; 9: 515–520.

[53] Joshi M, Misra A. Dry powder inhalation of liposomal Ketotifen fumarate: formulation and characterization. International Journal of Pharmaceutics. 2001; 223: 15–27.

[54] Skalko-Basnet N, Pavelic Z, Becirevic-Lacan M. Liposomes containing drug and cyclodextrin prepared by the one-step spray-drying method. Drug Development and Industrial Pharmacy. 2000; 26: 1279–1284.

[55] Koshkina NV, Waldrep JC, Roberts LE, Golunski E, Melton S, Knight V. Paclitaxel liposome aerosol treatment induces inhibition of pulmonary metastases in murine renal carcinoma model. Clinical Cancer Research. 2001; 7: 3258–3262.

[56] Omri A, Beaulac C, Bouhajib M, Montplaisir S, Sharkawi M, Lagacé J. Pulmonary retention of free and liposome-encapsulated tobramycin after intratracheal administration in uninfected rats and rats infected with Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy. 1994; 38: 1090–1095.

[57] Woodle MC, Collins LR, Sponsler E, Kossovsky N, Papahadjopoulos D, Martin FJ. Sterically stabilized liposomes. Reduction in electrophoretic mobility but not electrostatic surface potential. Biophysical Journal. 1992; 61: 902–910.

[58] Deol P, Khuller GK. Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. Biochimica Et Biophysica Acta (BBA) - General Subjects. 1997; 1334: 161–172.

[59] Hutchinson FG, Furr BJ. Biodegradable polymers for controlled release of peptides and proteins. Horizons in Biochemistry and Biophysics. 1989; 9: 111–129.

[60] Ehrhardt C, Fiegel J, Fuchs S, Abu-Dahab R, Schaefer UF, Hanes J, *et al.* Drug absorption by the respiratory mucosa: cell culture models and particulate drug carriers. Journal of Aerosol Medicine. 2002; 15: 131–139.

[61] Kawashima Y, Yamamoto H, Takeuchi H, Fujioka S, Hino T. Pulmonary delivery of insulin with nebulized DL-lactide/glycolide copolymer (PLGA) nanospheres to prolong hypoglycemic effect. Journal of Controlled Release. 1999; 62: 279–287.

[62] Cheng YS, Yazzie D, Gao J, Muggli D, Etter J, Rosenthal GJ. Particle characteristics and lung deposition patterns in a human airway replica of a dry powder formulation of polylactic acid produced using supercritical fluid technology. Journal of Aerosol Medicine. 2003; 16: 65–73.

[63] Dhiman N, Khuller GK. Protective efficacy of mycobacterial 71-kDa cell wall associated protein using poly (DL-lactide-co-glycolide) microparticles as carrier vehicles. FEMS Immunology and Medical Microbiology. 1998; 21: 19–28.

[64] Evora C, Soriano I, Rogers RA, Shakesheff KN, Hanes J, Langer R. Relating the phagocytosis of microparticles by alveolar macrophages to surface chemistry: the effect of 1,2-dipalmitoylphosphatidylcholine. Journal of Controlled Release. 1998; 51: 143–152.

[65] Fiegel J, Ehrhardt C, Schaefer UF, Lehr C, Hanes J. Large porous particle impingement on lung epithelial cell monolayers–toward improved particle characterization in the lung. Pharmaceutical Research. 2003; 20: 788–796.

[66] Bittner B, Kissel T. Ultrasonic atomization for spray drying: a versatile technique for the preparation of protein loaded biodegradable microspheres. Journal of Microencapsulation. 1999; 16: 325–341.

[67] Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Advanced Drug Delivery Reviews. 2001; 47: 39–54.

[68] Langer R. Drug delivery and targeting. Nature. 1998; 392: 5–10.

[69] Tsapis N, Bennett D, Jackson B, Weitz DA, Edwards DA. Trojan particles: large porous carriers of nanoparticles for drug delivery. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99: 12001–12005.

[70] Edwards DA, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew ML, *et al.* Large porous particles for pulmonary drug delivery. Science. 1997; 276: 1868–1871.

[71] Chao P, Deshmukh M, Kutscher HL, Gao D, Rajan SS, Hu P, *et al.* Pulmonary targeting microparticulate camptothecin delivery system: anticancer evaluation in a rat orthotopic lung cancer model. Anti-Cancer Drugs. 2010; 21: 65–76.

[72] Cannon GJ, Swanson JA. The macrophage capacity for phagocytosis. Journal of Cell Science. 1992; 101: 907–913.

[73] Steckel H, Bolzen N. Alternative sugars as potential carriers for dry powder inhalations. International Journal of Pharmaceutics. 2004; 270: 297–306.

[74] Kobayashi S, Kondo S, Juni K. Pulmonary delivery of salmon calcitonin dry powders containing absorption enhancers in rats. Pharmaceutical Research. 1996; 13: 80–83.

[75] Winters MA, Knutson BL, Debenedetti PG, Sparks HG, Przybycien TM, Stevenson CL, *et al.* Precipitation of proteins in supercritical carbon dioxide. Journal of Pharmaceutical Sciences. 1996; 85: 586–594.

[76] Sellers SP, Clark GS, Sievers RE, Carpenter JF. Dry powders of stable protein formulations from aqueous solutions prepared using supercritical CO(2)-assisted aerosolization. Journal of Pharmaceutical Sciences. 2001; 90: 785–797.

[77] Hickey WA. Factors influencing the distortion of sex ratio in Aedes aegypti. Journal of Medical Entomology. 1970; 7: 727–735.

[78] Maa YF, Nguyen PA, Sweeney T, Shire SJ, Hsu CC. Protein inhalation powders: spray drying vs spray freeze drying. Pharmaceutical Research. 1999; 16: 249–254.

[79] Klink DT, Chao S, Glick MC, Scanlin TF. Nuclear translocation of lactosylated poly-L-lysine/cDNA complex in cystic fibrosis airway epithelial cells. Molecular Therapy. 2001; 3: 831–841.

[80] . Kawashima Y, Serigano T, Hino T, Yamamoto H, Takeuchi H. A new powder design method to improve inhalation efficiency of pranlukast hydrate dry powder aerosols by surface modification with hydroxypropylmethylcellulose phthalate nanospheres. Pharmaceutical Research. 1998; 15: 1748–1752.

[81] Rajewski RA, Stella VJ. Pharmaceutical applications of cyclodextrins. 2. *in vivo* drug delivery. Journal of Pharmaceutical Sciences. 1996; 85: 1142–1169.

[82] Woodle MC, Scaria P, Ganesh S, Subramanian K, Titmas R, Cheng C, *et al*. Sterically stabilized polyplex: ligand-mediated activity. Journal of Controlled Release. 2001; 74: 309–311.

[83] Klonne DR, Dodd DE, Losco PE, Troup CM, Tyler TR. Two-week aerosol inhalation study on polyethylene glycol (PEG) 3350 in F-344 rats. Drug and Chemical Toxicology. 1989; 12: 39–48.

[84] Zhang L, Zhu W, Song L, Wang Y, Jiang H, Xian S, *et al*. Effects of hydroxylpropyl-beta-cyclodextrin on *in vitro* insulin stability. International Journal of Molecular Sciences, 2009; 10: 2031–2040.

[85] Brück A, Abu-Dahab R, Borchard G, Schäfer UF, Lehr CM. Lectin-functionalized liposomes for pulmonary drug delivery: interaction with human alveolar epithelial cells. Journal of Drug Targeting. 2001; 9: 241–251.

[86] Abu-Dahab R, Schäfer UF, Lehr CM. Lectin-functionalized liposomes for pulmonary drug delivery: effect of nebulization on stability and bioadhesion. European Journal of Pharmaceutical Sciences. 2001; 14: 37–46.

[87] Yi SM, Harson RE, Zabner J, Welsh MJ. Lectin binding and endocytosis at the apical surface of human airway epithelia. Gene Therapy. 2001; 8: 1826–1832.

[88] Kloss A, Henklein P, Siele D, Schmolke M, Apcher S, Kuehn L, *et al*. The cell-penetrating peptide octa-arginine is a potent inhibitor of proteasome activities. European Journal of Pharmaceutics and Biopharmaceutics. 2009; 72: 219–225.

[89] Strayer MS, Guttentag SH, Ballard PL. Targeting type II and Clara cells for adenovirus-mediated gene transfer using the surfactant protein B promoter. American Journal of Respiratory Cell and Molecular Biology. 1998; 18: 1–11.

[90] Goren D, Horowitz AT, Tzemach D, Tarshish M, Zalipsky S, Gabizon A. Nuclear delivery of doxorubicin via folate-targeted liposomes with bypass of multidrug-resistance efflux pump. Clinical Cancer Research. 2000; 6: 1949–1957.

[91] Cristiano RJ, Roth JA. Epidermal growth factor mediated DNA delivery into lung cancer cells via the epidermal growth factor receptor. Cancer Gene Therapy. 1996; 3: 4–10.

[92] Lundberg M, Wikström S, Johansson M. Cell surface adherence and endocytosis of protein transduction domains. Molecular Therapy. 2003; 8: 143–150.

[93] Derossi D, Calvet S, Trembleau A, Brunissen A, Chassaing G, Prochiantz A. Cell Internalization of the third Helix of the Antennapedia Homeodomain is Receptor-independent. Journal of Biological Chemistry. 1996; 271: 18188–18193.

[94] Kurten RC. Sorting motifs in receptor trafficking. Advanced Drug Delivery Reviews. 2003; 55: 1405–1419.

[95] Hasegawa S, Hirashima N, Nakanishi M. Microtubule involvement in the intracellular dynamics for gene transfection mediated by cationic liposomes. Gene Therapy. 2001; 8: 1669–1673.

[96] Leslie EM, Deeley RG, Cole SPC. Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicology and Applied Pharmacology. 2005; 204: 216–237.

[97] Sharom FJ. ABC multidrug transporters: structure, function and role in chemoresistance. Pharmacogenomics. 2008; 9: 105–127.

[98] Zhang L, Strong JM, Qiu W, Lesko LJ, Huang S. Scientific Perspectives on Drug Transporters and their Role in Drug Interactions. Molecular Pharmaceutics. 2006; 3: 62–69.

[99] Lechapt-Zalcman E, Hurbain I, Lacave R, Commo F, Urban T, Antoine M, *et al*. MDR1-Pgp 170 expression in human bronchus. The European Respiratory Journal. 1997; 10: 1837–1843.

[100] Scheffer GL, Pijnenborg ACLM, Smit EF, Müller M, Postma DS, Timens W, *et al*. Multidrug resistance related molecules in human and murine lung. Journal of Clinical Pathology. 2002; 55: 332–339.

[101] van der Valk P, van Kalken CK, Ketelaars H, Broxterman HJ, Scheffer G, Kuiper CM, *et al*. Distribution of multi-drug resistance-associated P-glycoprotein in normal and neoplastic human tissues. Analysis with 3 monoclonal antibodies recognizing different epitopes of the P-glycoprotein molecule. Annals of Oncology. 1990; 1: 56–64.

[102] Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. The Journal of Histochemistry and Cytochemistry. 1990; 38: 1277–1287.

[103] Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Advanced Drug Delivery Reviews. 2003; 55: 3–29.

[104] Hsia T, Lin C, Wang J, Ho S, Kao A. Relationship between chemotherapy response of small cell lung cancer and P-glycoprotein or multidrug resistance-related protein expression. Lung. 2002; 180: 173–179.

[105] Lallemand JY, Stoven V, Annereau JP, Boucher J, Blanquet S, Barthe J, *et al*. Induction by antitumoral drugs of proteins that functionally complement CFTR: a novel therapy for cystic fibrosis? The Lancet. 1997; 350: 711–712.

[106] Naumann N, Siratska O, Gahr M, Rösen-Wolff A. P-glycoprotein expression increases ATP release in respiratory cystic fibrosis cells. Journal of Cystic Fibrosis. 2005; 4: 157–168.

[107] Ye S, MacEachran DP, Hamilton JW, O'Toole GA, Stanton BA. Chemotoxicity of doxorubicin and surface expression of P-glycoprotein (MDR1) is regulated by the Pseudomonas aeruginosa toxin Cif. American Journal of Physiology. Cell Physiology. 2008; 295: C807–C818.

[108] van der Deen M, Marks H, Willemse BWM, Postma DS, Müller M, Smit EF, *et al*. Diminished expression of multidrug resistance-associated protein 1 (MRP1) in bronchial epithelium of COPD patients. Virchows Archiv. 2007; 449: 682–688.

[109] Blokzijl H, Vander Borght S, Bok LIH, Libbrecht L, Geuken M, van den Heuvel FAJ, *et al*. Decreased P-glycoprotein (P-gp/MDR1) expression in inflamed human intestinal epithelium is independent of PXR protein levels. Inflammatory Bowel Diseases. 2007; 13: 710–720.

[110] Demeule M, Jodoin J, Beaulieu E, Brossard M, Béliveau R. Dexamethasone modulation of multidrug transporters in normal tissues. FEBS Letters. 1999; 442: 208–214.

[111] Dinis-Oliveira RJ, Duarte JA, Remião F, Sánchez-Navarro A, Bastos ML, Carvalho F. Single high dose dexamethasone treatment decreases the pathological score and increases the survival rate of paraquat-intoxicated rats. Toxicology. 2006; 227: 73–85.

[112] Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, *et al*. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94: 2031–2035.

[113] Langmann T, Mauerer R, Zahn A, Moehle C, Probst M, Stremmel W, *et al*. Real-Time Reverse Transcription-PCR Expression Profiling of the Complete Human ATP-Binding Cassette Transporter Superfamily in Various Tissues. Clinical Chemistry. 2003; 49: 230–238.

[114] Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ,

Juijn JA, *et al*. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Research. 1997; 57: 3537–3547.

[115] Flens MJ, Zaman GJ, van der Valk P, Izquierdo MA, Schroeijers AB, Scheffer GL, *et al*. Tissue distribution of the multidrug resistance protein. The American Journal of Pathology. 1996; 148: 1237–1247.

[116] Bréchot J, Hurbain I, Fajac A, Daty N, Bernaudin J. Different Pattern of MRP Localization in Ciliated and Basal Cells from Human Bronchial Epithelium. Journal of Histochemistry & Cytochemistry. 1998; 46: 513–517.

[117] van der Deen M, de Vries EGE, Visserman H, Zandbergen W, Postma DS, Timens W, *et al*. Cigarette smoke extract affects functional activity of MRP1 in bronchial epithelial cells. Journal of Biochemical and Molecular Toxicology. 2008; 21: 243–251.

[118] van der Deen M, Homan S, Timmer-Bosscha H, Scheper RJ, Timens W, Postma DS, *et al*. Effect of COPD treatments on MRP1-mediated transport in bronchial epithelial cells. International Journal of Chronic Obstructive Pulmonary Disease. 2008; 3: 469–475.

[119] Garbuzenko OB, Saad M, Pozharov VP, Reuhl KR, Mainelis G, Minko T. Inhibition of lung tumor growth by complex pulmonary delivery of drugs with oligonucleotides as suppressors of cellular resistance. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107: 10737–10742.

[120] Bleasby K, Castle JC, Roberts CJ, Cheng C, Bailey WJ, Sina JF, *et al*. Expression profiles of 50 xenobiotic transporter genes in humans and pre-clinical species: a resource for investigations into drug disposition. Xenobiotica. 2006; 36: 963–988.

[121] Horvath G, Mendes ES, Schmid N, Schmid A, Conner GE, Salathe M, *et al*. The effect of corticosteroids on the disposal of long-acting beta2-agonists by airway smooth muscle cells. The Journal of Allergy and Clinical Immunology. 2007; 120: 1103–1109.

[122] Lips KS, Volk C, Schmitt BM, Pfeil U, Arndt P, Miska D, *et al*. Polyspecific Cation Transporters Mediate Luminal Release of Acetylcholine from Bronchial Epithelium. American Journal of Respiratory Cell and Molecular Biology. 2005; 33: 79–88.

[123] Horvath G, Schmid N, Fragoso MA, Schmid A, Conner GE, Salathe M, *et al*. Epithelial organic cation transporters ensure pH-dependent drug absorption in the airway. American Journal of Respiratory Cell and Molecular Biology. 2007; 36: 53–60.

[124] Kummer W, Lips KS, Pfeil U. The epithelial cholinergic system of the airways. Histochemistry and Cell Biology. 2008; 130: 219–234.

[125] Kummer W, Wiegand S, Akinci S, Schinkel AH, Wess J, Koepsell H, *et al*. Role of acetylcholine and muscarinic receptors in serotonin-induced bronchoconstriction in the mouse. Journal of Molecular Neuroscience. 2006; 30: 67–68.

[126] Groneberg DA, Fischer A, Chung KF, Daniel H. Molecular mechanisms of pulmonary peptidomimetic drug and peptide transport. American Journal of Respiratory Cell and Molecular Biology. 2004; 30: 251–260.

[127] Søndergaard HB, Brodin B, Nielsen CU. HPEPT1 is responsible for uptake and transport of Gly-Sar in the human bronchial airway epithelial cell-line Calu-3. Pflugers Archiv. 2008; 456: 611–622.

[128] Groneberg DA. Distribution and function of the peptide transporter PEPT2 in normal and cystic fibrosis human lung. Thorax. 2002; 57: 55–60.

[129] Endter S, Francombe D, Ehrhardt C, Gumbleton M. RT-PCR analysis of ABC, SLC and SLCO drug transporters in human lung epithelial cell models. Journal of Pharmacy and Pharmacology. 2009; 61: 583–591.

[130] Saito H, Terada T, Okuda M, Sasaki S, Inui K. Molecular cloning and tissue distribution of rat peptide transporter PEPT2. Biochimica Et Biophysica Acta. 1996; 1280: 173–177.

[131] Bosquillon C. Drug transporters in the lung–do they play a role in the biopharmaceutics of inhaled drugs? Journal of Pharmaceutical Sciences. 2010; 99: 2240–2255.

[132] Miyazaki H, Sekine T, Endou H. The multispecific organic anion transporter family: properties and pharmacological significance. Trends in Pharmacological Sciences. 2004; 25: 654–662.

[133] Hagenbuch B, Gui C. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. Xenobiotica. 2008; 38: 778–801.

[134] Adachi H, Suzuki T, Abe M, Asano N, Mizutamari H, Tanemoto M, *et al*. Molecular characterization of human and rat organic anion transporter OATP-D. American Journal of Physiology. Renal Physiology. 2003; 285: F1188–F1197.

[135] Zarogoulidis P, Eleftheriadou E, Sapardanis I, Zarogoulidou V, Lithoxopoulou H, Kontakiotis T, *et al*. Feasibility and effectiveness of inhaled carboplatin in NSCLC patients. Investigational New Drugs. 2012; 30: 1628–1640.

[136] Levet V, Merlos R, Rosière R, Amighi K, Wauthoz N. Platinum pharmacokinetics in mice following inhalation of cisplatin dry powders with different release and lung retention properties. International Journal of Pharmaceutics. 2017; 517: 359–372.

[137] Wittgen BPH, Kunst PWA, Perkins WR, Lee JK, Postmus PE. Assessing a system to capture stray aerosol during inhalation of nebulized liposomal cisplatin. Journal of Aerosol Medicine. 2006; 19: 385–391.

[138] Selting K, Waldrep JC, Reinero C, Branson K, Gustafson D, Kim DY, *et al*. Feasibility and safety of targeted cisplatin delivery to a select lung lobe in dogs via the AeroProbe intracorporeal nebulization catheter. Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2008; 21: 255–268.

[139] Anderson K, Lawson KA, Simmons-Menchaca M, Sun L, Sanders BG, Kline K. A-TEA Plus Cisplatin Reduces Human Cisplatin-Resistant Ovarian Cancer Cell Tumor Burden and Metastasis. Experimental Biology and Medicine. 2004; 229: 1169–1176.

[140] El-Gendy N, Berkland C. Combination Chemotherapeutic Dry Powder Aerosols via Controlled Nanoparticle Agglomeration. Pharmaceutical Research. 2009; 26: 1752–1763.

[141] Tatsumura T, Yamamoto K, Murakami A, Tsuda M, Sugiyama S. New chemotherapeutic method for the treatment of tracheal and bronchial cancers–nebulization chemotherapy. Gan No Rinsho. 1983; 29: 765–770. (In Japanese)

[142] Wattenberg LW, Wiedmann TS, Estensen RD. Chemoprevention of cancer of the upper respiratory tract of the Syrian golden hamster by aerosol administration of difluoromethylornithine and 5-fluorouracil. Cancer Research. 2004; 64: 2347–2349.

[143] Tatsumura T, Koyama S, Tsujimoto M, Kitagawa M, Kagamimori S. Further study of nebulisation chemotherapy, a new chemotherapeutic method in the treatment of lung carcinomas: fundamental and clinical. British Journal of Cancer. 1993; 68: 1146–1149.

[144] Hitzman CJ, Wattenberg LW, Wiedmann TS. Pharmacokinetics of 5-fluorouracil in the hamster following inhalation delivery of lipid-coated nanoparticles. Journal of Pharmaceutical Sciences. 2006; 95: 1196–1211.

[145] Hitzman CJ, Elmquist WF, Wiedmann TS. Development of a respirable, sustained release microcarrier for 5-fluorouracil II: in vitro and in vivo optimization of lipid coated nanoparticles. Journal of Pharmaceutical Sciences. 2006; 95: 1127–1143.

[146] Hohenforst-Schmidt W, Zarogoulidis P, Darwiche K, Vogl T, Goldberg EP, Huang H, *et al*. Intratumoral chemotherapy for lung cancer: re-challenge current targeted therapies. Drug Design, Development and Therapy. 2013; 7: 571–583.

[147] Faiyazuddin M, Mujahid M, Hussain T, Siddiqui HH, Bhatnagar A, Khar RK, *et al*. Aerodynamics and deposition effects of inhaled submicron drug aerosol in airway diseases. Recent Patents on Inflammation & Allergy Drug Discovery. 2013; 7: 49–61.

[148] Hershey AE, Kurzman ID, Forrest LJ, Bohling CA, Stonerook M, Placke ME, *et al*. Inhalation chemotherapy for macroscopic primary or metastatic lung tumors: proof of principle using dogs with spontaneously occurring tumors as a model. Clinical Cancer Research. 1999; 5: 2653–2659.

[149] Koshkina NV, Golunski E, Roberts LE, Gilbert BE, Knight V. Cyclosporin a Aerosol Improves the Anticancer Effect of Paclitaxel Aerosol in Mice. Journal of Aerosol Medicine. 2004; 17: 7–14.

[150] Koshkina V, Koshkina NV, Golunski E, Roberts LE, Gilbert BE. Cyclosporin a Aerosol Improves the Anticancer Effect of Paclitaxel Aerosol in Mice. Transactions of the American Clinical and Climatological Association. 2004; 115: 395–404.

[151] Azarmi S, Tao X, Chen H, Wang Z, Finlay WH, Löbenberg R, *et al*. Formulation and cytotoxicity of doxorubicin nanoparticles carried by dry powder aerosol particles. International Journal of Pharmaceutics. 2006; 319: 155–161.

[152] Otterson GA, Villalona-Calero MA, Hicks W, Pan X, Ellerton JA, Gettinger SN, *et al*. Phase I/II study of inhaled doxorubicin combined with platinum-based therapy for advanced non-small cell lung cancer. Clinical Cancer Research. 2010; 16: 2466–2473.

[153] Wang L, Wang Z, Chen X, Li Y, Wang K, Xia Y, *et al*. First-line combination of gemcitabine, oxaliplatin, and L-asparaginase (GELOX) followed by involved-field radiation therapy for patients with stage IE/IIE extranodal natural killer/T-cell lymphoma. Cancer. 2013; 119: 348–355.

[154] Rodriguez CO, Crabbs TA, Wilson DW, Cannan VA, Skorupski KA, Gordon N, *et al*. Aerosol Gemcitabine: Preclinical Safety andIn VivoAntitumor Activity in Osteosarcoma-Bearing Dogs. Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2010; 23: 197–206.

[155] Gagnadoux F, Le Pape A, Urban T, Montharu J, Vecellio L, Dubus J, *et al*. Safety of pulmonary administration of gemcitabine in rats. Journal of Aerosol Medicine. 2005; 18: 198–206.

[156] Min R, Li T, Du J, Zhang Y, Guo J, Lu W. Pulmonary gemcitabine delivery for treating lung cancer: pharmacokinetics and acute lung injury aspects in animals. Canadian Journal of Physiology and Pharmacology. 2008; 86: 288–298.

[157] Riedel SB, Fischer SM, Sanders BG, Kline K. Vitamin E analog, alpha-tocopherol ether-linked acetic acid analog, alone and in combination with celecoxib, reduces multiplicity of ultraviolet-induced skin cancers in mice. Anti-Cancer Drugs. 2008; 19: 175–181.

[158] Reinmuth N, Bryl M, Bondarenko I, Syrigos K, Vladimirov V, Zereu M, *et al*. PF-06439535 (a Bevacizumab Biosimilar) Compared with Reference Bevacizumab (Avastin®), Both Plus Paclitaxel and Carboplatin, as First-Line Treatment for Advanced Non-Squamous Non-Small-Cell Lung Cancer: A Randomized, Double-Blind Study. Randomized Controlled Trials. 2019; 33: 555–570.

[159] Chen S, Karnezis T, Davidson TM. Safety of intranasal Bevacizumab (Avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. The Laryngoscope. 2011; 121: 644–646.

[160] Amedee RG. Efficacy of intranasal bevacizumab (avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. American Journal of Rhinology & Allergy. 2011; 25: 368.

[161] Zarogoulidis P, Athanasiou E, Tsiouda T, Hatzibougias D, Huang H, Bai C, *et al*. Immunotherapy "Shock" a case series of PD-L1 100% and pembrolizumab first-line treatment. Respiratory Medicine Case Reports. 2017; 22: 197–202.

[162] Sapalidis K, Zarogoulidis P, Pavlidis E, Laskou S, Katsaounis A, Koulouris C, *et al*. Aerosol Immunotherapy with or without Cisplatin for metastatic lung cancer non-small cell lung cancer disease: *in vivo* Study. a more efficient combination. Journal of Cancer. 2018; 9: 1973–1977.

[163] Schizas N, Lazopoulos A, Krimiotis D, Rallis T, Paliouras D, Gogakos A, *et al*. Beware of hemopneumothorax following core needle breast biopsy. Respiratory Medicine Case Reports. 2018; 25: 49–51.

[164] Medical Device Brand Name: Blowfish Micro-Infusion Catheter (model MDL09-0616-145-34). 2019. Available at: https://www.hipaaspace.com/medical_billing/coding/global.unique.medical.device.identification/M973BH201M1 (Accessed: 12 January 2022).

[165] Parody R, Sánchez-Ortega I, Ferrá C, Guardia R, Talarn C, Encuentra M, *et al*. Mobilization of Hematopoietic Stem Cells into Peripheral Blood for Autologous Transplantation Seems less Efficacious in Poor Mobilizers with the Use of a Biosimilar of Filgrastim and Plerixafor: a Retrospective Comparative Analysis. Oncology and Therapy. 2020; 8: 311–324.

[166] Hayden PJ, Roddie C, Bader P, Basak GW, Bonig H, Bonini C, *et al*. Management of Adults and Children receiving CAR T-cell therapy: 2021 Best Practice Recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). Annals of Oncology. 2021. (in press)