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Nonviral Pulmonary Delivery of siRNA

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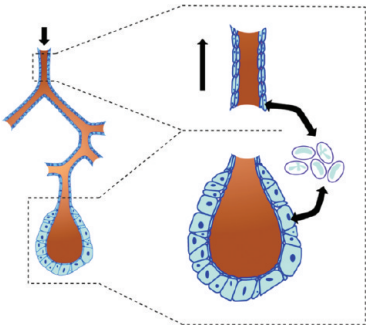

RNA interference (RNAi) is an important part of the cell's defenses against viruses and other foreign genes. Moreover, the biotechnological exploitation of RNAi offers therapeutic potential for a range of diseases for which drugs are currently unavailable. Unfortunately, the small interfering RNAs (siRNAs) that are central to RNAi in the cytoplasm are readily degradable by ubiquitous nucleases, are inefficiently targeted to desired organs and cell types, and are excreted quickly upon systemic injection. As a result, local administration techniques have been favored over the past few years, resulting in great success in the treatment of viral infections and other respiratory disorders.

Because there are several advantages of pulmonary delivery over systemic administration, two of the four siRNA drugs currently in phase II clinical trials are delivered intranasally or by inhalation. The air–blood barrier, however, has only limited permeability toward large, hydrophilic biopharmaceuticals such as nucleic acids; in addition, the lung imposes intrinsic hurdles to efficient siRNA delivery. Thus, appropriate formulations and delivery devices are very much needed. Although many different formulations have been optimized for in vitro siRNA delivery to lung cells, only a few have been reported successful in vivo. In this Account, we discuss both obstacles to pulmonary siRNA delivery and the success stories that have been achieved thus far.

The optimal pulmonary delivery vehicle should be neither cytotoxic nor immunogenic, should protect the payload from degradation by nucleases during the delivery process, and should mediate the intracellular uptake of siRNA. Further requirements include the improvement of the pharmacokinetics and lung distribution profiles of siRNA, the extension of lung retention times (through reduced recognition by macrophages), and the incorporation of reversible or stimuli-responsive binding of siRNA to allow for efficient release of the siRNAs at the target site. In addition, the ideal carrier would be biodegradable (to address difficulties with repeated administration for the treatment of chronic diseases) and would contain targeting moieties to enhance uptake by specific cell types. None of the currently available polymer- and lipid-based formulations meet every one of these requirements, but we introduce here several promising new approaches, including a biodegradable, nonimmunogenic polyester.

We also discuss imaging techniques for following the biodistribution according to the administration route. This tracking is crucial for better understanding the translocation and clearance of nanoformulated siRNA subsequent to pulmonary delivery.

In the literature, the success of pulmonary siRNA delivery is evaluated solely by relief from or prophylaxis against a disease; side effects are not studied in detail. It also remains unclear which cell types in the lung eventually take up siRNA. These are critical issues for the translational use of pulmonary siRNA formulations; accordingly, we present a flow cytometry technique that can be utilized to differentiate transfected cell populations in a mouse model that expresses transgenic enhanced green fluorescence protein (EGFP). This technique, in which different cell types are identified on the basis of their surface antigen expression, may eventually help in the development of safer carriers with minimized side effects in nontargeted tissues.

1. Introduction

In 2006, Andrew Fire and Greg Mello were awarded the Nobel Prize in Physiology for discovering gene silencing by long double-stranded RNA (dsRNA) in *Caenorhabditis elegans*.[1] Based on their findings that mRNA is degraded by sequence-complementary dsRNA,[1] a catalytic mechanism of a multiprotein complex was identified which incorporates short dsRNA.[2] This evolutionary conserved defense process for inactivation of foreign, for example, transposable, viral, or bacterial, dsRNA[3] can also be exploited biotechnologically. Long dsRNAs which reach the cytoplasm are degraded by "Dicer", an RNase III-like enzyme, into small interfering RNAs (siRNAs) of 21–25 nucleotides in length.[4] Subsequently, siRNA is incorporated into the RNA-induced

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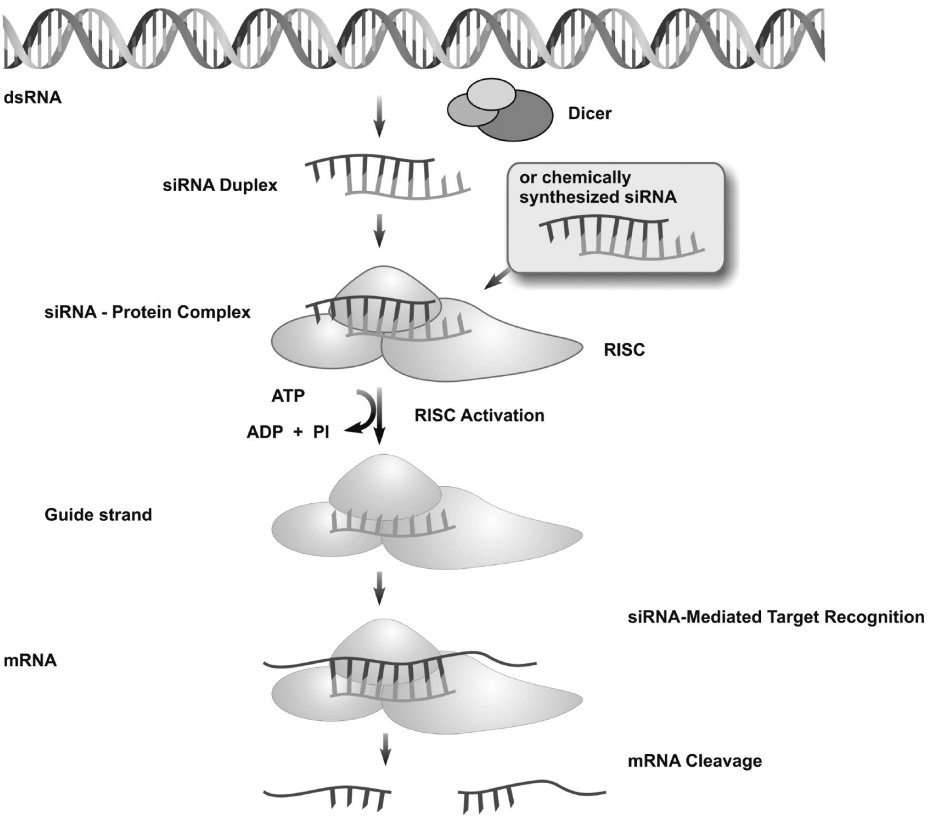

FIGURE 1. Endogenous and induced RNA interference: siRNA is incorporated into the RISC where complementary mRNA is cleaved after it binds to the guide strand of siRNA.

silencing complex (RISC) and cleaved upon activation of RISC. Argonaut, an endonuclease in the RISC, degrades the base-complementary mRNA after it binds to the antisense strand of siRNA, leading to down-regulation of the target gene expression (Figure 1).

After it was demonstrated in 2001 that siRNA can also knock down gene expression in mammalian cells,[5] RNAi has been routinely used in functional genomics and drug development.[6] Since siRNA can induce transient and reversible knockdown, it offers therapeutic potential for the treatment of currently "undruggable" diseases. Nevertheless, RNAi-based therapeutics are rather sparse with currently 11 siRNA drugs in clinical trials.[7] Due to the inefficient targeting of siRNA to the desired organs and cell types, rapid degradation by nucleases, and fast excretion upon systemic injection,[8] five of the clinical trials involving siRNA-based drugs utilize local administration to the eyes targeting age-related macular degeneration and diabetic retinopathy.[7] Other regional or local delivery routes include intradermal and pulmonary application. In fact, two of the four siRNA drugs currently in phase II clinical trials are delivered intranasally or by inhalation.[7]

Inhalation therapy goes back to the Ebers medical papyrus of about 1550 B.C. Since there are several advantages of aerosol delivery over systemic administration, such as local targeting, immediate availability, decreased systemic side effects, and noninvasive application, several obstacles encountered in systemic delivery of siRNA can be overcome by delivery via inhalation.[9] Additionally, the required dose of siRNA for efficient therapy can be substantially decreased. Most importantly, from a clinical point of view, pulmonary delivery of siRNA mediates direct access to lung epithelial cells which are affected in lung diseases such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), asthma, and pulmonary fibrosis. Besides, various viruses, including respiratory syncytial virus (RSV), parainfluenza virus (PIV), influenza, rhinoviruses, and severe acute respiratory syndrome (SARS) corona virus (SCV), infect the lung epithelium.[10] According to the World Health Organization (WHO), lung cancer is the eighth cause of death worldwide, tuberculosis is number 7, COPD number 4, and lower respiratory infections number 3.[11] In the light of this variety of lethal lung disorders, efficient therapies that lead to high and prolonged local drug concentration in the lung are much-needed.

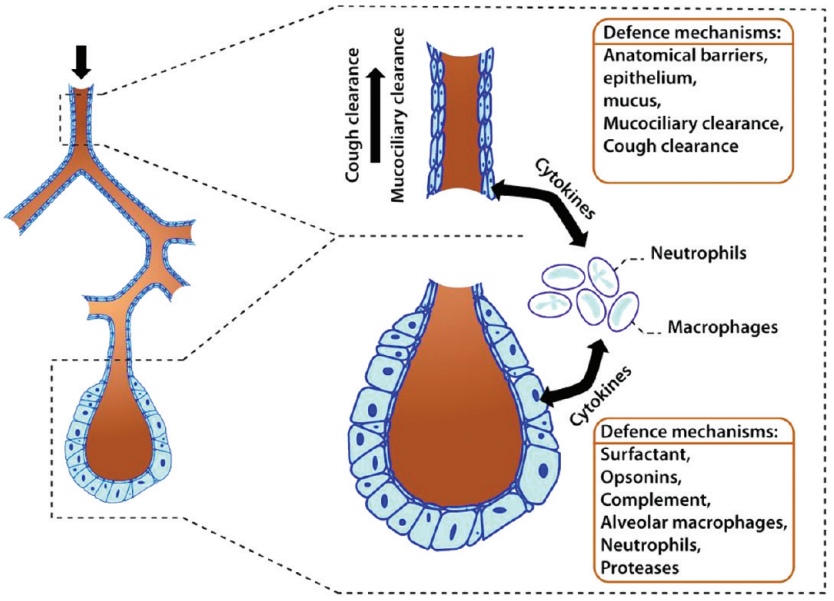

FIGURE 2. Lung-intrinsic barriers to efficient pulmonary siRNA delivery. Upper magnification, trachea; lower magnification, alveoli.

2. Biological Barriers to Pulmonary Delivery of siRNA

Since localized aerosol delivery allows for noninvasive ease of access, pulmonary formulations feature a history of patient acceptability and compliance.[12] The lung is well suited for the uptake of small, hydrophobic drug molecules due to its vast surface area and strong perfusion.[13] However, the air–blood barrier is only permeable to a limited extent for large, hydrophilic biopharmaceuticals such as nucleic acids. Although siRNA is small compared to plasmid DNA, siRNA duplexes with 7 nm in length and 13 kDa in weight are approximately 50 times larger in terms of molecular mass than typical small-molecule drugs.[14] Additionally, siRNA is strongly negatively charged with about 40 phosphate groups in the siRNA backbone leading to poor cellular uptake, which limits the use of siRNA even in local application approaches. While pulmonary delivery avoids interactions with serum proteins that can degrade siRNA after intravenous administration, the lung imposes intrinsic hurdles to efficient siRNA delivery. Lung-specific barriers include the reticulate pulmonary architecture ranging from the trachea to the alveoli, active clearance processes, such as mucociliary clearance and cough clearance, and effective immune responses, mainly mediated by macrophages and the influx of polymorphic neutrophils (PMNs),[15] that prevent the invasion of foreign material into the lung.[12] Additionally, the presence of respiratory mucus in the upper airways and airway surface liquid (surfactant) in the lower airways constitutes major physical and chemical barriers hampering the cellular delivery of siRNA (Figure 2) by entrapping siRNA-loaded nanoparticles and by slowing down the velocity of their transport.[16] For efficient therapeutic effects, siRNA needs to be directed to its target region[17] and should be taken up by the target cell populations.[18] Therefore, appropriate carrier systems need to be developed that address the improvement of the poor cellular uptake of siRNA and difficulties with repeated administration for the treatment of chronic diseases.

3. Formulations for Pulmonary Delivery

The bottlenecks in RNAi are the translocation of siRNA across the plasma membrane and its subsequent release from the endosomal compartment. Due to their susceptibility to degradation by ubiquitous nucleases[19] and their strongly negative surface charge, siRNA molecules require effective formulation that additionally maintains prolonged local drug concentrations and prevents siRNA from fast renal clearance in vivo.[8] The ideal delivery system should be biodegradable and biocompatible, should protect the payload from degradation by nucleases during the delivery process, should maintain long circulation times after systemic administration or long retention times after local administration by avoiding recognition by macrophages, and should reversibly (or stimuli-responsively) bind siRNA to allow for efficient release of the siRNAs at the target site.

Despite all barriers, successful local siRNA therapy, such as reduction of RSV and PIV titers in the lung,[20] attenuation of local pulmonary chemokine production after acute lung

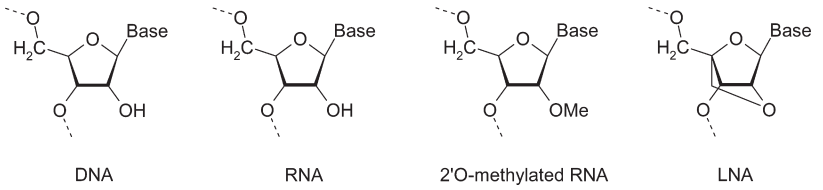

FIGURE 3. Modifications of RNA used for pulmonary delivery in comparison to unmodified DNA and RNA.

injury (ALI)[21,22] and infection with *M. tuberculosis*,[23] and protection from SARS infection,[24] has been achieved with naked siRNA,[23,25−28] or after administration with only isotonic dextrose solution,[24] saline,[21] or PBS[22,29] as carrier. As it was reported that surfactant produced by type II pneumocytes may facilitate nucleic acid delivery,[30] it may seem that for pulmonary siRNA delivery additional carriers are not necessary.

However, in the light of a recent report, the retrospective interpretation of some of these results is impossible because some control sequences used[21,22,31] were less immunostimulatory than the siRNA sequences targeting the gene of interest.[32] The results described in the aforementioned studies may therefore also be due to immunostimulation rather than to sequence-specific RNAi effects.

Consequently, from today's perspective, smart formulations are needed which stabilize siRNA, improve its pharmacokinetics and lung distribution profiles, and enhance cellular uptake and cytoplasmic localization of siRNA in target tissues and specific lung cell types in vivo. Such favorable delivery systems would additionally minimize potential side effects in nontargeted tissues.

3.1. Chemical Stabilization of siRNA. Since siRNA is easily enzymatically degraded, chemical modifications of the siRNA backbone and/or ribose[33] were applied to improve in vivo stability. The additional benefit of methylation of the ribose 2′-hydroxyl group (2′-OMe, Figure 3), in particular, is the diminishing of immunostimulatory effects.[34] A further modification to siRNA concerns the length of the double strands. While siRNA is usually a 21mer duplex comparable to naturally occurring products of Dicer,[2] so-called Dicer substrate inhibitory RNA (DsiRNA) of partly 2′-OMe 27mer blunt double strands can be up to 100-fold more potent than 21mer duplexes.[35] Formulated with PEGylated polyethylenimine (PEG-PEI), DsiRNA efficiently downregulated enhanced green fluorescent protein (EGFP) in bronchial and alveolar tissue post intratracheal instillation.[36] In the same animal model, intranasal delivery of chitosan nanoparticles containing siLNA efficiently reduced EGFP protein expression in the bronchial epithelium.[37] Alternatively, siRNA-expressing plasmids (shRNA)[18,38−40] can be complexed by carriers which have been optimized for DNA delivery, such as PEI[40] or chitosan.[39]

Many of the clinical trials also use chemically modified siRNA. While it is unknown whether ZaBeCor's Excellair is modified, Alynlam's ALN-RSV01 that succeeded in the phase II GEMINI study is cholesterol-attached siRNA.

3.2. Polymers and Lipids. In the first publication describing protection of mice against lethal challenges of highly pathogenic avian influenza A virus by intranasal coadministration of siRNA, the well-known commercially available transfection reagent Oligofectamine was used.[41] In one of the following reports about pulmonary siRNA delivery which was the basis to take siRNA against RSV into the clinic, it was shown that pretreatment could abolish RSV infection, while naked siRNA was only about 70% as effective as upon formulation with the cationic lipid transfection reagent TransIT-TKO.[20] In this study, 70 μg of siRNA was administered intranasally with no interferon (IFN) response and no adverse effects of the delivery system observed. To reduce the efficient dose, to improve the aerosolizability at maintained biocompatibility of the carrier, and to meet other requirements of an optimal delivery vehicle stated above, various nanocarriers were characterized. Intranasally applied siRNA formulated with Genzyme Lipid GL67, however, was mostly captured by alveolar macrophages. Therefore, siRNAs specific to β-galactosidase did not reduce protein expression and only knocked down mRNA levels by one-third in the airway epithelium of K18-lacZ transgenic mice.[42] Interestingly, intratracheal instillation of a Lipofectamine formulation of an ENaC-shRNA in a CF model required only very low doses of 32 μg/kg to downregulate mRNA by 50% and 59 μg/kg to reach the IC_{50} concentration for protein knockdown.[38] The biodistribution of siRNA-loaded 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) liposomes was compared after intravenous and intratracheal administration, revealing clear advantages of the local administration route due to longer retention of the liposomes and of siRNA in the lung as compared to systemic administration.[43] Atu-PLEX lipoplexes currently in phase I clinical trial in patients with advanced solid tumors were administered as aerosols,

leading to translocation into the systemic circulation and inflammation, but no significant reduction of the target gene expression at 2 and 10 mg/kg siRNA. Despite some successful approaches with liposomes and lipoplexes, their stability in terms of hydrolysis, oxidation, and enzymatic degradation but especially under shear forces is variable and their release profiles unpredictable.[44]

Similarly to cationic lipids, surfactants also have an amphiphilic character and have been exploited for intranasal siRNA delivery to prevent and treat influenza virus infection[31] and to determine the role of angiotensin-converting-enzyme-2 in fibrosis.[45]

Additionally, natural and synthetic polycations, especially mucoadhesive chitosan, were applied for pulmonary siRNA delivery. "Nanochitosan" was used for intranasal delivery of siNS1-shRNA resulting in decreased viral titers, inflammation, and airway reactivity in RSV-infected mice[39] and rats.[46] Daily intranasal application of 30 μg of siRNA formulated with 114 kDa chitosan with a deacetylation degree of 84% for 5 days to EGFP mice led to 37% and 43% reduction of EGFP-positive cells in bronchial epithelial cells on day 6 compared to mismatch and untreated control, respectively.[47] EGFP-positive cells were counted in paraffin section and expressed as percentage of 200 epithelial cells. Later, chitosan nanoparticles with EGFP siLNA were administered both systemically and intranasally to the same animal model in the same dosing regime, and EGFP-positive cells, counted by means of a physical fractionator probe, were found to be efficiently reduced in comparison to the mismatch control.[37] The same group later characterized an aerosol made of chitosan/siRNA particles, treated a different EGFP mouse strain by intratracheal aerosolization, and determined EGFP knockdown by flow cytometry of lung homogenates. They found that siRNA doses as low as 0.26 μg administered on day 1 and 3 were deposited throughout the entire lung in alveolar and bronchiolar regions, and significant EGFP gene silencing (68% reduction compared to mismatch control) was achieved on day 5.[48]

Another polymer that was first described for pulmonary gene delivery[30] which is now used for siRNA delivery is the synthetic polycation PEI. For pulmonary siRNA delivery, shRNA targeting Wilms' tumor gene 1 (WT1) was complexed with 25 kDa branched PEI and administered intratracheally as aerosol in a B16-F10 mouse melanoma lung metastasis model. The treatment with 25 μg of PEI/DNA complexes twice a week over 4 weeks decreased angiogenesis, number and size of lung tumor foci, lung weight, and tumor index.[40] Contrarily, intratracheal instillation of complexes containing 25 μg of siRNA and jetPEI at the charge ratio of N/P 10 (PEI amines per siRNA phosphates) caused rapid death of the mice in a different study,[49] while full deacylation of 87 kDa PEI resulted in a reduction of influenza viral titers in infected mice by 94%.[50] Another approach to decrease the toxicity of PEI is PEGylation. In a study using an EGFP mouse model, biodistribution, absorption, and clearance of DsiRNA formulated with PEI and PEG-PEI was investigated by noninvasive nuclear imaging after intratracheal instillation of 50 μg per mouse. However, PEI most easily released siRNA in vivo, leading to short lung retention but rapid excretion of PEI-formulated siRNA, while PEG-PEIs were stable in the lung with 12−15% of the instilled doses of both PEG-PEI and siRNA remaining in the lung 48 h after administration. Pulmonary histology did not indicate signs of inflammation, and significant knockdown of EGFP protein expression was achieved as measured by flow cytometry.[36] Unfortunately, PEG-PEI elicited moderate immunostimulatory effects reflected in increased levels of six cytokines in the brochoalveloar lavage fluid (BALF).[36] Another study demonstrated that not only PEGylated PEI 25 kDa but also fatty acid-modified PEI 8.3 kDa exhibited remarkably increased cytokine levels in the BALF despite reduced cytotoxicity.[51] Additionally, recent investigations showed that PEG-PEIs can induce the expression of apoptosis-related genes[52] and cause complement activation.[53] Therefore, a biodegradable polymer, DEAPA-PVA-*g*-PLGA, previously used for pulmonary gene delivery, which was shown to cause inflammatory responses and PMN recruiting in lungs not higher than the negative isotonic glucose control[54] was now adopted for pulmonary siRNA delivery after it was shown that the formulation is suitable for aerosolization.[55] Interestingly, DEAPA-PVA-*g*-PLGA nanoparticles were stable in the lung with little systemic availability and very good lung retention of encapsulated siRNA after pulmonary administration (Figure 4), also reflected in the SPECT images: Even 48 h after administration, the siRNA remained mostly in the lung and did not accumulate in other organs (Figure 5).

3.3. Surface Modifications and Active Targeting. Although targeted gene delivery to specific cell types utilizing cell-penetrating peptides or receptor-mediated endocytosis is a broad field of interest,[56] only three targeted pulmonary siRNA carriers have been described so far. The first two were TAT (48−60) and penetratin conjugates directly coupled to the 5′-end of the sense strand of thiol-modified siRNA via Npys-activated C-terminal cysteine-containing TAT and penetratin with N-terminal acetyl and C-terminal amide functions. Unfortunately, these conjugates

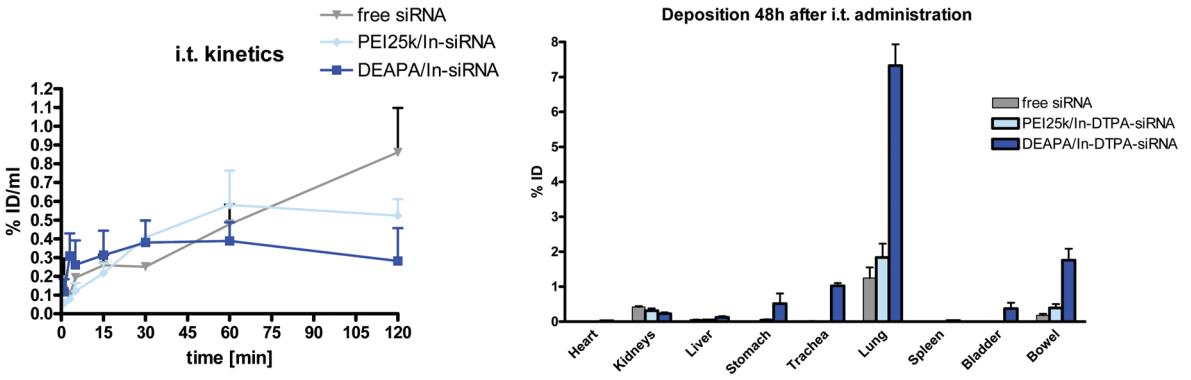

FIGURE 4. Pharmacokinetics and biodistribution of siRNA-loaded nanoparticles compared to free siRNA and PEI complexes after intratracheal administration as measured by scintillation counting of blood samples and dissected organs. DEAPA nanoparticles lead to prolonged lung retention of siRNA mirrored in low systemic availability.

did not achieve improved effects over naked siRNA, while the penetratin–siRNA conjugate activated innate immune responses.[57] The third one is a triblock of folate–chitosan-*graft*-polyethylenimine prepared by an imine reaction between periodate-oxidized folate–chitosan and low molecular weight PEI which was used to deliver shRNA to a lung cancer model.[18]

4. Therapeutic Models

With all of these formulations in hand, various diseases were treated. The first reports about successful RNAi in the lung described protection from influenza infection in 2004.[31,41] Later, prophylaxis and therapies for other viral infections such as SARS[24] and RSV[20,58] were studied, but influenza A retained interest.[59] Additionally, the focus for siRNA therapeutics moved toward other pulmonary disorders such as acute lung injury (ALI),[21,22,29,60] asthma,[61,62] fibrosis,[26,28,45,63] cystic fibrosis,[38] tuberculosis,[23,27] lung cancer,[43] or lung mestastases.[40] Several studies optimizing new carriers were concentrated to the investigation of the biodistribution post pulmonary administration,[42,43,64] and a number of publications are based on demonstrating proof of principle in EGFP-expressing mice as reporter gene model.[36,37,47,48,51]

4.1. Administration Techniques. In all of the above-mentioned in vivo studies, different administration routes were favored for specific reasons. While intranasal administration[28,29,37,47,65] is the least invasive technique, its drawback is that a certain amount of siRNA is lost in the nasal mucosa and a greater amount can be swallowed than after intratracheal application (Figure 6). In a study comparing intranasal versus intravenous routes, naked siLNA was only efficient if administered intravenously but not intranasally.[37] The most frequently applied technique is intratracheal instillation,[21,22,36,38,43,45,48,57,60,61,63] and the comparison between intratracheal versus intravenous administration of siRNA-containing DOTAP liposomes revealed advantages especially concerning lung retention for local administration.[43] Additionally, three different devices have been applied for intratracheal aerosolization: the Penn-Century microsprayer,[23,26,27,64] the Micro-Mist nebulizer,[40] or the AeroProbe nebulizing catheter.[48] However, after aerosolization, the biologic activity of the formulation which is exposed to severe shear forces has to be maintained,[48,55] because preferential nebulization of the solute and adhesion to plastic can strongly limit the siRNA dose emitted through the mouthpiece.[66] For clinical application in man, instillation cannot be realized, and therefore, nebulization of nanoparticle suspensions, aerosolization of lyophilized formulations, or pressurized expulsion from a propellant dispersion needs to be applied. Although metered dose inhalers (MDIs) and dry powder inhalers (DPIs) can improve stability, rapid administration, and ease of transportation, nebulizers have so far received the most attention in preclinical work. This most probably reflects the straightforwardness in the development of nebulizable suspensions compared to the more sophisticated development of MDIs and DPIs. One step toward the clinic was however just recently undertaken by optimizing conditions for lyophilization of DNA-containing polyplexes.[67]

4.2. Detection of Biodistribution. The biodistribution of siRNA formulations can be detected at various levels and by means of various techniques. If fluorescently labeled siRNA is utilized, fluorescence microscopy or confocal laser scanning microscopy[62] can detect the distribution into certain cell types and assess the subcellular distribution. Ex vivo fluorescence imaging with the IVIS Xenogen system[43] or even in vivo fluorescence imaging is possible with near-infrared probes. The lung can hardly be imaged by MRI if it is

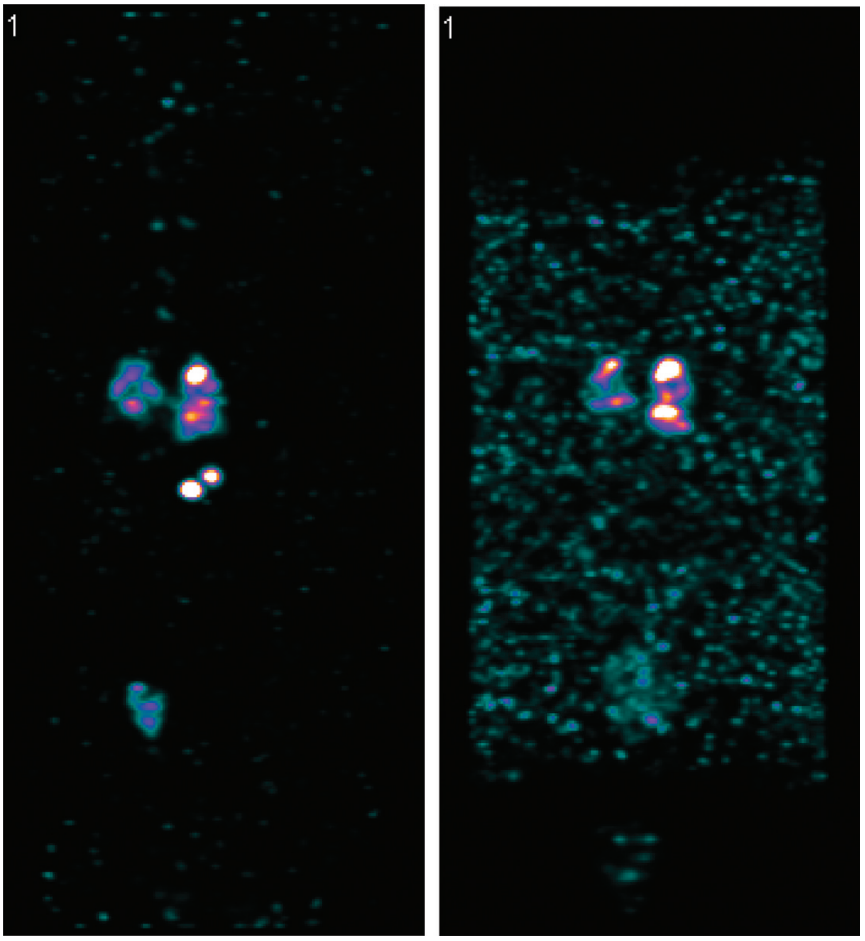

FIGURE 5. SPECT images of biodistribution of siRNA-loaded nanoparticles 2 h (left) and 48 h (right) after intratracheal administration. Swallowed siRNA can be found in the stomach 2 h post instillation (two dots below lung), and excretion into the bladder (signal in the bottom) can be observed at both time points.

not vented with gases such as hyperpolarized helium-3.[68] But radioactive imaging such as PET or SPECT allows for noninvasive detection of the whole body distribution of radiolabeled siRNA.[36] With dual-isotope approaches, the lung vasculature can additionally be visualized and colocalized, changes of the distribution within the same animal over time can be observed by planar gamma camera imaging,[36] and dynamic courses can be recorded.[69]

4.3. Measurement of Therapeutic Effects. Since the majority of studies concerning pulmonary siRNA so far investigated viral infections, the therapeutic outcome was most frequently measured by quantification of viral titers,[20,41,46] protection against lethal challenge,[41] airway hyperreactivity,[46] and body weight gain.[20] For evaluation of therapeutic effects in ALI models, pulmonary inflammation was measured concerning levels of cytokines and PNM influx.[21] Additionally, apoptosis and caspase 3 activity were investigated,[25] and survival was demonstrated.[60] In fibrosis, accumulation of collagen was an indicator,[28,45] as well as the expression of matrix metalloprotease 2 and transforming growth factor beta (TGF-β).[26] Lung cancer models investigated tumorigenesis,[18] number and size of lung metastases, angiogenesis, and lung weight,[40] and in tuberculosis models the number of T-lymphocytes, the IFN-γ response,[23] TGF-β levels, expression of antimicrobial mediators (NO and iNOS), and the bacterial load[27] were determined.

Although EGFP mouse models are only reporter gene models,[36,37,47,48] their potential in determining the nature of the epithelial cells that are transfected and whether other lung cell types also take up siRNA has not yet been fully exhausted. In a recent study, CD45-positive cells from EGFP-expressing mice were sorted by flow cytometry to find that knockdown by 25% and 36% was found in leucocytes and lung macrophages, respectively.[51] A similar technique can be utilized to differentiate CD45-positive myeloid cells from CD31-positive endothelial cells and surfactant-producing type II pneumocytes (Figure 7). The EGFP knockdown in the single cell populations can accordingly be quantified by

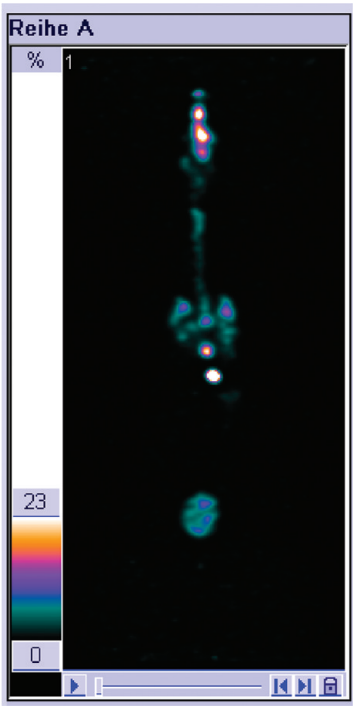

FIGURE 6. Biodistribution of radiolabeled siRNA/PEG-PCL-PEI polyplexes after intranasal administration.

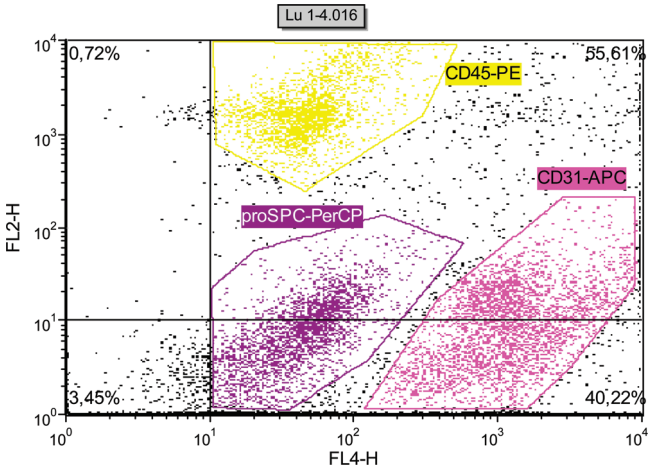

FIGURE 7. Differentiation of transfected lung cells in EGFP mice by flow cytometry after counterstaining various populations with differently fluorescently labeled antibodies.

measuring the EGFP fluorescence in the FITC-channel of each population.

5. Concluding Remarks

Herein, we discuss success stories and obstacles to pulmonary siRNA delivery in the light of the formulation chosen, the administration route, and the animal model or clinical trial. While many reports describe successful therapy or prophylaxis of lung diseases, only little is known about the biodistribution of the administered siRNA. It remains especially unclear which cell types in the lung can be transfected depending on the delivery route, although this aspect appears crucial for translation into the clinic. Techniques to fill theses gaps have been introduced. For administration in man, novel formulations will be needed that maintain practicability and patient compliance. Therefore, MDIs and DPIs will have to be developed in the future.

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Supporting Information. Table summarizing nonviral pulmonary siRNA vectors. This material is available free of charge via the Internet at http://pubs.acs.org.

BIOGRAPHICAL INFORMATION

Olivia M. Merkel studied pharmacy at Philipps-Universität Marburg, Germany, and graduated in 2004. She also received her M.S. and Ph.D. in pharmaceutics from the same university for investigating image-guided nonviral siRNA delivery under Thomas Kissel and has been a postdoc in Dr. Kissel's lab since 2009.

Thomas Kissel is the chairman of the Department of Pharmaceutics and Biopharmacy at Philipps-Universität Marburg, Germany. He studied pharmacy at Albert-Ludwigs-Universität Freiburg, Germany, and chemistry at Philipps-Universität Marburg, where he also earned a Ph.D. in medicinal chemistry. After receiving postdoctoral training in A. P. Schaap's lab in the Department of Chemistry at Wayne State University, he joined Sandoz Pharma Ltd. in Basle, Switzerland. In 1991, he was appointed a full professor of pharmaceutics at Philipps-Universität Marburg and the director of the department.

FOOTNOTES

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