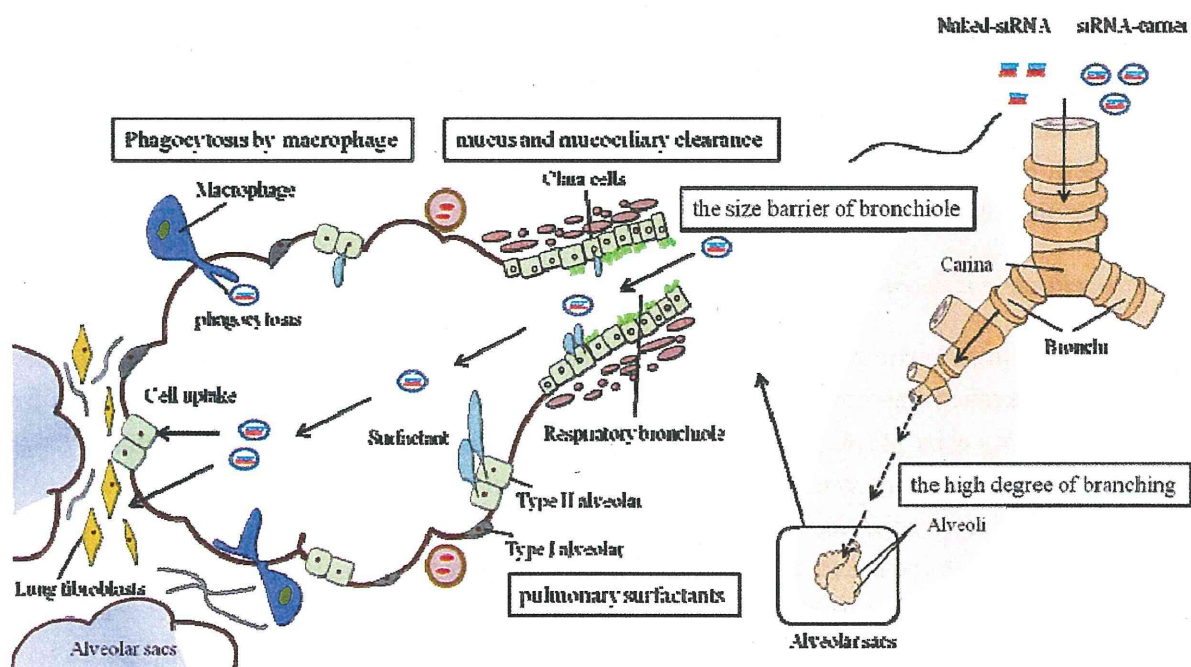


present major barriers to targeted pulmonary delivery. Therefore, delivery systems usually require delivery vectors, and these vectors need to be designed in order to maximize the siRNA deposition to the diseased area of the respiratory tract. Besides, the extracellular barriers to siRNA delivery also depend on physiological features of the respiratory tract, which may change with the disease stage and characteristics of the patient. At the active stage of lung disease, the physiological conditions of the airways might change and have significant impact on the efficiency of the pulmonary delivery system. During infection, inflammation, and allergic reaction, there is an increase in mucus secretion along with the impaired mucociliary clearance [43]. Moreover, asthma and COPD are both chronic inflammatory conditions of the lung associated with structural “remodeling” that is inappropriate to the maintenance of normal lung function [44]. The airway wall thickness, the high viscosity, and the composition of the mucus layer might be altered in patients who have inflammatory lung diseases.

**Figure 2.** Extracellular barriers to pulmonary siRNA delivery. The anatomical feature of the respiratory tract is its high degree of branching. The mucus lines the respiratory epithelium from the nasal cavity to the terminal bronchioles. The deposited particles on the ciliated epithelial cells are rapidly cleared by the mucociliary clearance actions. Mucus and mucociliary clearance of mucus-trapped particles is a pulmonary defense mechanism as a physiological barrier. In the alveolar, clara cells and type II alveolar cells secrete on the surface of the alveolar epithelium, forming a thin layer of pulmonary surfactants. The surfactants act as the main barrier for siRNA delivery because they reduce the transfection efficiency. In addition, the macrophages located in the alveoli rapidly engulf the foreign particles by phagocytosis. The particles taken up into the macrophages are subsequently degraded inside the cells. These factors present major barriers to targeted pulmonary delivery.

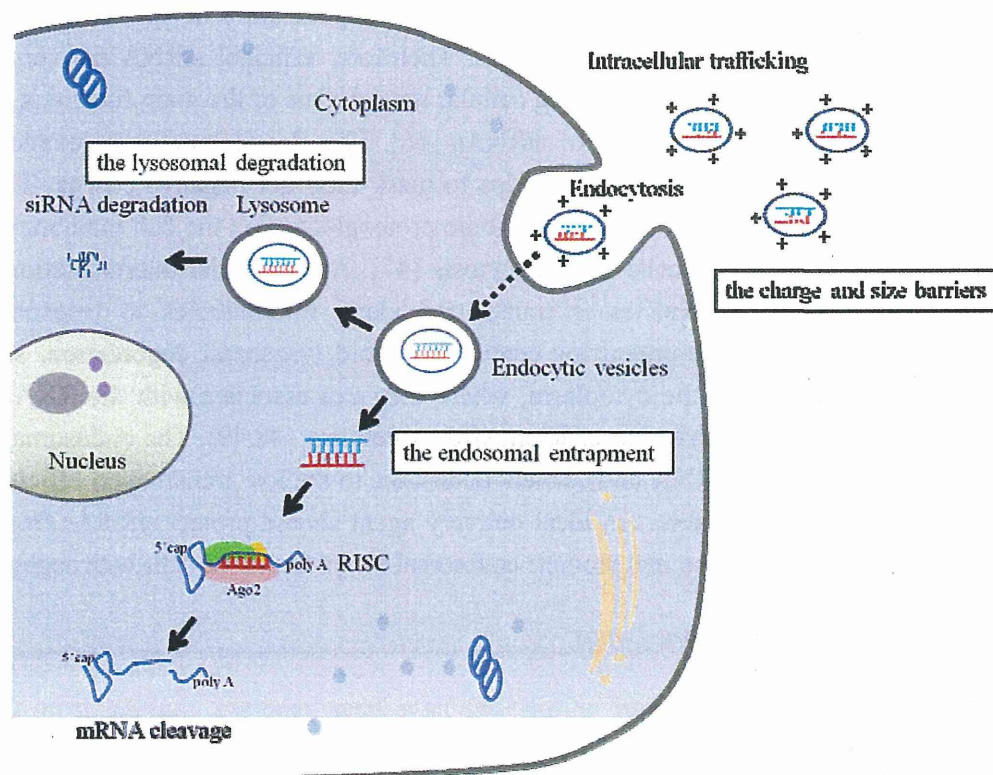


The second is the airway cell membrane and its intracellular barriers (Figure 3). For efficient gene silencing in the lungs, siRNAs must be delivered to their site of action, be stable, enter the target cells, and be present in the cytoplasm at sufficient concentration. Once the siRNAs reach the target cells, they must be trafficked into the cytoplasm and taken up by Argonaute (Ago)2/RNA-induced silencing complex (RISC), which degrades mRNAs and, subsequently, suppresses the sequence-specific gene expression. For efficient endocytosis to occur, particles should be under 150 nm in size. Particles within this size range could also avoid macrophage uptake and delayed lung clearance [45]. The physicochemical properties of siRNAs also play a significant role in crossing the biological membrane. Despite their small size, the negative charge and chemical degradability of siRNA molecules prevent them from readily crossing biological membranes. Therefore, efficient siRNA delivery approaches need to overcome this limitation by facilitating cellular uptake. One of the main functions of a delivery vector is to facilitate the cellular uptake of siRNAs [46]. The electrostatic complexation of siRNA molecules with cationic lipids and polymers helps to mask their net negative charge. The positively charged siRNA carrier complex interacts with anionic proteoglycans on the cell membrane, forms an endocytic vesicle, and enters the cells by endocytosis [47]. After cellular internalization, the siRNA carrier complex in endocytic vesicles is transported along microtubules to lysosomes that are co-localized with the microtubule-organizing center. To avoid lysosomal degradation, siRNAs must escape from the endosome into the cytoplasm, where they can associate with the RNAi machinery. Endosomal escape is a major barrier for efficient siRNA delivery [48,49]. The endosomal entrapment and lysosomal degradation of siRNA and carriers contribute to the low transfection efficiency and is a major difficulty for delivery vectors. An ideal delivery agent should protect siRNAs from enzymatic degradation, facilitate cellular uptake, and promote endosomal escape inside the cells with negligible toxicity.

### 2.3. Delivery Method of siRNA to the Lungs

Multiple approaches for the delivery of siRNAs have been reported, ranging from the relatively simple direct administration of saline-formulated siRNAs to lipid-based and polymer-based nanoparticle approaches and siRNA conjugation and complexation approaches [50]. The negative charge and chemical degradability of siRNAs under physiologically relevant conditions make its delivery a major challenge. Accordingly, the delivery of siRNAs usually requires a vector or carriers for their transfection into the target cells. In general, both viral and non-viral vectors are being assessed for siRNA delivery to lung cells. Some viral vectors, such as retroviruses and adenoviruses, have been demonstrated to mediate gene silencing in an *in vitro* lung model [51] and to induce RNAi in a range of animal tissues [52]. Recently, Guo *et al.* showed that lentivirus-mediated siRNA was used to specifically knock down the expression of nuclear protein 1 (NUPR1) *in vivo*, which resulted in inhibited tumor growth [53]. However, viral-based delivery has several disadvantages. The immune response to viruses not only impedes gene delivery but also has the potential to cause severe complications [54]. Recent well-documented cases, such as the death of Jesse Gelsinger due to complications related with an adenoviral delivery vector, highlight this problem [55]. In addition, some viral vectors may insert their genome at random positions in the host chromosome, which eventually restrict the gene function [56].

**Figure 3.** Intracellular barriers to pulmonary siRNA delivery. Barriers to cellular internalization are dependent on the surface properties of siRNA and carriers (e.g., charge and size). After siRNAs are successfully taken into the target cells by endocytosis, the main barriers for delivering siRNAs to its site of action are the endosomal entrapment and lysosomal degradation of siRNA and carriers. To direct target-gene silencing, the siRNAs need to escape from the endosome into the cytoplasm, where they associate with the Ago2/RNA-induced silencing complex (RISC) to direct the cleavage of mRNAs bearing complementary binding sites.



As an alternative to viral vectors, non-viral vectors, including lipid and polymer-based vectors, have been generally used for the delivery of siRNAs to the lungs due to their reduced toxicity [57]. Ongoing research into the transfection of primary cells and whole organisms with siRNA using non-viral transfection agents has produced some promising results. Lipid-based delivery vectors are successfully used to deliver siRNA *in vitro* and *in vivo* [58]. Cationic lipids are composed of positively charged head, a linker and hydrophobic. In general, lipid-based complexes are easy to formulate and good transfection efficacy is achieved due to interaction with negative charged cell membrane. Many commercial siRNA transfection agents are lipid-based delivery system, some of which are also employed for pulmonary delivery—DharmFECT [30], Oligofectamine [59], Lipofectamine [60] and TransIT-TKO [35]. Similarly, cationic polymers have also been assessed for siRNA delivery to lung cells. Cationic polymer polyethylenimine (PEI) is widely used for siRNA delivery [13,61]. PEI is considered as the gold standard for *in vitro* gene delivery and its transfection efficiency depends on the molecular weight and degree of branching.

On the other hand, lipid-based vectors can also induce toxicity and non-specific activation of inflammatory cytokine and interferon responses [62,63]. Although polymer-based vectors elicit a relatively less strong immune response than lipid-based vectors, effective siRNA delivery to a local area in lung diseases requires more attention to the development of non-toxic delivery vectors. An important point for siRNA-mediated inhibition of gene expression is whether the observed effects are specific rather than due to off-target effects and free from potential interferon responses [64,65]. Interestingly, some studies have shown that it was possible to administer “naked siRNAs” to mice and down-regulate an endogenous or exogenous target without inducing an interferon response [66].

The term “naked siRNAs” refers to the delivery of siRNAs without any delivery vectors. Naked siRNAs are degraded by serum endonucleases and are usually removed by glomerular filtration, resulting in a short plasma half-life of < 10 min. Thus, some studies of systemic delivery of naked siRNAs have failed to achieve the downregulation of the targeted gene [67,68]. In contrast, there have also been some successes of locally delivering naked siRNAs to the lungs [15,16,20,31]. A few of them reported that the use of delivery vectors showed no significant difference in gene silencing efficiency compared to that of naked siRNAs [16,35]. Indeed, in one clinical trial, the delivery of naked siRNAs for the treatment of RSV has been used [17,38]. This successful evidence can be because that naked siRNAs for clinical applications are highly chemically modified to prevent nuclease-induced degradation and presumably minimize immune stimulatory effects. Although it is unclear how the naked siRNAs cross the cell membrane, gain access to the cytoplasm, and remain intact to perform their biological action, both animal and human trials have been conducted successfully, showing the efficacy of naked siRNAs (ALN-RSV01) that were administered intranasally. This explanation has not been confirmed, but the physiological damage of respiratory epithelial cells caused by viral infection may have possibly influenced the mystery. The active change in airway epithelial cell membrane caused by infectious disease might affect cellular internalization. Naked siRNA delivery has some advantages, such as simple formation and the absence of toxicity or inflammatory responses that are usually associated with delivery vectors. Nevertheless, the advantage of naked siRNAs over delivery vectors in the treatment of lung diseases is controversial [69,70]. Further *in vivo* investigations about both naked siRNAs and non-viral vectors are required.

### 3. RNAi Medicine in Lung Diseases

Lung disease is a major cause of death, and diminished quality of life is responsible for the suffering of many patients. Various lung diseases make life extremely difficult for the patients, and severe cases of these lung diseases can result in death. The high death rates associated with lung cancer are partially due to the fact that it is unfortunately difficult to cure. Above all, COPD is the fourth-leading cause of death in most industrialized countries and is predicted to become third by 2020 [71]. Therefore, decisive action is needed to stem the rising health and economic burden this represents. Chronic lung diseases, such as COPD and asthma, are disorders of the airways largely related to the presence of persistent inflammation. The approval of inhaled corticosteroids pioneered a new generation of therapy in treating chronic inflammatory diseases. This was the first time that an anti-inflammatory product was available to reduce the characteristic lung inflammation in airways and the associated obstruction. Corticosteroids are still an important therapeutic intervention. However,

they are used with limitations in COPD and moderate to severe asthma. Likewise, the treatment of various refractory lung diseases also depends on systemic corticosteroid therapy. Many of these patients also suffered various side effects from systemic corticosteroid use, such as weight gain and uncontrolled hyperglycemia. Treatment of lung disease using cell-specific targeting as well as RNAi techniques represents a novel strategy and could possibly provide new opportunities in nanomedicine. Pulmonary applications of siRNA in *in vivo* conditions are frequently studied and often result in clinical trials [57,72]. The findings of recent clinical studies of pulmonary RNAi therapeutics are discussed.

### 3.1. Therapeutic siRNAs for Lung Diseases

Since the discovery of RNAi, the therapeutic potential of siRNAs has been rapidly recognized. In 2004, the first human clinical trial of RNAi-based therapy was initiated for the treatment of age-related macular degeneration with a siRNA targeting VEGF-receptor 1 delivered intravitreally [73]. Many studies have been conducted over the past few years that involve the delivery of siRNAs to the lungs for the treatment of various lung diseases. Delivery to the lungs will be most important to moving siRNA technology into the clinic. A number of siRNA-based therapies are being evaluated in clinical trials for the treatment of different conditions, including lung diseases such as asthma and RSV infection. Table 1 is a summary of clinical trials of siRNA-based therapeutics [74].

**Table 1.** Summary of siRNA-based therapeutics in clinical trials.

Drug	Route of Administration	Delivery Agent	Disease	Target	Stage of Clinical Trial
Excellair™	Inhalation	Unknown	Asthma	Syk kinase	II
ALN-RSV01	Intranasal spray	Naked siRNA	RSV infection	RSV nucleocapsid	I Ib
Atu027	IV	Lipid nanoparticles	Advanced solid cancer (Metastatic lung cancer)	PKN3	I
TKM-ApoB	IV	Lipid nanoparticles	Hypercholesterolemia	ApoB	I
TKM-PLK1	IV	Lipid nanoparticles	Cancer	Polo-like-kinase1	I
ALN-VSP02	IV	Lipid nanoparticles	Solid cancers with liver involvement	KSP and VEGF	I
ALN-TTR01	IV	Lipid nanoparticles	Transthyretin-mediated amyloidosis (ATTR)	Transthyretin (TTR)	I
CALAA-01	IV	Cyclodextrin nanoparticles	Solid tumor	RRM2	I
siG12D LODER	EUS biopsy needle	Miniature biodegradable polymer matrix	Pancreatic ductal adenocarcinoma	KRAS	I
I5NP	IV	Naked siRNA	Acute kidney injury	p53	I/II

Table 1. Cont.

Drug	Route of Administration	Delivery Agent	Disease	Target	Stage of Clinical Trial
QPI-1007	IVT	Naked siRNA	Glaucoma and acute eye injury	Caspase-2	I
TD101	Intradermal injection	Naked siRNA	Pachyonychia congenita	Keratin 6a N171Kmutant mRNA	Ib
SYL040012	Ophthalmic drops	Naked siRNA	Ocular hypertension and glaucoma	Adrenergic receptor beta-2	I/II
AGN-745	IVT	Naked siRNA	AMD	VEGF-receptor1	II
PF-655	IVT	Naked siRNA	AMD and diabetic macular edema	RTP801 (pro-angiogenic factor)	II
Bevasiranib	IVT	Naked siRNA	AMD	VEGF	III

IV: Intravenous injection; IVT: Intravitreal injection; RSV: Respiratory syncytical virus; AMD: Age-related macular degeneration; Syk: spleen tyrosine kinase; PKN3: protein kinase N3; KSP: kinesin spindle protein; RRM2: M2 submit of ribonucleotidereductase; KRAS: V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog; VEGF: vascular endothelial growth factor.

### 3.1.1. Pulmonary Viral Infections

SiRNA shows potential for the treatment of various pulmonary viral infections, and it has been reported that siRNA-based therapeutics can also be used in the treatment of influenza [13], parainfluenza virus [35], severe acute respiratory syndrome (SARS) [14], and RSV [35]. Above all, RSV is the most promising therapeutic target of siRNAs.

RSV is a common cause of serious respiratory infections in infants and children. It also produces significant morbidity and mortality in adult immunocompromised or elderly populations [75]. An RSV vaccine is not available, and the only approved antiviral therapy for RSV is undesirable for pediatric patients due to its potential teratogenicity and limited effectiveness. Thus, a safe and efficacious RSV therapy has long been awaited for both pediatric and adult patients. RNAi-based therapy has shown promising effects in murine models of RSV infection [35]. The siRNA, ALN-RSV01, is directed against the mRNA encoding the N-protein of RSV that exhibits specific *in vitro* and *in vivo* anti-RSV activity. It is delivered without a delivery vector as a nasal spray and targets the upper respiratory tract instead of the lower lung area. ALN-RSV01 has undergone complete phase I intranasal and inhalation studies in healthy adults and has been found to be generally well tolerated [38]. Additionally, ALN-RSV01 has been evaluated in a randomized, double-blind, placebo-controlled phase II trial in lung transplant patients with RSV respiratory tract infection [76]. The administration of ALN-RSV01 to RSV infected lung transplant patients was safe and well tolerated and associated with a statistically significant improvement in symptoms. Based on these results, a larger multinational, randomized,

double-blind Phase IIb trial of ALN-RSV01 has been initiated in lung transplant patients to confirm and extend these findings.

### 3.1.2. Lung Cancer

Cancer is a major target of RNAi-based therapy, as oncogenes, mutated tumor suppressor genes, and several other genes contributing to tumor progression are potentially important targets for gene silencing by RNAi. Lung cancer is one of the most frequent tumors worldwide with regard to incidence rates and mortality. Patients with lung cancer are commonly diagnosed at an advanced stage of the disease and have limited therapeutic options. Although the knowledge regarding the genetic and molecular basis of lung cancer has regularly increased, the median survival rates of individuals with advanced lung cancer are still poor.

RNAi-based therapy is an attractive strategy for the development of more effective anticancer therapies with reduced treatment-related toxicity. The major advantage of RNAi therapeutics in cancer might be the simultaneous targeting of multiple genes belonging to different cellular pathways that are involved in tumor progression. The simultaneous inhibition of several genes would also minimize the risk of drug resistance normally encountered with small molecule-based therapies, involving siRNAs and miRNAs. There have already been significant improvements in siRNAs for primary or metastatic lung cancer treatment by targeting oncogenes such as Akt1 [9], Wilms tumor 1 (WT1) [12], overexpressed genes such as the insulin-like growth factor receptor 1 (IGF-1R) [77], NUPR1 [53] and EZH2 [78]. Some of these studies have successfully shown the efficacy of RNAi-based therapy through intrapulmonary administration of siRNAs with non-viral vectors. Although strategies to minimize off-target and nonspecific immune stimulatory effects must be devised, these data suggest that the silencing of the target gene with siRNAs is an attractive strategy for the prevention and treatment of primary and metastatic lung cancer. There are currently some clinical trials in progress estimating the safety and efficacy of siRNA-based drugs for cancer treatment. Atu027, a siRNA-lipoplex targeted against protein kinase N3 (PKN3), prevented lung metastasis in a phase I trial of various cancer models [79]. PKN3 is a downstream effector of the phosphoinositide 3-kinase (PI3K) signaling pathway [80], which regulates diverse cellular responses, including development, growth, and survival [81]. Recently, PKN3 has also been considered as a suitable therapeutic target for modulating tumor angiogenesis because loss of function analysis with Atu027 in cultured primary endothelial cells showed an essential role of PKN3 for endothelial tube formation and migration [79]. Atu027 can be considered as a potential siRNA for preventing lung metastasis and might be suitable for preventing hematogenous metastasis combined with conventional cancer therapy.

### 3.1.3. Inflammatory Lung Diseases

Inflammatory lung disease, also called COPD, includes a wide range of lung ailments. These related diseases include asthma, pulmonary fibrosis, and chronic bronchitis. They are influenced by a combination of environmental, genetic, and epigenetic components [82]. COPD is a chronic inflammatory disease of the airways. This disease is hallmarked by airflow that is not fully reversible. Systemic and local airway inflammation has been implicated in the pathogenesis of COPD [83]. COPD is mainly associated with tobacco smoking, and recent studies investigating the pathophysiology of

emphysema have demonstrated that cigarette smoke can cause cells to enter cellular senescence. Smoking might cause cells to senesce due to DNA damage through increased cell turnover, which in turn leads to accelerated telomere shortening [84]. Lately, a lot of studies have investigated the role of cellular senescence in the development and progression of COPD [85]. Although several medication classes, including inhaled corticosteroids, are used for COPD treatment, none of these medications have been shown to significantly improve long-term lung function during the progression of the disease. Current interventions that have been shown to improve mortality in COPD are cessation of smoking and delivery of supplemental oxygen when hypoxemia is present.

Many people are developing COPD, and the cause of this condition is complicated and not thoroughly understood. One key factor is genetic susceptibility. Some studies have shown a large genetic contribution to the variability in pulmonary function and COPD [86,87]. Polymorphisms in multiple genes have been reported to be associated with COPD [87], such as transcription factor [e.g. nuclear factor-kappa B (NFκB)] [88], extracellular matrix (e.g., matrix metalloproteinase-12 (MMP-12)) [89,90], cytokines [e.g. tumor necrosis factor (TNF)-α] [91], chemokines [e.g. interleukins (IL)-8, IL-8 receptor and chemokine receptor (CCR)1] [92,93], and apoptosis (e.g., caspase-3 and vascular endothelial growth factor (VEGF)) [94,95]. Many of these have been identified as possible targets for therapeutic intervention using molecule inhibitors or antagonists. Although several new treatments that target the inflammatory process are now in clinical development, such as TNF-α inhibitors and I-kappaB kinase complex 2 (IKK2) inhibitors [96,97], clinical trials with siRNAs have never been performed in COPD. The delay of drug development for COPD might be due to the relatively recent emergence of research addressing the molecular basis of COPD. Furthermore, more research is needed to understand the essential molecular mechanisms about the pathogenesis of COPD and to develop monitoring techniques to support the development of RNAi therapies. Currently, no available treatments reduce the progression of COPD or suppress the inflammation in small airways and lung parenchyma. The RNAi-based approach for the key molecules also has potential implications for the treatment of COPD.

Asthma is also a chronic inflammatory disease of the airways characterized by variable and recurring symptoms and reversible airflow obstruction. The World Health Organization estimates that 300 million people are currently affected and that, by the year 2025, another 100 million will be affected by the disease [98]. Inhaled corticosteroids are very effective in mild asthma because they improve symptoms and decrease exacerbations. However, in moderate and severe asthma, inhaled corticosteroids have important therapeutic limitations. Although corticosteroids remain an important therapeutic intervention for inflammatory lung diseases, their use is not always completely effective and is associated with side effects. Due to such limitations, it is clear that there is a need for new types of medications that can treat and improve the prognosis of moderate to severe asthma.

Many target genes have been identified that participate in the pathogenesis of asthma. The most promising targets include genes coding for cytokines (IL-4, IL5, and IL-13), cytokine and chemokine receptors (IL-4 receptor and CCR3), and tyrosine kinases [spleen tyrosine kinase (Syk) and LCK/YES-related novel tyrosine kinase (Lyn)], as well as for transcription factors [signal transducers and activators of transcription 1 (STAT1), STAT6, GATA3, and NFκB] that are involved in asthma [19,99,100]. The genes that have been assessed as siRNA targets for the treatment of asthma in preclinical models are reported [101]. Currently, in a clinical trial for asthma, Excellair™ (ZaBeCor, Bala Cynwyd, PA,



USA), a siRNA that targets Syk, is being used. The kinase is involved in signaling from a B cell receptor and is a key regulator of downstream signaling cascades that ultimately lead to the activation of several pro-inflammatory transcription factors. It has been reported that antisense oligonucleotides administered by aerosol were potent to decrease Syk expression, mediator release from alveolar macrophages, and Syk-dependent pulmonary inflammation [102]. Moreover, inhibition of inflammatory mediators was shown in a study using siRNA targeting Syk in airway epithelial cells [103]. Following the successful results of the company's Phase I clinical trial, a Phase II trial for its asthma drug candidate Excellair™ has already been initiated. Some of the current treatments for asthma and other inflammatory conditions, such as TNF- $\alpha$  inhibitors or leukotriene inhibitors, inhibit only one of the mediators of inflammation. In contrast, siRNA targeting Syk seeks to inhibit an initial signaling step of inflammation and, thereby, prevent the release of multiple inflammatory mediators. Overall, recent progress of siRNAs to the lungs has also improved the therapeutic feasibility of RNAi for inflammatory lung diseases. The rapid progress will put siRNA-based therapeutics on a fast track to the clinic.

### 3.2. Therapeutic microRNA/Anti-microRNA for Lung Diseases

MiRNAs are small endogenous noncoding RNAs that regulate gene expression by repressing translation or promoting the degradation of their target mRNA. MiRNAs regulate gene expression by binding to the 3' untranslated region (UTR) of their target mRNAs and mediating mRNA degradation or translational inhibition. In the human genome, transcripts of approximately 60% of all mRNAs are estimated to be targeted by miRNAs [104]. According to their function, miRNAs play an important role in cellular processes as development, proliferation, and apoptosis of pulmonary pathologies [105]. A growing number of miRNAs have been shown to be involved in different lung diseases. This evidence makes miRNAs a promising technology for current and future therapeutic development. We discuss the role of some miRNAs in various lung diseases as well as the possible future of these discoveries in clinical applications. Table 2 shows the summary of miRNAs in therapeutic development. At this point, a miRNA-based therapy has already entered a phase II clinical trial.

**Table 2.** miRNAs in therapeutic development.

miRNA	Disease	Stage of clinical trial	Reference
miRNA replacement			
<i>let-7</i>	Lung cancer	Preclinical	[106]
miR-34	Lung cancer, Prostate cancer	Preclinical	[107,108]
miR-29	Cardiac fibrosis	Preclinical	[109]
miRNA antagonists			
miR-122	Hepatitis C virus	II	[110]
miR-208/499	Chronic heart failure	Preclinical	[111]
miR-15/195	Post-myocardial infarction remodeling	Preclinical	[112,113]
miR-206	Amyotrophic lateral sclerosis	Preclinical	[114]
miR-451	Myeloproliferative diseases	Preclinical	[115]

## 3.2.1. Role of microRNA in Inflammatory Lung Diseases

There is evidence that upregulation or downregulation of miRNAs is critical for lung homeostasis and, thus, may contribute to the development of pathological pulmonary conditions. Many studies have focused on the role of miRNAs in inflammatory lung diseases, such as COPD [116,117], pulmonary fibrosis [118–121], and asthma [122–125] (Table 3).

**Table 3.** miRNAs in inflammatory lung diseases.

Lung diseases	Expression of specific miRNA	Reference
COPD	miR-223/1274a	↑ [126]
	<i>let-7</i>	↓ [117]
	miR-1	↓ [116]
	miR-146a	↓ [127]
	miR-150	↓ [117]
Asthma	miR-21	↑ [123]
	miR-126	↑ [124]
	miR-155	↑ [122]
	miR-133a	↓ [125]
Pulmonary fibrosis	miR-21	↑ [118]
	miR-155	↑ [128]
	<i>let-7d</i>	↓ [119]
	miR-29	↓ [120]
	miR-200	↓ [121]
Smoking-related miRNA	<i>let-7</i>	↓ [129]
	miR-10a	↓ [129]
	miR-34	↓ [129]
	miR-123	↓ [129]
	miR-145	↓ [129]
	miR-150	↓ [117]
	miR-199b	↓ [130]
	miR-218	↓ [130]
miR-222	↓ [117,129]	

The pathogenesis of COPD is attributed to not only chronic inflammation in the airways but also systemic inflammation [131]. Cigarette smoking is the main risk factor for the development of COPD. Smoking has been shown to cause biological change in the gene expression of the lungs [132], and there are some reports about smoking-related miRNAs [117,129,130]. However, there are few reports that focus on the miRNAs related to the pathogenesis of this disease with systemic inflammatory components. Recent study on pulmonary fibroblasts of COPD patients presents less expression of miR-146a after stimulation with proinflammatory cytokines when compared with non-COPD subjects with similar smoking histories [127]. The downregulation of miR-146a resulted in a prolonged mRNA half-life of cyclooxygenase-2, thus increasing prostaglandin E2 in fibroblasts from COPD subjects.

Moreover, Ezzie *et al.* researched the difference of miRNA profiles expressed in the lungs of smokers with and without COPD. They concluded that miR-223 and miR-1274a were the most affected miRNAs in subjects with COPD [126]. Yet, COPD is a complex, multi-component, and heterogeneous disorder with a number of different pathological processes and subgroups with their own characteristics and natural history [133]. A better understanding of the complexity of the disease and potential clinical relevance of the identified miRNAs is needed.

Pulmonary fibrosis can be caused by an identifiable irritation to the lungs, but, in many cases, the cause is unknown, and the therapeutic possibilities are limited. Cigarette smoking is one of the most recognized risk factors for the development of pulmonary fibrosis. This disorder is mainly accompanied by increased expression of the key fibrotic mediator transforming growth factor  $\beta$  (TGF- $\beta$ ) and other cytokines produced at the lesion of active fibrosis [128]. Recently, it was reported that miRNAs may play an important regulatory role in the pulmonary fibrotic change in the lungs. The downregulation of *let-7d* in idiopathic pulmonary fibrosis (IPF) resulted in increased collagen deposition and alveolar septal thickening [119]. In addition, Liu *et al.* reported that the oncogenic miR-21 was found to be upregulated in IPF patients and in the murine lungs with bleomycin-induced fibrosis [118]. Although these miRNAs may be potential therapeutic targets because their expression is related to the regulation of TGF- $\beta$ , the factor is necessary but not sufficient for pathologic fibrosis of the lungs. Pulmonary fibrosis is also a complicated illness that can have many different causes.

Focus on the role of miRNAs in asthma has recently increased. Asthma is an inflammatory disease of the airway that is characterized by an abnormal response of T helper-2 (Th2)-type CD4<sup>+</sup>T lymphocytes against inhaled allergens [134]. In a different asthmatic mouse model, there was an observed increase in the expression of miR-21 in the lungs [123]. This report might contribute to the understanding of the inflammatory mechanism in the airway through the inhibition of IL-12, favoring the Th2 lymphocyte response. A toll-like receptor 4 (TLR4)-induced Th2 lymphocyte induces high expression of miR-126, and selective blockade of miR-126 suppressed the asthmatic phenotype [124]. In addition, airway remodeling is a characteristic feature of asthma and has important functional implications. Rodriguez *et al.* have shown that miR-155 is related to the development of inflammatory infiltration into the lung and airway remodeling [122]. Thus, some studies present a functional connection between miRNA expression and asthma pathogenesis and suggest that targeting miRNAs in the airways may lead to anti-inflammatory treatments for allergic asthma. Despite the evidence from experimental models, the expression profiling of miRNAs in airway biopsies from patients with mild asthma before and after treatment with inhaled corticosteroids and in healthy volunteers revealed no differences in miRNA expression [135]. Further investigations about the role of miRNAs related to asthma pathogenesis are required.

Although the basic evidence of miRNA biology is still providing new insights, applications of miRNA-based therapy for inflammatory lung diseases are less advanced than those for lung cancer [136]. One reason for this could be that the disease heterogeneity is caused by the effects of many environmental air pollutants, including smoke and volatile organic compounds. The presence of several risk factors makes the understanding of the pathogenesis of inflammatory lung diseases complicated. Understanding the role that miRNAs play in the modulation of gene expression, leading to sustain the pathogenesis of lung diseases, is important for the development of new therapies that focus on the prevention of disease progression and symptom relief.

### 3.2.2. microRNA/Anti-microRNA Delivery for Lung Cancer Therapy

Given the significant roles that miRNAs play in multiple pathways of lung carcinogenesis, increasing efforts are dedicated to the research and development of miRNA-based therapies, including restoring functions of tumor suppressive miRNAs or inhibiting oncogenic miRNAs. The development of miRNA-based therapies for lung cancer is growing prosperously with the help of new RNAi technologies. Compared to siRNA-based therapies, which are already in clinical trials, miRNAs are less toxic and have the potential to target multiple genes. The difficulty associated with miRNA delivery is mainly equal to that of siRNAs. The critical problems for the development of this therapy are effective delivery into target sites, potency of the therapy, and elimination of off-target effects [137].

There are two strategies as the therapeutic applications of miRNAs for lung cancer [138]. One strategy is miRNA replacement therapy, which involves the re-introduction of a tumor suppressor miRNA mimic to restore a loss of the function. MiRNA mimics are synthetic RNA duplexes designed to mimic the endogenous functions of miRNAs with chemical modifications for stability and cellular uptake. The concept of miRNA replacement therapy is most exemplified by the *let-7* miRNA. *let-7* is a tumor-suppressor miRNA in non-small-cell lung cancer that inversely correlates with the expression of the RAS oncoprotein, a key cancer gene [139]. Intranasal administration of *let-7* mimic into mouse models of lung cancer significantly reduced tumor growth, suggesting that miRNA replacement therapy is indeed promising [106,140,141]. Another miRNA that shows the value of miRNA replacement is provided by miR-34a [107,142]. Local and/or systemic delivery of a synthetic miR-34a mimic led to accumulation of miR-34a in the tumor tissue and inhibition of lung tumor growth. Lately, Ling *et al.* also showed that tumor suppressor miR-22 exhibited anti-lung cancer activity through post-transcriptional regulation of ErbB3 [143]. Thus, therapeutic miRNA mimics have a powerful potential by attacking multiple genes relevant to several diseases. However, it is necessary to pay attention to the potential toxicity in normal tissues under conditions in which the therapeutic delivery of miRNA mimics will lead to an accumulation of exogenous miRNAs in normal cells [138]. Although the assumptions are well founded, there is still insufficient evidence for toxicity caused by miRNA mimics. Indeed, several *in vivo* studies failed to reveal side effects caused by the miRNA mimics and suggested that delivery of miRNA mimics to normal tissues was well tolerated [107,141]. It will be important to research miRNA mimic-induced effects in normal cells and to carefully assess toxicity before using them in clinical practice.

The second strategy is directed toward a gain of function and aims to inhibit oncomiRs by using anti-miRNAs. Chemical modifications, such as 2'-O-methyl-group and locked nucleic acid (LNA), would increase oligo stability against nucleases [144]. Antisense oligonucleotides contained in these modifications are termed antagomirs or "LNA-antimiRs" [144,145]. They are oligonucleotides with sequences complementary to the endogenous miRNA and inhibit the specific miRNA function. An LNA-antimiR against miR-122 has been shown to effectively silence miR-122 in non-human primates [145], and the findings support the potential of these compounds as a new class of therapeutics. Moreover, it has also been reported that anti-miR-150 delivered into lung tumor xenografts in mice led to inhibited tumor growth [146]. Relative to studies on miRNA mimics, studies with antisense oligonucleotides have shown effective evidence with naked oligonucleotides. This illustrates the potential of chemical modifications of oligonucleotides to improve their stability, resistance to RNase, and pharmacologic

properties. Therefore, inhibition of miRNA function by chemically modified antimiR oligonucleotides has become an important and widely used approach. Recent data from the first phase II study in patients with chronic HCV infection treated with the LNA-modified antimiR-122 showed that this compound was well tolerated and provided continuing viral suppression.

An increasing number of studies have examined the therapeutic potential of miRNAs. Recently, the evidence of roles for miRNAs in determining drug resistance has emerged [147]. Cytotoxic and molecular target drugs have been widely used in the treatment of advanced lung cancer; unfortunately, many cases are still refractory to chemotherapy. In this situation, combining miRNA mimics or antimiR with chemotherapy may potentiate the efficacy of the cancer treatment in the future. In addition, miRNAs related with cancer stem cells may significantly broaden the field of miRNA-based therapy and suggest that miRNAs can be potential tools to kill cancer cells associated with therapy resistance, recurrence, and metastasis [108,148]. Hence, the main challenge is the successful delivery and chemical modifications of the therapeutic miRNAs to the target tissue without harming normal tissues.

#### **4. Conclusions and Prospects**

RNAi-based approaches provide a promising therapeutic modality for the treatment of various lung diseases. One of the greatest challenges in RNAi-based therapy continues to be the delivery method of the therapeutic siRNAs and miRNAs to the target cells. Pulmonary delivery applications are very attractive, since they tend to be non-invasive, are locally restricted, and can be administered by the patient. A realistic therapeutic intervention, such as aerosolization, can enhance drug delivery to the site of action and decrease systemic exposure of the patient to the therapy, thereby reducing off-target effects. The advancement of pulmonary siRNA delivery to the clinic illustrates that RNAi-based therapy holds a central place in the future treatment of lung diseases. On the other hand, miRNAs have the opportunity to target multiple genes in a fine-tuned manner, and the miRNA-based therapy will provide an attractive anti-tumor and anti-inflammatory approach for various lung diseases. In particular, anti-miRNA therapy by chemically modified antimiR oligonucleotides has become a potential therapy for lung diseases because the oligonucleotides can be successfully delivered without delivery vectors. Increased evidence has indicated that miRNAs fulfill causative roles in a variety of lung diseases and have prompted investigations into their potential as therapeutic targets. Further understanding of the detailed mechanisms of RNAi-based therapy and investigations of more effective delivery methods are required for future development. These novel approaches could open new avenues for various lung diseases and improve the clinical outcome of the patients.

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## Conflict of Interest

The authors declare that there are not conflicts of interest to report.

## References

1. Kim, D.H.; Rossi, J.J. Strategies for silencing human disease using RNA interference. *Nat. Rev. Genet.* **2007**, *8*, 173–184.
2. Bumcrot, D.; Manoharan, M.; Koteliansky, V.; Sah, D.W. RNAi therapeutics: A potential new class of pharmaceutical drugs. *Nat. Chem. Biol.* **2006**, *2*, 711–719.
3. Giladi, H.; Ketzinel-Gilad, M.; Rivkin, L.; Felig, Y.; Nussbaum, O.; Galun, E. Small interfering RNA inhibits hepatitis B virus replication in mice. *Mol. Ther.* **2003**, *8*, 769–776.
4. Xia, H.; Mao, Q.; Eliason, S.L.; Harper, S.Q.; Martins, I.H.; Orr, H.T.; Paulson, H.L.; Yang, L.; Kotin, R.M.; Davidson, B.L. RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. *Nat. Med.* **2004**, *10*, 816–820.
5. Judge, A.D.; Robbins, M.; Tavakoli, I.; Levi, J.; Hu, L.; Fronda, A.; Ambegia, E.; McClintock, K.; MacLachlan, I. Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. *J. Clin. Invest.* **2009**, *119*, 661–673.
6. Chernolovskaya, E.L.; Zenkova, M.A. Chemical modification of siRNA. *Curr Opin Mol. Ther.* **2010**, *12*, 158–167.
7. Schlee, M.; Hornung, V.; Hartmann, G. siRNA and isRNA: Two edges of one sword. *Mol. Ther.* **2006**, *14*, 463–470.
8. Li, S.D.; Huang, L. Targeted delivery of antisense oligodeoxynucleotide and small interference RNA into lung cancer cells. *Mol. Pharm.* **2006**, *3*, 579–588.
9. Xu, C.X.; Jere, D.; Jin, H.; Chang, S.H.; Chung, Y.S.; Shin, J.Y.; Kim, J.E.; Park, S.J.; Lee, Y.H.; Chae, C.H.; *et al.* Poly(ester amine)-mediated, aerosol-delivered Akt1 small interfering RNA suppresses lung tumorigenesis. *Am. J. Respir. Crit. Care Med.* **2008**, *178*, 60–73.
10. Jere, D.; Xu, C.X.; Arote, R.; Yun, C.H.; Cho, M.H.; Cho, C.S. Poly(beta-amino ester) as a carrier for si/shRNA delivery in lung cancer cells. *Biomaterials* **2008**, *29*, 2535–2547.
11. Ren, X.L.; Xu, Y.M.; Bao, W.; Fu, H.J.; Wu, C.G.; Zhao, Y.; Li, Z.K.; Zhang, J.; Li, S.Q.; Chen, W.Q.; *et al.* Inhibition of non-small cell lung cancer cell proliferation and tumor growth by vector-based small interfering RNAs targeting HER2/neu. *Cancer Lett.* **2009**, *281*, 134–143.
12. Zamora-Avila, D.E.; Zapata-Benavides, P.; Franco-Molina, M.A.; Saavedra-Alonso, S.; Trejo-Avila, L.M.; Resendez-Perez, D.; Mendez-Vazquez, J.L.; Isaias-Badillo, J.; Rodriguez-Padilla, C. WT1 gene silencing by aerosol delivery of PEI-RNAi complexes inhibits B16-F10 lung metastases growth. *Cancer Gene Ther.* **2009**, *16*, 892–899.
13. Ge, Q.; Filip, L.; Bai, A.; Nguyen, T.; Eisen, H.N.; Chen, J. Inhibition of influenza virus production in virus-infected mice by RNA interference. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 8676–8681.
14. Li, B.J.; Tang, Q.; Cheng, D.; Qin, C.; Xie, F.Y.; Wei, Q.; Xu, J.; Liu, Y.; Zheng, B.J.; Woodle, M.C.; *et al.* Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. *Nat. Med.* **2005**, *11*, 944–951.

15. Rosas-Taraco, A.G.; Higgins, D.M.; Sanchez-Campillo, J.; Lee, E.J.; Orme, I.M.; Gonzalez-Juarrero, M. Intrapulmonary delivery of XCL1-targeting small interfering RNA in mice chronically infected with *Mycobacterium tuberculosis*. *Am. J. Respir. Cell Mol. Biol.* **2009**, *41*, 136–145.
16. Fulton, A.; Peters, S.T.; Perkins, G.A.; Jarosinski, K.W.; Damiani, A.; Brosnahan, M.; Buckles, E.L.; Osterrieder, N.; Van de Walle, G.R. Effective treatment of respiratory alphaherpesvirus infection using RNA interference. *PLoS One* **2009**, *4*, 5.
17. DeVincenzo, J.; Lambkin-Williams, R.; Wilkinson, T.; Cehelsky, J.; Nochur, S.; Walsh, E.; Meyers, R.; Gollob, J.; Vaishnav, A. A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 8800–8805.
18. Lee, C.C.; Chiang, B.L. RNA interference: new therapeutics in allergic diseases. *Curr. Gene Ther.* **2008**, *8*, 236–246.
19. Seguin, R.M.; Ferrari, N. Emerging oligonucleotide therapies for asthma and chronic obstructive pulmonary disease. *Expert Opin. Investig. Drugs* **2009**, *18*, 1505–1517.
20. Senoo, T.; Hattori, N.; Tanimoto, T.; Furonaka, M.; Ishikawa, N.; Fujitaka, K.; Haruta, Y.; Murai, H.; Yokoyama, A.; Kohno, N. Suppression of plasminogen activator inhibitor-1 by RNA interference attenuates pulmonary fibrosis. *Thorax* **2010**, *65*, 334–340.
21. D'Alessandro-Gabazza, C.N.; Kobayashi, T.; Boveda-Ruiz, D.; Takagi, T.; Toda, M.; Gil-Bernabe, P.; Miyake, Y.; Yasukawa, A.; Matsuda, Y.; Suzuki, N.; *et al.* Development and preclinical efficacy of novel transforming growth factor-beta1 short interfering RNAs for pulmonary fibrosis. *Am. J. Respir. Cell. Mol. Biol.* **2012**, *46*, 397–406.
22. Lam, J.K.; Liang, W.; Chan, H.K. Pulmonary delivery of therapeutic siRNA. *Adv. Drug Deliv. Rev.* **2012**, *64*, 1–15.
23. Agu, R.U.; Ugwoke, M.I.; Armand, M.; Kinget, R.; Verbeke, N. The lung as a route for systemic delivery of therapeutic proteins and peptides. *Respir. Res.* **2001**, *2*, 198–209.
24. Jensen, D.M.; Cun, D.; Maltesen, M.J.; Frokjaer, S.; Nielsen, H.M.; Foged, C. Spray drying of siRNA-containing PLGA nanoparticles intended for inhalation. *J. Control. Release* **2010**, *142*, 138–145.
25. Jensen, D.K.; Jensen, L.B.; Koocheki, S.; Bengtson, L.; Cun, D.; Nielsen, H.M.; Foged, C. Design of an inhalable dry powder formulation of DOTAP-modified PLGA nanoparticles loaded with siRNA. *J. Control. Release* **2012**, *157*, 141–148.
26. Perl, M.; Chung, C.S.; Lomas-Neira, J.; Rachel, T.M.; Biffi, W.L.; Cioffi, W.G.; Ayala, A. Silencing of Fas, but not caspase-8, in lung epithelial cells ameliorates pulmonary apoptosis, inflammation, and neutrophil influx after hemorrhagic shock and sepsis. *Am. J. Pathol.* **2005**, *167*, 1545–1559.
27. Lomas-Neira, J.L.; Chung, C.S.; Wesche, D.E.; Perl, M.; Ayala, A. In vivo gene silencing (with siRNA) of pulmonary expression of MIP-2 *versus* KC results in divergent effects on hemorrhage-induced, neutrophil-mediated septic acute lung injury. *J. Leukoc Biol* **2005**, *77*, 846–853.

28. Merkel, O.M.; Beyerle, A.; Librizzi, D.; Pfestroff, A.; Behr, T.M.; Sproat, B.; Barth, P.J.; Kissel, T. Nonviral siRNA delivery to the lung: investigation of PEG-PEI polyplexes and their *in vivo* performance. *Mol. Pharm.* **2009**, *6*, 1246–1260.
29. Garbuzenko, O.B.; Saad, M.; Betigeri, S.; Zhang, M.; Vetcher, A.A.; Soldatenkov, V.A.; Reimer, D.C.; Pozharov, V.P.; Minko, T. Intratracheal *versus* intravenous liposomal delivery of siRNA, antisense oligonucleotides and anticancer drug. *Pharm. Res.* **2009**, *26*, 382–394.
30. Wang, J.C.; Lai, S.; Guo, X.; Zhang, X.; de Crombrughe, B.; Sonnylal, S.; Arnett, F.C.; Zhou, X. Attenuation of fibrosis *in vitro* and *in vivo* with SPARC siRNA. *Arthritis Res. Ther.* **2010**, doi: 10.1186/ar2973.
31. Gutbier, B.; Kube, S.M.; Reppe, K.; Santel, A.; Lange, C.; Kaufmann, J.; Suttorp, N.; Witzenrath, M. RNAi-mediated suppression of constitutive pulmonary gene expression by small interfering RNA in mice. *Pulm. Pharmacol. Ther.* **2010**, *23*, 334–344.
32. Driscoll, K.E.; Costa, D.L.; Hatch, G.; Henderson, R.; Oberdorster, G.; Salem, H.; Schlesinger, R.B. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol. Sci.* **2000**, *55*, 24–35.
33. Bivas-Benita, M.; Zwier, R.; Junginger, H.E.; Borchard, G. Non-invasive pulmonary aerosol delivery in mice by the endotracheal route. *Eur. J. Pharm. Biopharm.* **2005**, *61*, 214–218.
34. Lu, D.; Hickey, A.J. Pulmonary vaccine delivery. *Expert Rev. Vaccines* **2007**, *6*, 213–226.
35. Bitko, V.; Musiyenko, A.; Shulyayeva, O.; Barik, S. Inhibition of respiratory viruses by nasally administered siRNA. *Nat. Med.* **2005**, *11*, 50–55.
36. Massaro, D.; Massaro, G.D.; Clerch, L.B. Noninvasive delivery of small inhibitory RNA and other reagents to pulmonary alveoli in mice. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2004**, *287*, L1066–L1070.
37. Hickey, A.J.; Garcia-Contreras, L. Immunological and toxicological implications of short-term studies in animals of pharmaceutical aerosol delivery to the lungs: relevance to humans. *Crit. Rev. Ther. Drug Carrier. Syst.* **2001**, *18*, 387–431.
38. DeVincenzo, J.; Cehelsky, J.E.; Alvarez, R.; Elbashir, S.; Harborth, J.; Toudjarska, I.; Nechev, L.; Murugaiah, V.; Van Vliet, A.; Vaishnav, A.K.; *et al.* Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV). *Antiviral. Res.* **2008**, *77*, 225–231.
39. Mastrandrea, L.D.; Quattrin, T. Clinical evaluation of inhaled insulin. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1061–1075.
40. Bai, S.; Gupta, V.; Ahsan, F. Inhalable lactose-based dry powder formulations of low molecular weight heparin. *J. Aerosol. Med. Pulm. Drug Deliv.* **2010**, *23*, 97–104.
41. Sanders, N.; Rudolph, C.; Braeckmans, K.; De Smedt, S.C.; Demeester, J. Extracellular barriers in respiratory gene therapy. *Adv. Drug Deliv. Rev.* **2009**, *61*, 115–127.
42. Roy, I.; Vij, N. Nanodelivery in airway diseases: Challenges and therapeutic applications. *Nanomedicine* **2010**, *6*, 237–244.
43. Groneberg, D.A.; Eynott, P.R.; Lim, S.; Oates, T.; Wu, R.; Carlstedt, I.; Roberts, P.; McCann, B.; Nicholson, A.G.; Harrison, B.D.; *et al.* Expression of respiratory mucins in fatal status asthmaticus and mild asthma. *Histopathology* **2002**, *40*, 367–373.



44. Jeffery, P.K. Remodeling in asthma and chronic obstructive lung disease. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, S28–S38.
45. Lehardt, T.; Roesler, S.; Beck-Broichsitter, M.; Kissel, T.; Polymeric nanocarriers for drug delivery to the lung. *J. Drug Deliv. Sci. Technol.* **2010**, *20*, 171–180.
46. Akhtar, S.; Benter, I.F. Nonviral delivery of synthetic siRNAs *in vivo*. *J. Clin. Invest.* **2007**, *117*, 3623–3632.
47. Goula, D.; Becker, N.; Lemkine, G.F.; Normandie, P.; Rodrigues, J.; Mantero, S.; Levi, G.; Demeneix, B.A. Rapid crossing of the pulmonary endothelial barrier by polyethylenimine/DNA complexes. *Gene Ther.* **2000**, *7*, 499–504.
48. Wang, J.; Lu, Z.; Wientjes, M.G.; Au, J.L. Delivery of siRNA therapeutics: Barriers and carriers. *AAPS J.* **2010**, *12*, 492–503.
49. Endoh, T.; Ohtsuki, T. Cellular siRNA delivery using cell-penetrating peptides modified for endosomal escape. *Adv. Drug Deliv. Rev.* **2009**, *61*, 704–709.
50. Novobrantseva, T.I.; Akinc, A.; Borodovsky, A.; de Fougerolles, A. Delivering silence: advancements in developing siRNA therapeutics. *Curr. Opin. Drug Discov. Devel.* **2008**, *11*, 217–224.
51. Shen, C.; Buck, A.K.; Liu, X.; Winkler, M.; Reske, S.N. Gene silencing by adenovirus-delivered siRNA. *FEBS Lett.* **2003**, *539*, 111–114.
52. Narvaiza, I.; Aparicio, O.; Vera, M.; Razquin, N.; Bortolanza, S.; Prieto, J.; Fortes, P. Effect of adenovirus-mediated RNA interference on endogenous microRNAs in a mouse model of multidrug resistance protein 2 gene silencing. *J. Virol.* **2006**, *80*, 12236–12247.
53. Guo, X.; Wang, W.; Hu, J.; Feng, K.; Pan, Y.; Zhang, L.; Feng, Y. Lentivirus-mediated RNAi knockdown of NUPR1 inhibits human nonsmall cell lung cancer growth *in vitro* and *in vivo*. *Anat. Rec.* **2012**, *295*, 2114–2121.
54. Scanlon, K.J. Cancer gene therapy: Challenges and opportunities. *Anticancer Res.* **2004**, *24*, 501–504.
55. Teichler Zallen, D. US gene therapy in crisis. *Trends Genet.* **2000**, *16*, 272–275.
56. Musiyenko, A.; Bitko, V.; Barik, S. RNAi-dependent and -independent antiviral phenotypes of chromosomally integrated shRNA clones: role of VASP in respiratory syncytial virus growth. *J. Mol. Med.* **2007**, *85*, 745–752.
57. Thomas, M.; Lu, J.J.; Chen, J.; Klibanov, A.M. Non-viral siRNA delivery to the lung. *Adv. Drug Deliv. Rev.* **2007**, *59*, 124–133.
58. Tseng, Y.C.; Mozumdar, S.; Huang, L. Lipid-based systemic delivery of siRNA. *Adv. Drug Deliv. Rev.* **2009**, *61*, 721–731.
59. Tompkins, S.M.; Lo, C.Y.; Tumpey, T.M.; Epstein, S.L. Protection against lethal influenza virus challenge by RNA interference *in vivo*. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 8682–8686.
60. Han, S.W.; Roman, J. Fibronectin induces cell proliferation and inhibits apoptosis in human bronchial epithelial cells: pro-oncogenic effects mediated by PI3-kinase and NF-kappa B. *Oncogene* **2006**, *25*, 4341–4349.
61. Thomas, M.; Lu, J.J.; Ge, Q.; Zhang, C.; Chen, J.; Klibanov, A.M. Full deacylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5679–5684.

62. Sioud, M.; Sorensen, D.R. Cationic liposome-mediated delivery of siRNAs in adult mice. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1220–1225.
63. Wu, S.Y.; McMillan, N.A. Lipidic systems for *in vivo* siRNA delivery. *AAPS J.* **2009**, *11*, 639–652.
64. Jackson, A.L.; Bartz, S.R.; Schelter, J.; Kobayashi, S.V.; Burchard, J.; Mao, M.; Li, B.; Cavet, G.; Linsley, P.S. Expression profiling reveals off-target gene regulation by RNAi. *Nat. Biotechnol.* **2003**, *21*, 635–637.
65. Bridge, A.J.; Pebernard, S.; Ducraux, A.; Nicoulaz, A.L.; Iggo, R. Induction of an interferon response by RNAi vectors in mammalian cells. *Nat. Genet.* **2003**, *34*, 263–264.
66. Heidel, J.D.; Hu, S.; Liu, X.F.; Triche, T.J.; Davis, M.E. Lack of interferon response in animals to naked siRNAs. *Nat. Biotechnol.* **2004**, *22*, 1579–1582.
67. Urban-Klein, B.; Werth, S.; Abuharbeid, S.; Czubayko, F.; Aigner, A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA *in vivo*. *Gene Ther.* **2005**, *12*, 461–466.
68. Pal, A.; Ahmad, A.; Khan, S.; Sakabe, I.; Zhang, C.; Kasid, U.N.; Ahmad, I. Systemic delivery of RafsiRNA using cationic cardioliipin liposomes silences RAF-1 expression and inhibits tumor growth in xenograft model of human prostate cancer. *Int. J. Oncol.* **2005**, *26*, 1087–1091.
69. Nielsen, E.J.; Nielsen, J.M.; Becker, D.; Karlas, A.; Prakash, H.; Glud, S.Z.; Merrison, J.; Besenbacher, F.; Meyer, T.F.; Kjems, J.; Howard, K.A. Pulmonary gene silencing in transgenic EGFP mice using aerosolised chitosan/siRNA nanoparticles. *Pharm. Res.* **2010**, *27*, 2520–2527.
70. Akinc, A.; Zumbuehl, A.; Goldberg, M.; Leshchiner, E.S.; Busini, V.; Hossain, N.; Bacallado, S.A.; Nguyen, D.N.; Fuller, J.; Alvarez, R.; *et al.* A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. *Nat. Biotechnol.* **2008**, *26*, 561–569.
71. Cosio, M.G.; Saetta, M.; Agusti, A. Immunologic aspects of chronic obstructive pulmonary disease. *New Engl. J. Med.* **2009**, *360*, 2445–2454.
72. Xu, L.; Anchordoquy, T. Drug delivery trends in clinical trials and translational medicine: challenges and opportunities in the delivery of nucleic acid-based therapeutics. *J. Pharm. Sci.* **2011**, *100*, 38–52.
73. Shen, J.; Samul, R.; Silva, R.L.; Akiyama, H.; Liu, H.; Saishin, Y.; Hackett, S.F.; Zinnen, S.; Kossen, K.; Fosnaugh, K.; *et al.* Suppression of ocular neovascularization with siRNA targeting VEGF receptor 1. *Gene Ther.* **2006**, *13*, 225–234.
74. The US National Institutes of Health. ClinicalTrials.gov. Available on line: <http://www.clinicaltrials.gov/> (accessed on 30 November 2012).
75. Falsey, A.R.; Hennessey, P.A.; Formica, M.A.; Cox, C.; Walsh, E.E. Respiratory syncytial virus infection in elderly and high-risk adults. *New Engl. J. Med.* **2005**, *352*, 1749–1759.
76. Zamora, M.R.; Budev, M.; Rolfe, M.; Gottlieb, J.; Humar, A.; Devincenzo, J.; Vaishnav, A.; Cehelsky, J.; Albert, G.; Nochur, S.; *et al.* RNA interference therapy in lung transplant patients infected with respiratory syncytial virus. *Am. J. Respir. Crit. Care Med.* **2011**, *183*, 531–538.
77. Dong, A.Q.; Kong, M.J.; Ma, Z.Y.; Qian, J.F.; Xu, X.H. Down-regulation of IGF-IR using small, interfering, hairpin RNA (siRNA) inhibits growth of human lung cancer cell line A549 *in vitro* and in nude mice. *Cell Biol. Int.* **2007**, *31*, 500–507.

78. Xia, H.; Yu, C.H.; Zhang, Y.; Yu, J.; Li, J.; Zhang, W.; Zhang, B.; Li, Y.; Guo, N. EZH2 silencing with RNAi enhances irradiation-induced inhibition of human lung cancer growth *in vitro* and *in vivo*. *Oncol. Lett.* **2012**, *4*, 135–140.
79. Aleku, M.; Schulz, P.; Keil, O.; Santel, A.; Schaeper, U.; Dieckhoff, B.; Janke, O.; Endruschat, J.; Durieux, B.; Roder, N.; *et al.* Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression. *Cancer Res.* **2008**, *68*, 9788–9798.
80. Leenders, F.; Mopert, K.; Schmiedeknecht, A.; Santel, A.; Czauderna, F.; Aleku, M.; Penschuck, S.; Dames, S.; Sternberger, M.; Rohl, T.; *et al.* PKN3 is required for malignant prostate cell growth downstream of activated PI 3-kinase. *EMBO J.* **2004**, *23*, 3303–3313.
81. Katso, R.; Okkenhaug, K.; Ahmadi, K.; White, S.; Timms, J.; Waterfield, M.D. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu. Rev. Cell Dev. Biol.* **2001**, *17*, 615–675.
82. Hao, S.; Baltimore, D. The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules. *Nat. Immunol.* **2009**, *10*, 281–288.
83. Falk, J.A.; Minai, O.A.; Mosenifar, Z. Inhaled and systemic corticosteroids in chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.* **2008**, *5*, 506–512.
84. Tsuji, T.; Aoshiba, K.; Nagai, A. Cigarette smoke induces senescence in alveolar epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **2004**, *31*, 643–649.
85. Tuder, R.M.; Kern, J.A.; Miller, Y.E. Senescence in chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.* **2012**, *9*, 62–63.
86. Molfino, N.A. Genetics of COPD. *Chest* **2004**, *125*, 1929–1940.
87. Chen, Z.H.; Kim, H.P.; Ryter, S.W.; Choi, A.M. Identifying targets for COPD treatment through gene expression analyses. *Int. J. Chron. Obstruct. Pulmon. Dis.* **2008**, *3*, 359–370.
88. Edwards, M.R.; Bartlett, N.W.; Clarke, D.; Birrell, M.; Belvisi, M.; Johnston, S.L. Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol. Ther.* **2009**, *121*, 1–13.
89. Shapiro, S.D.; Goldstein, N.M.; Houghton, A.M.; Kobayashi, D.K.; Kelley, D.; Belaaouaj, A. Neutrophil elastase contributes to cigarette smoke-induced emphysema in mice. *Am. J. Pathol.* **2003**, *163*, 2329–2335.
90. Yoshida, T.; Tuder, R.M. Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. *Physiol. Rev.* **2007**, *87*, 1047–1082.
91. Huang, S.L.; Su, C.H.; Chang, S.C. Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. *Am. J. Respir. Crit. Care Med.* **1997**, *156*, 1436–1439.
92. Stevenson, C.S.; Coote, K.; Webster, R.; Johnston, H.; Atherton, H.C.; Nicholls, A.; Giddings, J.; Sugar, R.; Jackson, A.; Press, N.J.; *et al.* Characterization of cigarette smoke-induced inflammatory and mucus hypersecretory changes in rat lung and the role of CXCR2 ligands in mediating this effect. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2005**, *288*, 29.
93. Stevenson, C.S.; Docx, C.; Webster, R.; Battram, C.; Hynx, D.; Giddings, J.; Cooper, P.R.; Chakravarty, P.; Rahman, I.; Marwick, J.A.; *et al.* Comprehensive gene expression profiling of rat lung reveals distinct acute and chronic responses to cigarette smoke inhalation. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2007**, *293*, L1183–L1193.

94. Aoshiba, K.; Yokohori, N.; Nagai, A. Alveolar wall apoptosis causes lung destruction and emphysematous changes. *Am. J. Respir. Cell Mol. Biol.* **2003**, *28*, 555–562.
95. Kasahara, Y.; Tuder, R.M.; Taraseviciene-Stewart, L.; Le Cras, T.D.; Abman, S.; Hirth, P.K.; Waltenerger, J.; Voelkel, N.F. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J. Clin. Invest.* **2000**, *106*, 1311–1319.
96. Barnes, P.J.; Stockley, R.A. COPD: current therapeutic interventions and future approaches. *Eur. Respir. J.* **2005**, *25*, 1084–1106.
97. Banerjee, A.; Koziol-White, C.; Panettieri, R., Jr. p38 MAPK inhibitors, IKK2 inhibitors, and TNFalpha inhibitors in COPD. *Curr. Opin. Pharmacol.* **2012**, *12*, 287–292.
98. World Health Organization. Chronic respiratory disease—Asthma. Available online: <http://www.who.int/respiratory/asthma/en/> (accessed on 30 November 2012).
99. Huang, H.Y.; Chiang, B.L. siRNA as a therapy for asthma. *Curr. Opin. Mol. Ther.* **2009**, *11*, 652–663.
100. Meinicke, H.; Darcan, Y.; Hamelmann, E. Targeting allergic airway diseases by siRNA: An option for the future? *Curr. Mol. Med.* **2009**, *9*, 483–494.
101. Popescu, F.D. Antisense- and RNA interference-based therapeutic strategies in allergy. *J. Cell Mol. Med.* **2005**, *9*, 840–853.
102. Stenton, G.R.; Kim, M.K.; Nohara, O.; Chen, C.F.; Hirji, N.; Wills, F.L.; Gilchrist, M.; Hwang, P.H.; Park, J.G.; Finlay, W.; *et al.* Aerosolized Syk antisense suppresses Syk expression, mediator release from macrophages, and pulmonary inflammation. *J. Immunol.* **2000**, *164*, 3790–3797.
103. Ulanova, M.; Puttagunta, L.; Marcet-Palacios, M.; Duszyk, M.; Steinhoff, U.; Duta, F.; Kim, M.K.; Indik, Z.K.; Schreiber, A.D.; Befus, A.D. Syk tyrosine kinase participates in beta1-integrin signaling and inflammatory responses in airway epithelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2005**, *288*, 19.
104. Winter, J.; Jung, S.; Keller, S.; Gregory, R.I.; Diederichs, S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell. Biol.* **2009**, *11*, 228–234.
105. Pagdin, T.; Lavender, P. MicroRNAs in lung diseases. *Thorax* **2012**, *67*, 183–184.
106. Trang, P.; Medina, P.P.; Wiggins, J.F.; Ruffino, L.; Kelnar, K.; Omotola, M.; Homer, R.; Brown, D.; Bader, A.G.; Weidhaas, J.B.; *et al.* Regression of murine lung tumors by the let-7 microRNA. *Oncogene* **2010**, *29*, 1580–1587.
107. Wiggins, J.F.; Ruffino, L.; Kelnar, K.; Omotola, M.; Patrawala, L.; Brown, D.; Bader, A.G. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Res.* **2010**, *70*, 5923–5930.
108. Liu, C.; Kelnar, K.; Liu, B.; Chen, X.; Calhoun-Davis, T.; Li, H.; Patrawala, L.; Yan, H.; Jeter, C.; Honorio, S.; *et al.* The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat. Med.* **2011**, *17*, 211–215.
109. Van Rooij, E.; Sutherland, L.B.; Thatcher, J.E.; DiMaio, J.M.; Naseem, R.H.; Marshall, W.S.; Hill, J.A.; Olson, E.N. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13027–13032.
110. Lanford, R.E.; Hildebrandt-Eriksen, E.S.; Petri, A.; Persson, R.; Lindow, M.; Munk, M.E.; Kauppinen, S.; Orum, H. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* **2010**, *327*, 198–201.