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The potential of siRNA based drug delivery in respiratory disorders: recent advances and progress

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Abstract

Lung diseases are the leading cause of mortality worldwide. The currently available therapies are not sufficient, leading to the urgent need for new therapies with sustained anti-inflammatory effects. Small/short or silencing interfering RNA (siRNA) has potential therapeutic implications through post-transcriptional downregulation of the target gene expression. siRNA is essential in gene regulation, so is more favorable over other gene therapies due to its small size, high specificity, potency and no or low immune response. In chronic respiratory diseases, local and targeted delivery of siRNA is achieved via inhalation. The effectual delivery can be attained by the generation of aerosols via inhalers and nebulizers which overcomes anatomical barriers, alveolar macrophage clearance and mucociliary clearance. In this review, we discuss the different siRNA nanocarrier systems for chronic respiratory diseases, for safe and effective delivery. siRNA mediated pro-inflammatory gene or miRNA targeting approach can be a useful approach in combating chronic respiratory inflammatory conditions and thus providing sustained drug delivery, reduced therapeutic dose, and improved patient compliance. This review will be of high relevance to the formulation, biological and translational scientists working in the area of respiratory diseases.

Keywords: siRNA; delivery systems; pulmonary; nanocarriers; RNA interference
Introduction

Chronic respiratory disease (CRD), in particular, asthma and chronic obstructive pulmonary disease (COPD), are amongst the leading causes of mortality and morbidity that also exert huge health and economic burden globally ("Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015," 2017). Asthma is a complex and heterogeneous chronic inflammatory disorder, primarily affecting the airways. The disease is largely ‘allergic’ in nature and is characterized by the upregulation of genes that lead to multiple inflammatory cascades implicated in asthma (Moheimani et al., 2016). Current asthma therapies focus on reduction of symptoms and limit exacerbations during the course of the disease. However, the proportion of asthmatics with the uncontrolled disease remain significantly high and utilizes the majority of healthcare expenses globally (P. M. Hansbro et al., 2017; Peters, Ferguson, Deniz, & Reisner, 2006). Despite the increasing classification/categorization of asthmatics into various endotypes/phenotypes (based on a number of molecular biomarkers, clinical presentation and responsiveness to common therapies)(Fajt & Wenzel, 2015), the key pathological feature of asthma is involvement of multiple inflammatory mediators that are often regulated by ‘key’ genes/proteins, which are also referred as master transcription regulators(Sel, Henke, Dietrich, Herz, & Renz, 2006). Thus, targeting these key pro-inflammatory genes would potentially improve the overall disease outcomes(Sel et al., 2006).

COPD is another complex and multi-factorial respiratory disease that is caused primarily due to chronic exposure to cigarette smoking(Rennard & Vestbo, 2006). In addition, exposures to biomass smoke, air pollution and a variety of occupational exposures to chemical dust and fumes also play an important role in the onset and progression of COPD(KC, Shukla, Gautam, Hansbro, & O’Toole, 2018). COPD constitutes multiple inflammatory pathways, that are induced by chronic exposure to irritants and, in addition, due to an imbalance between the oxidase/anti-oxidant ratio and/or proteases/anti-proteases ratio (Peter J. Barnes, 2016). The course of the disease is further complicated by frequent acute exacerbations, which are described as worsening of disease symptoms that require a change of daily medications and often requiring hospitalizations(Rodriguez-Roisin, 2000). Acute exacerbations are primarily caused by infections (bacterial and/or viral) which dramatically increase the risk of mortality and morbidity amongst COPD patients(Sapey & Stockley, 2006). In addition, the lack of effective therapies to limit the onset and/or progression of COPD further aids in increased global morbidity and mortality, especially in aging populations.
Idiopathic pulmonary fibrosis (IPF) is a life-threatening lung disease that involves the progressive loss of lung function along with clinically significant thickness/stiffness of lung tissues, generally accompanied by tissue scarring. The 3- and 5-year mortality rates in IPF patients are almost 50% (Meltzer & Noble, 2008). Integrative network analysis revealed a total of 27 genes (CHIT1, CXCL14, LPPR4, etc.) and 22 miRNAs (in particular, miR-409-5p and has-miR-376c) that are associated with the disease development (L. Wang, Huang, Zhang, Chen, & Zhao, 2018). Targeting these and similar nucleic acids that are crucial in the onset/progression of IPF would certainly lead to the development of novel therapies for treating IPF and enhancing the quality of life in IPF patients.

Lung cancer is one of the leading causes of cancer-related mortality globally (Bray et al., 2018). A number of genetic mutations have been linked to the pathogenesis of two major types of lung cancer, including non-small-cell lung cancer (e.g., KRAS, EGFR, ALK, MET exon 14, BRAF, PIK3CA, ROS1, HER2, and RET) ("Comprehensive molecular profiling of lung adenocarcinoma," 2014) and squamous cell carcinoma (PIK3CA, PTEN and amplification of FGFR1) (Rosell & Karachaliou, 2016). Importantly, circulating cell-free nucleic acids are now being investigated/utilized in both diagnosis and personalised treatments for lung cancers, which have been reviewed in detail by Sorber et al. (Sorber et al., 2017). Given the immense burden and limited treatment options for lung cancer, nucleic acid-based therapies may present a novel therapeutic front to develop personalised therapies.

Respiratory infections, especially with viruses and bacteria, further complicate the course of chronic respiratory diseases. In particular, viral pathogens, such as rhinovirus, influenza virus and respiratory syncytial virus, are often implicated in the exacerbations of respiratory diseases, including asthma, COPD and IPF (Wark, Tooze, Powell, & Parsons, 2013). In addition, bacterial pathogens (e.g., Haemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis), either on its own or as a secondary pathogen following prior viral infections seem to be more detrimental to the lung health and poorer quality of life (Didierlaurent, Goulding, & Hussell, 2007; Wark et al., 2013). A better understanding of the interactions between respiratory infections and chronic respiratory diseases (asthma, COPD) is essential for the design of more effective preventive and treatment strategies. For instance, tuberculosis seems to increase the risk of COPD and vice versa (O'Toole, Shukla, & Walters, 2015). Moreover, tuberculosis increases the oxidative burden in the lungs (K. Dua et al., 2018; Shastri et al., 2018), regulated by multiple inflammatory mediators (K. Dua et al., 2019), that could be targeted with the novel, nucleic acid-based therapies for reducing the treatment regimens.

Despite the huge burden of major respiratory disease, there is a lack of effective treatments that could limit the disease onset and/or disease progression (K Dua, DK Chellappan, et al., 2018; K Dua, Gupta,
Chellappan, Shukla, & Hansbro, 2018; Kamal Dua, Vamshi Krishna Rapalli, et al., 2018; Kamal Dua, Shukla, de Jesus Andreoli Pinto, & Hansbro, 2017; Rapalli et al., 2018). Thus, extensive research into potentially novel therapeutics is urgently required. Currently, the potential of novel classes of therapeutics, which are nucleic acid-based, for treating lung diseases is under intense investigation due to their crucial roles in regulating gene expression(Kamal Dua, Terezinha de Jesus Andreoli Pinto, et al., 2018; Kamal Dua, Hansbro, Foster, & Hansbro, 2017; Kamal Dua, Shukla, Tekade, & Hansbro, 2017). For instance, antisense oligonucleotides, which are single-strand DNAs or RNAs that selectively bind to complementary mRNAs and modulate their functions, could potentially result in up-/down-regulation of particular genes, thus aiding in limiting the disease progression (Bennett, Baker, Pham, Swayze, & Geary, 2017). Similarly, small interference RNAs (siRNAs) are double-strand RNA molecules (~21-23 base pairs in length) capable to ‘silence’ specific genes of a known sequence responsible for genome stability(J. K. Lam, Chow, Zhang, & Leung, 2015). siRNA works at two stages: post-transcriptional gene silencing (PTGS) resulting in direct sequence-specific cleavage causing repression of translation and degradation resulting in transcriptional gene silencing (TGS). siRNA is found associated with effector associations which are recognized by RNA-induced silencing complexes (RISCs)(Martinez, Patkaniowska, Urlaub, Lührmann, & Tuschl, 2002). Further, this siRNA uses its full sequence for the recognition of the target sequence and cleaves the target mRNA (Jie Wang, Lu, Wientjes, & Au, 2010).

Micro RNAs (miRNAs) are single-stranded, endogenous non-coding RNA molecules (~18–24 base pairs long). The miRNAs act as important regulators for a variety of immunological and cellular pathways (Awasthi, Madan, Malipeddi, Dua, & Kulkarni, 2019; Hansbro & Dua, 2018; D. D. Nguyen & Chang, 2017). Notably, siRNAs have been previously speculated to be an effective nucleic acid-based therapy in asthma(M. Choi, J. Gu, M. Lee, & T. Rhim, 2017; Luo et al., 2012), COPD(Luo et al., 2012), IPF(C. N. D'Alessandro-Gabazza et al., 2012), lung cancer(Merkel, Rubinstein, & Kisel, 2014) and respiratory infections(Merkel et al., 2014). We have attempted to primarily focus on the potential drug delivery strategies to enhance the efficacy of siRNAs in treating chronic respiratory diseases, because siRNA can result in multiple gene mutations (oncogenes and tumor suppressor genes) and acts as efficient and promising cure of disease in comparison to other therapies.

**Drug delivery systems for siRNA in pulmonary diseases**

siRNA has an enormous prospective for the treatment or avoidance of different lung infections. When the RNA particles effectively enter the target, they may obstruct the expression of specific gene series through RNA interference (RNAi) to produce remedial impacts(Amreddy et al., 2018; Ayatollahi et al.,
The greatest deterrent to translating siRNA treatment from the research facilities into the clinics is delivery. A perfect delivery operator ought to shield the siRNA from enzymatic debasement, encourage cell take-up and advance endosomal escape inside the cells, with irrelevant poisonous effect (V. Capel et al., 2018; Jin et al., 2018). Pulmonary targeting could be accomplished by fundamental delivery or lung delivery. The latter administrative route could possibly upgrade siRNA maintenance in the lungs and lessen fundamental poisonous impacts. The delivery design should be planned cautiously so as to boost the deposition of siRNA to the unhealthy region of the aviation routes. In majority of the lung siRNA treatment studies in vivo, siRNA was conveyed either intranasally or intratracheally (Figure: 1) (I. d'Angelo et al., 2018; Ding et al., 2018; He et al., 2018).

Limited studies have been reported on siRNA formulations via inhalation, although, it is expected for potential future prospects. Following are the delivery systems of siRNA in pulmonary diseases:

1. **Lipid-based delivery vectors**

The lipid-based delivery system is generally used to deliver siRNA in vitro and in vivo. Ordinarily, cationic lipids or liposomes utilizes negatively charged siRNA through electrostatic forces and are known as lipoplexes. Several commercially available agents are lipid-based, some of which are for in vivo lung delivery, for example, DharmFECT, lipofectamine, and Oligofectamine. The main difficulties of utilizing lipid-based conveyance vectors in the clinical setup are their harmfulness and their non-specific activation of inflammatory cytokines and interferon reactions. Lipid-based delivery vectors can be classified into five types of molecules: Cationic lipoplexes and liposomes; PEGylated lipids; Neutral lipids; Lipids particles and Lipid-like molecules(Kaczmarek et al., 2018; Liu et al., 2019; Mokhtarieh, Lee, Kim, & Lee, 2018; O. S. Muddineti, A. Shah, S. V. K. Rompicharla, B. Ghosh, & S. Biswas, 2018).

2. **Polymer-based delivery vectors**

The polymer-based delivery vectors have an adaptable nature, which enables their physicochemical qualities to change effectively to accommodate their purpose. Also, it has been recommended that polymers usually do not induce a strong immune response (Ni et al., 2018; Otsuka et al., 2017). Polymer-based vectors are separated into two classes: polycations and polymeric nanoparticles. Engineered polycations, for example, polyamidoamine dendrimers and polyethyleneimine and natural polycations, for example, chitosan are utilized for conveying DNA for a long time (Y. Qiu et al., 2017; Sasaki & Guo, 2018).

3. **Peptide-based delivery vectors**
Since the discovery of TAT protein from HIV-1, which assist in the uptake of the virus in the cell, a range of cell-penetrating peptides (CPPs) have been identified. CPPs are most commonly utilized as vehicles of restorative macromolecules. This technique can thus be used to deliver siRNA (Veilleux et al., 2018; P. Y. Xu et al., 2018). CPPs and subsidiaries have been explored for siRNA conveyance which include MPG, TAT, transportan, penetratin, CADY and LAH4. The peptides are either covalently connected to siRNA through disulfide bond development or electrostatically in a non-covalent way (Yuan et al., 2017; D. Zhang et al., 2018).

**Immune response in siRNA-mediated drug delivery to lungs**

Airway inflammation is the major contributing factor in the pathogenesis of lung diseases, which corresponds to the degree of symptoms, airways hyper-responsiveness, and obstruction. Therefore, siRNA mediated delivery vehicle, as well as the products of secondary RNAi, stimulate the immune system by enhancing the expression or suppressing pro-inflammatory cytokines, interferons, and toll-like receptors via signaling pathways (Sioud, 2015). This is evident from an in vivo study for intratracheal administration of penetratin (peptide) conjugated siRNA capable of penetrating cytosol, activating several biochemical pathways and elevates the expression of TNF-α, IL-12, and p40. It also activates the innate immune system to target p38 MAP kinase (Moschos et al., 2007).

The approach of siRNA based therapeutics against tuberculosis infections directs the gene expression modulation of the host rather than the bacilli (Man, Chow, Casettari, Gonzalez-Juarrero, & Lam, 2016). The genes that regulate autophagy in the host can be targets for *M. tuberculosis* infection such as Rap22a, Bfl-1/A1, Ras homologue enriched in brain (Rheb) and UV radiation resistance associated genes (UVRAG) (Kathania, Raje, Raje, Dutta, & Majumdar, 2011; Kim et al., 2015; Roberts, Chua, Kyei, & Deretic, 2006; Jinli Wang et al., 2013). The suppression of these genes can hinder bacterial growth and proliferation. Another strategy is immunosuppression to inhibit bacterial growth. TNF-α and IFNγ are essential for macrophage activation to initiate granuloma formation for the proliferation of *M. tuberculosis* (Ramakrishnan, 2012; Silva Miranda, Breiman, Allain, Deknuydt, & Altare, 2012). Chemokine and Lymphotacin/XCL-1 activate CD8+T cells during severe tuberculosis infection. In addition to this, XCL-1 regulates IFN-γ and CD4+T cells to maintain granuloma. When siRNA targeting XCL-1 is delivered in TB infected mice, the expression of XCL-1 is greatly reduced, therefore, decreasing T lymphocytes, INFγ, and granuloma (Rosas-Taraco, Higgins, Sanchez-Campillo, et al., 2009).

The intranasal siRNA mediated delivery of SOC3 in chronic asthma mouse model had been reported to improve eosinophil count and airways hyper-responsiveness. This has also been correlated to improved
mucus secretion, collagen reduction, and airway remodeling. siRNA mediated SOC3 reduces RhoA/Rho kinase signaling pathway, thereby, regulating inflammation, bronchial smooth muscle contractions and upregulating IL-13, IL-4 via RhoA protein and STAT6 activation. This reduces cytokine expression and airways hyper-responsiveness. It is known that IL-17 and IL-23 are responsible for neutrophilic and eosinophilic inflammation in mice (Figure.2)(Molet et al., 2001; Wakashin et al., 2008). Thus, siRNA mediated silencing of SOC3 regulates IL-17 expression (Staff, 2014).

siRNA drug delivery in asthma/allergic airway diseases

The combination therapy for asthma includes the use of inhaled corticosteroids, β₂-adrenergic receptor agonists, injected immunoglobulin E antibodies and quick-relief medications for effective control of asthma (Peter J Barnes, 2004). However, conventional inhaled corticosteroid therapies do not work in severe asthmatic patients (refractory asthma) and may cause adverse effects after long-term treatment (Kamal Dua, Hansbro, & Hansbro, 2017; Philip M Hansbro et al., 2017; Mealey, Kenyon, Avdalovic, & Louie, 2007). Therefore, more attention is needed in asthma research focused on the development of target-specific therapeutics. It has been previously reported that proteins such as chemokines (Rosenwasser, Zimmermann, Hershey, Foster, & Rothenberg, 2003) and cytokines (Peter J Barnes, 2008) are involved in the asthmatic-inflammatory processes. Leukotrienes are also involved in the pathogenesis of asthma (Ogawa & Calhoun, 2006).

Omalizumab (Xolair® - a monoclonal antibody against IgE), reslizumab (Cinqair® in the US and Cinqaero® in Europe -a humanized antibody against human interleukin-5) and mepolizumab (Nucala® -blocks interleukin-5) are commercially available potential targeted therapeutics to treat asthma (Catley, Coote, Bari, & Tomlinson, 2011; Corren, 2012). Due to the complex nature of the disease, there is no single medication available for effective control of the asthmatic symptoms. Such targeted therapies may provide a valuable breakthrough in the pathophysiology of asthma and increase the possibility of decreasing asthma burden (M Choi, J Gu, M Lee, & T Rhim, 2017; Cook & Bochner, 2010).

siRNA delivery to the pulmonary system has ameliorated the therapeutic benefits of RNA interference (RNAi) for asthma (H.-Y. Huang & Chiang, 2009). RNAi has been reported to be effective in blocking functions of molecular targets of asthma. siRNAs (21-23 bp in length) are involved in the sequence-specific degradation of messenger RNA (mRNA) and decrease the expression of the corresponding proteins (Agrawal et al., 2003; Xie & Merkel, 2015). Prolonged lung retention of siRNA administered via the pulmonary route can reduce systemic side effects and improve the therapeutic benefits in the
treatment of asthma (Deng et al., 2014; Rettig & Behlke, 2012; Xie & Merkel, 2015). Intranasal administration of siRNA formulation with or without a transfection agent has been reported to inhibit replication of the respiratory syncytial virus in mice models (Whitehead, Langer, & Anderson, 2009).

Xie et al., (2016) developed a siRNA based delivery system, transferring polyethylenimine (Tf-PEI), to target specific delivery of siRNAs and activated T cells in the lung. The optimized polyplexes possess ideal physical properties such as zeta-potential, size, distribution and siRNA condensation efficiency. Formulated polyplexes showed significant enhancement in cellular uptake and gene knockdown. Biodistribution studies in murine asthmatic model confirmed effective delivery of siRNA to the activated T cells (Xie et al., 2016). Based on the literature evidence, Xie and Merkel summarized that chemokines, cytokines, tyrosine kinases, transcription factors, and co-stimulatory factors are the target of siRNA-mediated asthma treatment. Further, the authors proposed potential applications of targeted siRNA delivery to macrophages, T cells, and dendritic cells in asthma therapy (Xie & Merkel, 2015).

Wang et al., 2008 reported imiquimod cream-chitosan nanoparticle system containing siRNA green indicator (siGLO) or siRNA for natriuretic peptide receptor (siNPRA). The formulation was applied to the mice skin. Fluorescence microscopy confirmed the delivery of SiGLO to the lungs via transdermal route. OVA-sensitized asthmatic BALB/c mice model treated with imiquimod cream-siNPRA chitosan nanoparticles showed significant (< 0.05) decrease in airway hyper-responsiveness, lung histopathology, eosinophilia and pro-inflammatory cytokines IL-4 and IL-5 (X. Wang et al., 2008).

Packaging RNAs (pRNAs), a component of the bacteriophage phi29-packaging motor, have been used to deliver signal transducer and activator of transcription (STAT5b) siRNA to asthmatic spleen lymphocytes. Reverse transcription-polymerase chain reaction (RT-PCR) study showed that the STAT5b gene mRNA expression was effectively inhibited by the pRNA dimer. It is suggested that pRNA dimer carrying aptamer (ligand to interact with receptors) and siRNA can deliver functional siRNA to cells (C. Qiu, Peng, Shi, & Zhang, 2012). The first report on lung alveolar epithelial A549 cell targeting by siRNA-generation four, amine-terminated poly (amidoamine) dendrimerdendrplexes and about 40% gene silencing via siRNA exposed to the propellant used in oral inhalation devices was given by Conti et al., (Conti, Brewer, Grashik, Avasarala, & da Rocha, 2014).

Choi et al., developed dexamethasone and vitamin D binding protein (VDBP) siRNA combination therapeutic system for the treatment of asthma. The results showed that the dexamethasone-conjugated polyethylenimine/ vitamin D binding protein (DEXA-PEI/VDBP) siRNA, reduced goblet cell hyperplasia, ovalbumin sensitization, challenge-induced enhancement of airway inflammation, and
expressions of interleukin-4 (IL-13), interleukin-4 (IL-4) and eosinophil mobilizing chemokine (CCL11) (M Choi et al., 2017). Wu et al., investigated the ability of c-kit silenced siRNA to decrease the inflammation caused by allergic asthma using asthmatic mouse model treated with intranasal anti-c-kit siRNA. The result showed that siRNA significantly inhibited c-kit gene expression and decreased airway mucus secretion. The production of stem cell factor, IL-4, and IL-5 declined significantly by c-kit siRNA. However, no effect was recorded on interferon-γ (IFN-γ) generation (Wu et al., 2014).

Ambient particulate matters (PMs) are the major causative agents for asthma and chronic obstructive pulmonary disease, by increasing mucus hypersecretion and inflammation. Wang et al., used ambient particulate matter (PM)-exposed human bronchial epithelial cells (HBEC) to determine the function of Amphiregulin (AREG), a ligand for epidermal growth factor receptor (EGFR), in PM-induced inflammation and mucus hypersecretion. The AREG-siRNA significantly suppressed the PM-induced inflammation and mucus hypersecretion. This also suppressed the activation of the EGFR-AKT/ERK pathway (Jian Wang, Zhu, Wang, Chen, & Song, 2019).

**siRNA drug delivery in lung cancer**

Lung cancer is the leading cause of death all over the world (Siegel, Naishadham, & Jemal, 2012). The widely available therapies include chemotherapy, radiotherapy, and surgery, while, NSCLC treatment is majorly dependent on chemotherapy. Conventionally, chemotherapy has been practiced by intravenous (IV) administration. However, IV administration has side effects like bioavailability of the drug throughout the body via the bloodstream, which affects both malignant as well as healthy cells, the death of healthy cells causes adverse side effects like hair loss, fatigue and infections(Gandhi et al., 2018; Moding, Kastan, & Kirsch, 2013). Dobashi et al., reported that the Akt/mTOR pathway is abnormally activated in NSCLC (Dobashi, Watanabe, Miwa, Suzuki, & Koyama, 2011). Several tumor suppressors have been found to be mutated in NSCLC, proposing the function of mTOR pathway. Furthermore, the use of mTOR inhibitors is responsible for adverse side effects on healthy cells. Several molecular targeting drugs such as gefitinibcause inhibition of phosphorylation and tyrosine kinase activity of intracellular ATP binding domain of EGFR via competitive inhibition, erlotinib also inhibits tyrosine kinase and bevacizumab inhibits angiogenesis (Y.K. Oh & Park, 2009; Tiseo, Bartolotti, Gelsomino, & Bordi, 2010). Due to numerous side effects, there is an emerging need for therapeutics for the treatment of lung cancer especially NSCLC (Sadowski, Kotulska, & Jóźwiak, 2016).

Taratula and his co-workers developed nanostructured lipid-based carriers (NLCs) for simultaneous delivery of anticancer drug and siRNA specifically for lung cancer. The drug encapsulated nanocarriers...
induce cell death, whereas, siRNA suppresses multi-drug resistance. Two types of siRNA were delivered to improve efficacy; the first siRNA was targeting the MRP1 (Multidrug resistance-associated protein) mRNA which is responsible for the suppression of main drug efflux transporter. The second siRNA was targeting Bcl2 (B-cell lymphoma) mRNA, which suppresses cellular anti-apoptotic defense. The drug was delivered to lungs by inhalation after encapsulation in NLCs. Inhalation leads to high accumulation of nanocarriers in lungs whereas, intravenous injection led to major accumulation in liver, spleen, and kidney compared to lungs. The developed NLC formulation effectively delivered the drug and siRNA into cancer cells. This induced cell death of lung tumor cells by targeted gene silencing (Taratula, Kuzmov, Shah, Garbuzenko, & Minko, 2013).

Xu et al., studied the combined effect of doxorubicin and Bcl2 siRNA using polyethylenimine as a carrier for pulmonary delivery. Confocal laser scanning microscopy and flow cytometry exhibited a high cellular uptake of drug and siRNA in B16F10 cell lines. Real-time polymerase chain reaction (RT-PCR) observations had high gene silencing, where, 70% of Bcl2 mRNA were bashed down. The combination has increased cell apoptosis and cell proliferation inhibition in B1F10 cells. The in-vivo studies in mice showed high accumulation of doxorubicin and siRNA in metastatic lung cancer upon pulmonary delivery (C.-N. Xu et al., 2017).

Hybrid lipid-polymer nanoparticles comprising of dipalmitoylphosphatidylcholine and poly(lactic-co-glycolic) acid were developed to encapsulate siRNA. siGENOMESMART pool siRNA was used to check their fate on the human epithelial airway barrier, which acts against α and β subunits of the sodium trans epithelial channel. The developed nanoparticles exhibited ~150nm hydrodynamic diameter, with -25mV zeta potential. In-vitro aerosolization studies were performed on a triple cell co-culture model which mimic human epithelial airway barrier. There were no changes in nanoparticulate structure by transmission electron microscopic imaging after nebulization. Nanoparticles were internalized in epithelial cells and there were no cytotoxic effects or acute inflammation towards cell components. In-vitro inhibition of sodium trans-epithelial channel protein expression was evaluated in A549 cell lines, which confessed prolonged inhibition (Ivana d'Angelo et al., 2018).

Capel et al., reported delivery of siRNA using water-soluble piperazine substituted chitosan derivatives for efficient delivery by inhalation. The piperazine derivative chitosan is water-soluble at physiological pH, and forms nano-complexes with siRNA up to the size of 300nm at a relatively low polymer to siRNA ratio (5:1). Glyceraldehyde-3-phosphate dehydrogenase (GADPH) targeting siRNA was complexed with siRNA, in-vitro studies performed on lung epithelial cells revealed chitosan and siRNA complexes exhibited silencing of a gene from 40-80%. There was no effect of the aerosolization by PenCenturyTM
microsprayer device on particle size and integrity. *In-vivo* studies performed to determine the potential of piperazine chitosan complex in subcutaneous bioluminescent tumor A540-lucxenograft model. There was a significant reduction of the tumor with absence of adverse effects. The modified chitosan siRNA complexes were found to be safe and had the potential to deliver by inhalation therapy (Victoria Capel et al., 2018).

Ihara *et al.*, developed dry powdered chitosan siRNA complexes, and its gene silencing efficiency was quantified histologically after intratracheal administration into murine lungs. EGFP-siRNA chitosan complex efficacy was studied in EGFP transgenic mice and mice carrying metastatic lung cancer of Lewis lung carcinoma. Transgenic mice are divided into three groups, where, one group was treated with targeting siRNA, one group treated with non-targeting siRNA and one was left without any treatment. The fluorescence in bronchus, bronchioles, alveolar walls of the group treated with targeting siRNA is reduced to a large extent in comparison to mice group treated with non-targeting siRNA and group without treatment. Similarly, the same results were observed in the metastatic lung tumor consisting of mice groups. The results conclude a high extent of gene silencing in proximal airways compared to peripheral lung tissues. The study proves pulmonary delivery of siRNA is a predominant approach to target gene expression in respiratory disorders involving airways, parenchyma and lung cancers (Ihara *et al.*, 2015).

A dry powder siRNA based formulation was developed targeting vascular endothelial growth factors (VEGF), which inhibits lung tumor growth in mice. *In-vivo* studies were performed on mice with metastatic lung cancer, which were induced by B16F10 melanoma cells or Lewis lung carcinoma cells. VEGF siRNA efficiency in gene suppression was evaluated by treating B16F10 and Lewis lung carcinoma cell lines. There was reduced VEGF protein and mRNA pertaining to VEGF. In-vivo studies were performed in tumor-bearing mice, where, chitosan was used to deliver siRNA. Prior to delivery of siRNA, VEGF levels were measured in bronchoalveolar lavage fluid (BALF) collected from mice bearing a tumor. The results showed reduced VEGF concentrations in BALF after single intratracheal administration of dry siRNA powder. Repeated intratracheal administration reduced tumor growth in the lungs. An *in-vitro* inhalation performance study was performed with jethaler single, which exhibited a low-pressure drop. These results suggest chitosan dry powder of siRNA is a novel strategy for lung cancer-specific and high gene silencing effect (Miwata *et al.*, 2018).

Bohr and his co-workers developed phosphorus-based dendrimers for delivery of siRNA. Pyrrolidinium and morpholinium were selected as protonated amino groups for better compatibility. The dendriplexes form strong complexes with siRNA targeting tumor necrosis factor-α (TNF-α). The *in-vitro* studies
revealed high cellular uptake and *in-vitro* silencing efficiency of TNF-α in RAW264.7 lipopolysaccharide activated macrophage cell line with pyrroloidiniumdendriplexes. The improved efficiency of pyrroloidinium complexes is expected due to high pKa value which improves stronger siRNA complex formation. Nasal administration of complexed dendriplexes has higher efficacy towards lung injury in comparison to non-complexed siRNA (Table 1) (Bohr *et al*., 2017).

The RNAi mechanism and siRNA provides the potential for designing therapeutics for the treatment of disease like cancer (Leung & Whittaker, 2005). siRNA has intrinsic efficacy as it utilizes the endogenous RNAi pathway, reduces the expression of disease-linked genes and can be used for any gene with its complementary sequence (Vogelstein & Kinzler, 2004). Several genes, their mutations, and pathways have been found to be associated in different cancers, thus it is evident that siRNA can provide therapeutic effect in cancers. siRNA mediated silencing of cancer associated proteins causes a remarkable apoptotic effect (Pai *et al*., 2006). The major barriers for effective delivery of siRNA to the lungs are complex branching of lungs associated with biomechanical barriers including mucus over the airways and the airways cell membrane. Gene silencing will be achieved only when siRNA delivered is stable, is of good concentration, penetrates the cell and reaches the cytoplasm (Durcan, Murphy, & Cryan, 2008).

Zhang *et al*., reported siRNA counters K-RAS mutants and observed the anti-cancer effect by reducing K-RAS in lung cancer cell line. Additionally, adenovirus-mediated siRNA precisely targets RAS and acts as a drug for lung cancer treatment (Z. Zhang, Jiang, Yang, & Wang, 2006). Han *et al*., proposed the use of p65 siRNA for anti-tumor effect by blocking PI3-kinase and NFκB (Han & Roman, 2006).

Presently, in clinical trials, siRNA was locally administered to the target site to bypass the systemic delivery but the systemic route is required for the treatment of cancers and other diseases. For an *in vivo* delivery system, it ought to be biocompatible, non-immunogenic and biodegradable. The siRNA should be effectively delivered to the target site and must be protected from the action of serum nucleases. The system must evade from immediate hepatic or renal clearance and foster endosomal siRNA release into the cytoplasm for endogenous RISC interaction (Juliano, Alam, Dixit, & Kang, 2008). Development and validation of different strategies of siRNA delivery are underway. Zhang *et al*., studies the *in vitro* delivery of encapsulated human double-minute gene 2-specific siRNA in arginine octamer surface-modified liposomes. The complex was reported to be stable for 24 hours in blood and had potentially good transfection in different lung cancer cell lines (C. Zhang *et al*., 2006). Another study based on siRNA against human survivin was coated with cationic liposomes containing DOTAP and cholesterol (1:1 molar ratio) which resulted in LPD (liposome-polycation-DNA) nanoparticles. Further, LPD was PEGylated for ligand targeting and steric stabilization for selective delivery to the lungs. Further analysis suggested that PEGylated LPD has an anti-cancer effect via surviving downregulation (Li & Huang,
In the *in vivo* mouse model, LPD nanoparticle encapsulating siRNA for epidermal growth factor showed anti-tumor activity in combination with cisplatin on intravenous injection (Li, Chen, Hackett, & Huang, 2008). Biodegradable cationic poly (amino-ether) (mPAE) were accessed as a carrier for mTOR siRNA for lower toxic effect, the prohibition against nuclease degradation and inhibition of cancer cell proliferation (Gandhi *et al*., 2018). Chono *et al*., reported on the immunotoxicity and organ defects of siRNA-LPD nanoparticle administration intravenously (Chono, Li, Conwell, & Huang, 2008). Cationic immune-liposomes conjugated with anti-transferrin receptor single chain antibody fragment with the fluorescent label have been studied for systemic delivery for lung cancer metastasis. It was observed that the labeled siRNA was distributed in lung metastasis rather than liver (Pirollo *et al*., 2006). Cationic single-walled carbon nanotubes having siRNA for telomerase reverse transcriptase are being currently studied as *in vitro* lung cancer models. *In vitro* internalization of siRNA suppresses target gene expression. Furthermore, this model has also been reported for mice model for subcutaneous Lewis lung tumors (Zhuohan Zhang *et al*., 2006). Xu and his colleagues developed a pH-sensitive nanoparticles system for co-delivery of doxorubicin and survivin siRNA. Doxorubicin was conjugated with polyethylenimine by a pH-sensitive hydrazine bond using 3-maleimidopropionic acid hydrazide. The formed polyethylenimine-doxorubicin -3- maleimidopropionic acid hydrazides are cationic in nature, and form complexes with anionic survivin siRNA with electrostatic interactions. On pulmonary delivery of these complexes in B16F10 tumor-bearing mice, resulted in the high accumulation of doxorubicin and siRNA in lungs. There was limited accumulation in case of normal lung tissues which indicates targeted drug delivery. The nanoparticulate system has improved anti-tumor efficacy in comparison to individual delivery of doxorubicin or surviving siRNA (C. Xu, Tian, Wang, Wang, & Chen, 2016). A similar study was performed using Bcl2 siRNA with pH-sensitive polyethyleneimine hydrazine doxorubicin complex. The *in-vitro* and *in-vivo* studies showed pH sensitive complex nanoparticles improved anti-tumor efficacy by pulmonary administration. There were high deposition and prolonged retention time in the lungs by pulmonary administration (C. Xu *et al*., 2015).

Self-assembled cholesterol conjugated chitosan nanoparticles were used to deliver curcumin and siRNA concurrently to achieve a synergistic effect against the cancer cells. Curcumin and siRNA were internalized by clathrin-dependent endocytosis in a time-dependent manner. This method was successful for A549 human lung carcinoma cell line for the co-delivery of siRNA and hydrophobic drug (Omkara Swami Muddineti, Aashma Shah, Sri Vishnu Kiran Rompicharla, Balaram Ghosh, & Swati Biswas, 2018). Similarly, cationic polyethyleneimine-polyactic acid (PEI-PLA) was synthesized for systemic delivery of paclitaxel and siRNA for the knockdown of survivin gene for lung carcinoma. Upon nanoparticle uptake by the A549 cells, they turn electrically neutral due to lower endosomal pH. These
nanoparticles have pH-responsive property as pH 5.5 leads to drug release while pH 7.4 for cellular uptake. This study proved tumor growth inhibition along with surviving mRNA knockdown. Such co-delivery systems provide large surface area for high drug loading, time-dependent drug release which allows extended exposure to tumor treatment, passive targeting, lower cytotoxicity increased the proliferative effect and anti-cancer activity (Jin et al., 2018) as depicted in the Figure:3.

Inhalation therapy can be potentially used for siRNA delivery due to high gene silencing effect and sequence specificity. In vivo study on mice with Lewis lung carcinoma reveals the effect of intratracheal vascular endothelial growth factor (VEGF) siRNA dry powder delivery downregulates the VEGF levels in both tumor tissue as well as broncho-alveolar lavage and reduces the metastatic loci in lungs (Miwata et al., 2018). The aerosol composed of PEA and siRNA for Akt1 was administered in mice with urethane-induced inhaled lung cancer. The use of aerosol for 4 weeks reduces Akt1 levels and inhibits the tumor progression (C.-X. Xu et al., 2008).

Clinical trial studies for siRNA drug delivery in pulmonary diseases

A drug called Excellair™ was developed by ZaBeCor Pharmaceuticals for the treatment of asthma. The drug targets mRNA of spleen tyrosine kinase (Syk) which is responsible for activation of several pro-inflammatory transcription factors. In the Phase I of the study, patients received siRNA Excellair™ via inhalation for 21 days (Watts & Corey, 2010). The drug did not cause any side effects to the asthma patients and almost 75% of the patients reported improved breathing and reduction in the use of inhalers while placebo patients showed no improvement. Further, in 2009, Excellair™ entered Phase II of clinical trials but in 2015 it was discontinued as Syk can act as both suppressor and promoter of cell growth(Krisenko & Geahlen, 2015).

Alnylan Pharmaceuticals developed ALN-RSV01, a siRNA therapeutic to target mRNA of viral protein in respiratory syncytial virus (RVS) (DeVincenzo et al., 2010). ALN-RSV01 targets nucleocapsid (N) protein of RSV which is essential for viral replication. In the Phase I of the clinical trial, 100 healthy males of 18 to 45 years age group were accounting to 65 having single and multiple dose of ALN-RSV01 while 36 were placebo. All the volunteers were given the therapeutic doses via nasal spray. No severe adverse effects were observed in different treated groups which lead ALN-RSV01 to enter Phase II (DeVincenzo et al., 2008). The Phase II study comprised of 85 healthy males of 18-45 years of age. All subjects received RSV01 inoculation at day 0, and the ALN-RSV01 treated cohorts received the siRNA intranasal spray at days −1, 0,
+1, +2, and +3. A statistically significant reduction in detected RSV by quantitative culture and real-time PCR was reported for patients receiving 150mg of study drug, the highest dosage tested. Averaged for all treated patients vs. placebo, an acquisition over time effect was observed by either PCR or quantitative culture. Indeed, the strongest effects of treatment with ALN-RSV01 were observed by its prophylactic efficacy. The drug provided an antiviral effect over an 11-day time course, which resulted in reduced infection over time noticeable within 3–4 days after inoculation (DeVincenzo et al., 2008). The safety trial for antiviral activity was conducted for lung transplant patients with RSV. In Phase II b it was showed the safety of ALN-RSV01 treated RSV infections across the broader groups for lung transplant (Alvarez et al., 2009).

Atu-027 is composed of siRNA with lipoplex delivery system represents RNAi mediated suppression of protein kinase N3 (PKN3) in vascular endothelial cells and prevents lung metastasis. Phase Ib trials for the safety of Atu-027 with gemcitabine have been completed (Schultheis et al., 2014).

**Future prospects of drug delivery with siRNA in pulmonary diseases**

RNA interference plays a key role in the treatment of several disorders. siRNA induces gene silencing by acting on sequence-specific cleavage of complementary mRNA (messenger RNA) and thereby inhibiting protein synthesis. siRNA based therapy was found to be a better strategy over the existing therapeutics, such as drug molecules, monoclonal antibodies and proteins (Fujita, Takeshita, Kuwano, & Ochiya, 2013). Apart from its advantages, administration and delivery of siRNA is the major challenge. siRNA undergoes degradation in the presence of serum nucleases when they are administered directly into the blood. Several strategies are utilized for delivery of siRNA to the target organ (T. Nguyen, Menocal, Harborth, & Fruehauf, 2008). siRNA demonstrated as potential therapeutic agents to treat pulmonary disorders including lung cancer, infectious diseases, airway inflammatory diseases, and cystic fibrosis. Delivery of siRNA directly by pulmonary route has added advantages such as reduced dose, reduced systemic side effects and reduced degradation due to a lower concentration of nuclease enzymes in airways. Pulmonary delivery of siRNA can also be helpful in systemic action, due to the large surface area, thin epithelium and high vascularization in alveoli which favors rapid absorption of siRNA(Fujita et al., 2013). Inhalation is the most preferred and easy mode of non-invasive administration which can be applied for siRNA by liquid aerosol or dry aerosol formulations. There is a need for a high attention regarding stability and biological activity of siRNA at the time of formulation development and delivery. However, the pulmonary delivery of these siRNA is challenging due to mucociliary clearance by ciliated
epithelium cells, mucus, alveolar fluid and macrophages along the airways. Particles inhaled get deposited on ciliated cells and eventually get cleared by cough and swallowed. The mucus secreted, form a thin film thereby restricting diffusion and penetration of siRNA into the cell membrane. The alveolar fluids form a thin film as a pulmonary surfactant (phospholipids and surfactant proteins) obstruct permeation efficiency of lipid-based formulations to some extent and there was no effect in case of polymer-based systems. Macrophages engulf the inhaled particles as part of the defense mechanism. There will be altered conditions like increased mucus secretion, viscosity and ciliary clearance in diseased conditions. siRNA with a negative charge and high molecular weight (13kDa), has also contributed to the poor ability to cross cell membrane even it reaches the target surface area. Viral vectors and non-viral vectors are used for the delivery of siRNA. Viral vectors are found to be more efficient to transfer genetic material into host cells. Despite of its advantages, activation of immune responses after repeated administration may lead to organ failure and chances of serious concerns. Non-viral vectors which include lipids, polymers, inorganic materials and transfection agents (i.e., Lipofectamine, Oligofectamine, TransIT-TKO and DharmaFECT) are more vastly utilized for siRNA delivery. The ideal characteristic feature for a siRNA delivery system should include a) Protection from enzymatic degradation, b) Ability to penetrate cell membrane (facilitate cell uptake) c) It should able to protect from endosomal degradation and induce gene silencing d) Should not affect siRNA activity and specificity e) Non-toxic (Feldmann & Merkel, 2015; J. K.-W. Lam, Liang, & Chan, 2012; Yingshan Qiu, Lam, Leung, & Liang, 2016; Youngren-Ortiz, Gandhi, España-Serrano, & Chougule, 2016, 2017). Several strategies are utilized to overcome stated challenges in the delivery of siRNA some of them are discussed below.

Conclusion

The potential siRNA based therapeutics for lung diseases have to be explored further. The nanoparticle-based inhalable and aerosols have been studied extensively for improved delivery and clinical efficiency. Therefore, ex vivo models for inhaled particulate distribution and in vivo models for pharmacokinetics/pharmacodynamics are being taken under consideration to understand their distribution, safety, and efficiency of siRNA in the pulmonary system. Different organic and inorganic nanoparticle with varied size, charge, and chemistry have been used as carriers of siRNA or siRNA drug conjugate delivery. Also, upon internalization at the target site siRNA should be able to escape the endosomal mechanism. Furthermore, a nanoparticle with small size have been preferred for longevity at the target site, non-specific interactions and to prevent the off-site toxic effect.

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References


**Table 1: siRNA delivery systems for targeted pulmonary therapy**

<table>
<thead>
<tr>
<th>Delivery system</th>
<th>siRNA delivered</th>
<th>Target</th>
<th>Route of administration</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylenimine-cis-aconitic anhydride-doxorubicin and (B cell) Bcl2 siRNA complex nanoparticles</td>
<td>Bcl-2 siRNA</td>
<td>Lung cancer</td>
<td>Intratracheal</td>
<td>The polyethyleneimine-cis-aconitic anhydride-doxorubicin/Bcl2 siRNA complex nanoparticles are for treating metastatic lung cancer by pulmonary delivery with low side effects on the normal tissues.</td>
<td>(C. Xu et al., 2015)</td>
</tr>
<tr>
<td>Arginine-glycine-aspartic acid peptide (RGD) gold nanoparticles</td>
<td>c-mycsiRNA</td>
<td>Lung cancer</td>
<td>Intratracheal</td>
<td>c-myc-RGD gold nanoparticles are capable of targeting tumor cells, significant tumor growth inhibition, as well as extended survival of mice bearing tumors.</td>
<td>(Conde et al., 2013)</td>
</tr>
<tr>
<td>Carrier/SiRNA</td>
<td>Additional Component</td>
<td>Disease</td>
<td>Route</td>
<td>Treatment Effect</td>
<td>Reference</td>
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<tr>
<td>Glycerol propoxylate triacrylate and spermine</td>
<td>Sodium-dependent phosphate co-transporter 2b(NPT2b) siRNA</td>
<td>Lung cancer</td>
<td>Intratracheal</td>
<td>siNPT2b successfully suppressed lung cancer growth and decreased cancer cell proliferation and angiogenesis, facilitating apoptosis.</td>
<td>(Hong et al., 2014)</td>
</tr>
<tr>
<td>Chitosan dry powder</td>
<td>Luciferase siRNA</td>
<td>Lung cancer</td>
<td>Intratracheal</td>
<td>siRNA/chitosan powder prepared using super critical carbon dioxide has an effective and specific gene silencing against the tumor cells metastasized in the lungs of mice.</td>
<td>(Okuda et al., 2013)</td>
</tr>
<tr>
<td>Naked siRNA or TransIT-TKO</td>
<td>PhosphoprteinsiRNA</td>
<td>Parainfluenza virus (PIV), Respiratory syncytial virus(RSV)</td>
<td>Intranasal</td>
<td>Animals were successfully protected from RSV and PIV infections specifically.</td>
<td>(Bitko, Musiyenko, Shulyayeva, &amp; Barik, 2005)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Nucleocapsid gene-specificsiRNA</td>
<td>Viral infections</td>
<td>Intranasal</td>
<td>Significant reductions of viral load were achieved in both prophylactic and therapeutic regimens.</td>
<td>(Alvarez et al., 2009)</td>
</tr>
<tr>
<td>Oligofectamine</td>
<td>Nucleocapsid protein and Polymerase acidic protein</td>
<td>Influenza type A</td>
<td>Intranasal and hydrodynamic injection</td>
<td>Treated animals lung virus titres were reduced and protected from lethal challenge with highly pathogenic viruses.</td>
<td>(Tompkins, Lo, Tumpey, &amp; Epstein, 2004)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Lymphotacticin (XCL1) siRNA</td>
<td>Tuberculosis</td>
<td>Intratracheal</td>
<td>XCL1 expression in the lungs was significantly suppressed; decreased T lymphocytes, IFN- response and disorganized granulomatous lesions and high fibrosis.</td>
<td>(Rosas-Taraco, Higgins, Sánchez-Campillo, et al., 2009)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Transforming growth factor-β1 siRNA</td>
<td>Tuberculosis</td>
<td>Intratracheal</td>
<td>Increased expression of antimicrobial mediators, with the reduced bacterial load in the lungs of treated mice.</td>
<td>(Rosas-Taraco et al., 2011)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Suppressor</td>
<td>Asthma</td>
<td>Intranasal</td>
<td>Decrease in lung.</td>
<td>(Staff, 2014)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>s of cytokine signaling protein 3 (SOCS) siRNA</td>
<td>Asthma</td>
<td>Intranasal</td>
<td>Eosinophilia, normalization of hyperresponsiveness, increase in mucus secretion and reduction in collagen deposition in the lungs.</td>
<td>(Khaitov et al., 2014)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Interleukin-4 siRNA and Phosphoprotein siRNA</td>
<td></td>
<td></td>
<td>Eosinophilia in bronchoalveolar lavage fluid, hyperresponsiveness and airway inflammation were significantly reduced</td>
<td></td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Signal transducer and activator of transcription factor 6 (STAT6) siRNA</td>
<td>Asthma</td>
<td>Intratracheal and intranasal</td>
<td>Allergen-induced lung inflammation was significantly reduced and Expression of key cytokines (IL-4, IL-13) and allergen-induced inflammation in lung tissues were significantly reduced</td>
<td>(Darcan-Nicolaisen et al., 2009)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Receptor-interacting protein 2 (Rip2) siRNA</td>
<td>Asthma</td>
<td>Intratracheal</td>
<td>Ovalbumin-induced cytokine release, inflammatory cell infiltration and mucus hypersecretion was inhibited, elevation of serum Ovalbumin-specific IgE level was markedly suppressed</td>
<td>(Goh et al., 2013)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>cluster of differentiation 86 (CD86) siRNA</td>
<td>Asthma</td>
<td>Intratracheal</td>
<td>Ovalbumin-induced airway eosinophilia, airway hyperresponsiveness, and cytokine production was reduced</td>
<td>(Asai-Tajiri et al., 2014)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Spleen tyrosine kinase (syk) siRNA</td>
<td>Asthma</td>
<td>Intranasal</td>
<td>siRNA administration by intranasal route inhibited inflammatory cells in the bronchoalveolar lavage fluid (BALF) of allergen sensitized mice.</td>
<td>(Z.-Y. Huang, Kim, Kim-Han, Indik, &amp; Schreiber, 2013)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>c-kit siRNA</td>
<td>Asthma</td>
<td>Intranasal</td>
<td>Airway mucus secretion and eosinophil infiltration in BALF was effectively reduced. C-kit further</td>
<td>(Wu et al., 2014)</td>
</tr>
<tr>
<td>Transferrin polyethylenimine (Tf-PEI)</td>
<td>Fluorescently labeled siRNA</td>
<td>Asthma</td>
<td>Intratracheal</td>
<td>Reduced the production of stem cell factor, IL-4, and IL-5, but had no effect on interferon-γ (IFN-γ) generation</td>
<td>(Xie et al., 2016)</td>
</tr>
<tr>
<td>R3V6 peptides were used as a carrier. (Ternary complex of siS1PLyase, HMGB1A, and R3V6 was produced by charge interaction)</td>
<td>siS1PLyase/HMGB1A/R3V6 ternary complex</td>
<td>Acute lung injury</td>
<td>Intratracheal</td>
<td>siS1PLyase/HMGB1A/R3V6 complex reduced the levels-6 and TNF-α more efficiently compared to HMGB1A alone and siS1PLyase/R3V6 complex in lipopolysaccharides activated macrophages and reduced the inflammatory response and apoptosis in acute lung injury</td>
<td>(B. Oh &amp; Lee, 2014)</td>
</tr>
<tr>
<td>Naked siRNA, i.v., liposomes</td>
<td>Tumor necrosis factor-α</td>
<td>Acute lung injury</td>
<td>Intratracheal</td>
<td>Systemic injection but not intratracheal delivery of TNF-α siRNA significantly reduced the incidence of acute lung injury. Results suggest pulmonary endothelial and/or other possible vascular resident cells, not epithelial cells, play a greater role in mediating the TNF-α priming response in hemorrhage/sepsis-induced acute lung injury.</td>
<td>(Lomas-Neira, Perl, Venet, Chung, &amp; Ayala, 2012)</td>
</tr>
<tr>
<td>Naked siRNA</td>
<td>Transforming growth factor-β1 (TGF-β1) siRNA</td>
<td>Pulmonary fibrosis</td>
<td>Intratracheal</td>
<td>Levels of inflammatory cytokines, including IFN-α and IFN-β, were not significantly affected, whereas TGF-β1 was significantly inhibited.</td>
<td>(Corina N D’Alessandro-Gabazza et al., 2012)</td>
</tr>
<tr>
<td>PEGylated poly(dimethylamino)ethylmethacrylate (PDMAEMA)</td>
<td>Connective tissue growth factor (CTGF) siRNA</td>
<td>Pulmonary fibrosis</td>
<td>Intratracheal</td>
<td>There was a reduction in collagen deposition, inflammatory cytokines production and drastic attenuation of pulmonary fibrosis</td>
<td>(Sung et al., 2013)</td>
</tr>
<tr>
<td>Self-assembled micelle interfering RNA (siRNA) nanoparticles</td>
<td>Amphiregulin (AR) and connective tissue growth factor (CTGF) targeting siRNA</td>
<td>Pulmonary fibrosis</td>
<td>Intratracheal/intra venous</td>
<td>Collagen accumulation was significantly reduced and lung function was substantially restored in TGF-β transgenic mice.</td>
<td>(Yoon et al., 2016)</td>
</tr>
<tr>
<td>Chitosan-based siRNA nanoparticle</td>
<td>siRNA specific to the BCR/ABL-1 junction sequence</td>
<td>Potential of chitosan nanocarriers</td>
<td>Nasal</td>
<td>In bronchiole epithelial cells of transgenic EGFP (endogenous enhanced green fluorescent protein) mice.</td>
<td>(Howard et al., 2006)</td>
</tr>
<tr>
<td>Spray dried naked siRNA using L-leucine as dispersion enhancer</td>
<td>siRNA targeting interleukin 10</td>
<td>2% W/W siRNA developed into an inhalable dry powder</td>
<td>Not applicable</td>
<td>The integrity of siRNA was successfully retained after spray drying. Spray dried powder were crystal in nature with low moisture levels traits stable formulation.</td>
<td>(Chow et al., 2017)</td>
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</table>