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Review Article

Nucleic Acid-Based Therapeutics for Pulmonary Diseases

Jing Chen,[1] Yue Tang,[1,2] Yun Liu,[1] and Yushun Dou[1]

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Abstract. Nucleic acid-based therapeutics present huge potential in the treatment of pulmonary diseases ranging from lung cancer to asthma and chronic pulmonary diseases, which are often fatal and widely prevalent. The susceptibility of nucleic acids to degradation and the complex structure of lungs retard the effective pulmonary delivery of nucleic acid drug. To overcome these barriers, different strategies have been exploited to increase the delivery efficiency using chemically synthesized nucleic acids, vector encapsulation, proper formulation, and administration route. However, several limitations regarding off-target effects and immune stimulation of nucleic acid drugs hamper their translation into the clinical practice. Therefore, their successful clinical application will ultimately rely on well-developed carriers and methods to ensure safety and efficacy. In this review, we provide a comprehensive overview of the nucleic acid application for pulmonary diseases, covering action mechanism of the nucleic acid drugs, the novel delivery systems, and the current formulation for the administration to lungs. The latest advances of nucleic acid drugs under clinical evaluation to treat pulmonary disorders will also be detailed.

KEY WORDS: nucleic acid; antisense oligonucleotide (ASO); short interfering RNA (siRNA); microRNA (miRNA); pulmonary diseases.

INTRODUCTION

Due to their location and physiological function, the lungs are directly accessible to pollutants and viruses from the outside, rendering them susceptible to diseases ranging from lung cancer to chronic pulmonary diseases. Among these pulmonary diseases, chronic obstructive pulmonary disease claimed 3.0 million lives in 2016, while lung cancer caused 1.7 million deaths (1). Since current treatments of these diseases have limited efficacy, many studies are being conducted to find novel effective treatments. Though most lung diseases are considered to be the product of a variety of endogenous and exogenous influences, and less obviously are associated with gene replacement therapy. Abnormal conditions are likely to arise from an imbalance between destructive and protective mechanisms. Nucleic acids can be a new class of therapeutics to reconstitute a homeostatic balance by over-expression of protective genes or the suppression of damaging genes, which offers new strategies for the treatment of respiratory diseases (2). The mesh-like network of blood vessels in the lungs, coupled with easy access through the pulmonary airways, enables the lungs to be targeted by both intravenous and topical routes. The latter fact makes the lung unique compared with other organs, allowing specific lung sites such as alveolar cells and bronchial epithelium to be exclusively targeted for different therapeutic applications (3). In this review, we focus on nucleic acid-based therapies for pulmonary diseases. We discuss the hurdles nucleic acids face for translation into clinics and recent progress in the product into clinical trials.

ACTION MECHANISMS OF NUCLEIC ACID-BASED THERAPEUTICS

Antisense oligonucleotides (ASOs) are single-strand DNAs or RNAs that selectively bind to complementary mRNAs to modulate their functions. Their hybridization could result in downregulation or upregulation of gene expression by diverse mechanisms. RNase H1-dependent ASOs could bind to target RNA to form hybrid through Watson-Crick base pairing and downregulate translation through RNase H-induced degradation of the mRNA. Splice switching oligonucleotides could control the way exons skipping, modulate pre-mRNA splicing, and generate novel proteins. ASOs can also interfere with other aspects of RNA functions, such as blocking association of specific transcription factors with mRNA, antagonizing microRNA activities, and inhibiting RNA-mediated telomerase activity (4–6). Antisense oligonucleotides are the first kind of nucleic acid drugs widely used in clinical trials. Among the FDA-approved nucleic acids, ASO-based drugs account for the majority as for now (Table I). As of August 2018, only one aptamer drug and siRNA drug have been approved by the

[1] Department of Pharmacy, China Pharmaceutical University, No.639, Longmian Avenue, Nanjing, 211198, People's Republic of China.
[2] To whom correspondence should be addressed. (e–mail: tangyue@cpu.edu.cn)

Table I. List of FDA-Approved Nucleic Acid Products

Drug name	Active ingredient	Year	Therapeutic agent	Indication	Route	Vehicle	Company
Vitravene	Fomivirsen	1998	Antisense oligonucleotide	Cytomegalovirus retinitis	Intravitreal injection	None	Isis Pharmaceuticals
Macugen	Pegatanib	2004	Aptamer	Age-related macular degeneration	Intravitreal injection	None	Eyetech Inc.
Kynamro	Mipomersen	2013	Antisense oligonucleotide	Homozygous familial hypercholesterolemia	Subcutaneous injection	None	Genzyme Corporation
Exondys 51	Eteplirsen	2016	Antisense oligonucleotide	Duchenne muscular dystrophy	Intravenous infusion	None	Sarepta Therapeutics
Spinraza	Nusinersen	2016	Antisense oligonucleotide	Spinal muscular atrophy	Intrathecal injection	None	Biogen Inc.
Defitelio	Defibrotide	2016	Oligonucleotide with profibrinolytic properties	Sinusoidal obstruction syndrome	Intravenous infusion	None	Jazz Pharmaceuticals, Inc.
Onpattro	Patisiran	2018	Small interference RNA	Polyneuropathy of hereditary transthyretin-mediated amyloidosis	Intravenous infusion	Lipid complex	Alnylam Pharma Inc.

FDA. The first clinically approved nucleic acid drug was ASO drug, Vitravene (fomivirsen), indicated for cytomegalovirus retinitis in 1998. Followed by Kynamro (mipomersen) targeting mRNA encoding apolipoprotein B for the treatment of familial hypercholesterolemia, Exondys 51 (eteplirsen) designed to skip exon 51 of the dystrophin protein for the treatment of Duchenne muscular dystrophy, Spinraza (nusinersen) inducing the inclusion of exon 7 in the SMN1 and SMN2 mRNA to treat spinal muscular atrophy and recently Luxturna (voretigeneneparvovec-rzyl) for biallelic RPE65 mutation-associated retinal dystrophy (7,8).

Small interference RNAs(siRNAs) are double-strand RNA molecules of 21 to 23 base pairs in length designed to silence target genes in a sequence-specific manner. After introduction into the cytoplasm, siRNAs interact with multifunctional protein Argonaute-2 and form the RNA-induced silencing complex (RISC), where one of the strands is degraded and the other strand (mostly antisense) is left as a guide to recognizing target mRNA sequences. Subsequently, mRNAs which are perfect or nearly perfectly complementary to the siRNA antisense strand are cleaved by the activated RISCs (9). The specific gene silencing effect of siRNAs makes them indispensable tools for target identification and validation in drug discovery and development (10). In 2018, Onpattro (patisiran) infusion became the first FDA-approved siRNA drug. It is for the treatment of peripheral nerve disease caused by hereditary transthyretin-mediated amyloidosis in adult patients. Onpattro is designed to interfere with RNA production of an abnormal form of the protein transthyretin. By preventing the production of transthyretin, the drug can help reduce the accumulation of amyloid deposits in peripheral nerves, improving symptoms, and helping patients better manage the condition.

Micro RNAs(miRNAs) are 18–24 nucleotides long, single-stranded, endogenous noncoding RNA molecules that act as key regulators for a variety of cellular pathways. They can regulate gene expression by complementary binding to the core sequence in the 3′-untranslated region(3′-UTR) of target mRNAs (11). Either siRNA or miRNA could associate into the RISC. Unlike siRNA, miRNA can recognize mRNA with partially complementary sequences, which means one miRNA may have multiple different mRNA targets (10). Hence, delivery of exogenous microRNAs or microRNA mimics could be particularly useful in diseases having multiple disease-relevant targets (7). miRNAs mediate multiple biological processes, and alterations in miRNA function have been associated with different diseases like cancer, metabolic disorders, and viral pathogenesis (12). miRNAs related to cancer are generally classified as tumor suppressor miRNAs or tumor-promoting miRNAs. Tumor suppressor miRNAs (*e.g.*, let-7, miR-34 families, and miR-15/16) are responsible for suppressing oncogenes and are mostly downregulated in cancer. Restoration of their normal function can be achieved by miRNA replacement *via* administration of synthetic miRNA mimics functioning similarly to the endogenous counterparts. Tumor-promoting miRNAs (*e.g.*, miR-21, miR-17-92 cluster, and miR-155) are known to downregulate tumor suppressor genes and have been reported to be overexpressed in cancer (13). ASOs and miRNA sponges targeting tumor-promoting miRNAs can be used to block aberrantly overexpressed miRNAs (14).

Aptamers are short oligonucleotides with unique three-dimension structures that enable them to specifically recognize and bind to targeted proteins. Aptamers of interest could be selected from a pool of randomized molecules by methods known as systematic evolution of ligands through exponential enrichment. Therapeutic aptamers could act as inhibitors of protein function, or as targeting moieties for drug delivery (15,16). The use of RNA-aptamers conjugates for targeted delivery of oligonucleotide molecules has been widely explored and well reviewed elsewhere (17,18). Pegaptanib, the only aptamer that has been approved by the FDA, is acting through the former way. Vascular endothelial growth factor (VEGF) induces angiogenesis, and increases vascular permeability and inflammation, playing a central role in the progression of age-related macular degeneration. Pegaptanib

could selectively bind to VEGF isoform, VEGF165, thereby preventing VEGF165 from activating its receptors and suppressing pathological neovascularization (19). The therapeutic and targeting properties of aptamers could be combined to construct multifunctional molecules. Using an aptamer that binds to and antagonizes the receptor tyrosine kinase Axl, an aptamer-miRNA conjugates was developed with synergistic therapeutic effects, owing to oncosuppressive effects of the miRNA and inhibitory function of the aptamer (20,21).

DELIVERY OF NUCLEIC ACID DRUGS FOR PULMONARY DISEASES

Barriers to Nucleic Acid-Based Therapies for Pulmonary Diseases

Physiological Barriers to Overcome

The treatments for pulmonary diseases are mainly by parenteral injection and pulmonary administration through intranasal instillation, aerosol, or inhalation. Hence, the first barriers that nucleic acid drugs *via* these two routes encounter are blood and respiratory tract (Fig. 1). Parenteral administration of unmodified nucleic acids has been set back by their very short half-life in the bloodstream, serum nuclease degradation, quick renal clearance, and poor biodistribution. The parenteral route also exposes the whole human body to nucleic acids, which may hamper the delivery efficiency to target tissues or organs (22). To avoid enzymatic degradation and renal clearance, local drug administration routes have been proposed to directly deliver the drugs to the site of interest. Pulmonary administration reveals a strong potentiality as it could transport therapeutic agents to diseased lung tissue in a non-invasive manner. While the degradation by nucleases is negligible comparing to systemic administration, delivery through the airway could be hampered by physiological barriers. The mucociliary clearance action, the surface liquid that covers the airway and macrophages along different parts of the airways, limits the transport of nucleic acids to the site of action (23). The highly viscous mucus layer in the airways traps and prevents nucleic acids reaching the underlying epithelium and propelled them out with the impact of cillated cells (24). Thus, the development of particles that could efficiently penetrate the mucus barrier, without compromising its protective properties, is a clear challenge for improving pulmonary drug delivery (25).

Intracellular Barriers to Overcome

Even if the nucleic acids successfully penetrate through and escape from all the extracellular barriers mentioned previously, they still face the challenge to cross the cell membrane and reach the site of action in the cytoplasm or nucleus. Negative charge and large molecular weight make it hard for naked nucleic acids to enter the cell. The endocytosis of nucleic acids could be improved with the help of cationic biomaterials or targeting moieties which interact with the negative proteins or receptors on the cellular surface (26). One of the most challenging intracellular barriers for nucleic acids delivery is their tendency to remain entrapped in endosomes. Intracellular nucleic acids are transported in early endosome vesicles where various nucleases exist and the pH further reduce to 4.5 in the process to late endosomes and lysosomes, and most nucleic acids degraded in the endosome before reaching the site of action (27). The classic approach has been to use small-molecule endosomolytic agents like chloroquine to disrupt endosomes and release entrapped oligonucleotides from endosomes. Two similar types of small molecules have been reported recently with

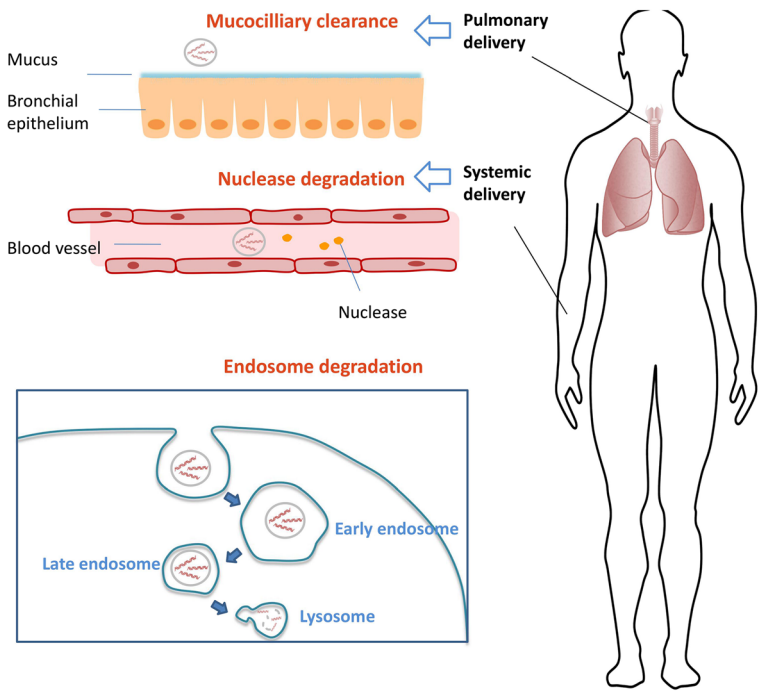

Fig. 1. Barriers to successful pulmonary delivery of nucleic acids

the help of a high-throughput screen of chemical libraries. These molecules substantially enhanced the pharmacological activities of oligonucleotides both in cell culture and murine model (28,29). Although these endosomolytic agents significantly enhanced the delivery efficiency, they currently display a narrow therapeutic window for clinical use.

To overcome these biological barriers, strategies like chemical modification, conjugation, vector encapsulation, and selection of administration route have been utilized to improve the delivery of nucleic acids to lungs.

Chemical Modification and Conjugation

Since naked nucleic acid is prone to degradation in the biological fluid, chemical modifications at the sugar, backbone, or the individual bases have been introduced to improve its stability and efficacy in biological systems. Phosphorothioate(PS)-modified backbone is the most widely used chemistry modification to increase the nuclease resistance. Based on PS backbones, nucleic acids designed with additional 2′-sugar modifications such as 2′-O-methyl (2′-OME) or 2′-O-methoxyethyl (2′-MOE) can not only further enhance stability and target affinity, but also largely block the activation of toll-like receptors and reduce immune responses (30). Besides PS modification, peptide nucleic acids and phosphoramide morpholino oligomers are nucleotide analogs with strong nuclease resistance as the phosphodiester linkage is completely substituted by a polyamide backbone or a phosphorodiamidate group (31). However, 2′-sugar modifications of ASOs might block the recruitment of RNaseH. Therefore, “gapmers” was developed, that is ASOs containing a sequence of PS-modified backbone residues(“gap”) to facilitate RNase H activity and sugar-modified residues(“flanks”) on either side of the gap to increase resistance to degradation and enhance binding to target mRNA (6).

Beside chemical modification, conjugation strategies are often exploited to enhanced stability and delivery efficiency. Representative biomolecules conjugated to nucleic acids conclude targeting ligands and membrane-active molecules, such as lipids, aptamers, peptides, carbohydrates, and polymers (32). Cholesterol attachment to nucleic acids facilitates cellular import and improves intracellular uptake *via* lipoproteins-mediated pathways (33). Intravenous and intraperitoneal injection of anti-MDR1 cholesterol-siRNA conjugate in healthy and tumor-bearing severe combined immune deficiency mice demonstrated efficient accumulation deep in the tissue and the cytoplasm of almost all the liver and tumor cells (34). siRNAs conjugated to *N*-acetylgalactosamine molecule, a high-affinity ligand for the hepatocyte-specific asialoglycoprotein, are undergoing clinical trials and provided promising results (32). Antibodies or aptamers could be conjugated directly to nucleic acids to realize targeted delivery to specific tissues or cell types. Because of the advantages like good reproducibility and low system toxicity, chemical modification and conjugation of nucleic acids have been paid great attention and all the four FDA-approved ASOs are chemically modified and used without a delivery vehicle. While compared to vector-based systems, poor delivery efficiency and limited orientation are still great concerns of nucleic acid-conjugates for their clinical translation.

Vectors

Besides chemical modification, vectors offer important opportunities for nucleic acids to overcome delivery challenges. Ideal nucleic acid delivery vectors are expected to condense and protect nucleic acids, facilitate their transport to target cells, and subcellular compartments. Viruses, as naturally evolved transfection agents, could enter the cells *via* endocytosis and release viral genome that could replicate and transcribe into proteins for producing multiple copies. Due to their higher transfection efficiency, three major classes of viral vectors, namely, adenovirus (35), adeno-associated virus (36), and lentivirus (37) have been extensively used in nucleic acid therapy. However, the limitation of payload, inherent immunogenicity, and the difficulty of large-scale production limited their clinical application.

The advantage of non-viral vectors lies in low immunogenicity and toxicity, ease of production, and the large payload over their viral counterparts. Widely investigated non-viral delivery vectors include polymers, lipids, polypeptides, and inorganic nanomaterials (such as calcium phosphate and quantum dots). Most of the vectors for nucleic acids possess cationic charges that assist in loading nucleic acids through charge interactions. Common non-viral delivery systems used in pulmonary diseases are listed in Table II. Based on various non-viral vectors, hybrid systems made up by condensed nucleic acid/polycation complexes as the core and lipid bilayer membrane as the shell have been developed. The use of endogenous phospholipids, such as dipalmitoylphosphatidylcholine, can be considered a valid approach to increase the compatibility of nanoparticles with the lung environment (46). Researchers combined a natural-derived pulmonary surfactant shell with a siRNA-loaded dextran nanogel to achieve effective siRNA delivery to murine alveolar macrophages, which are difficult to transfect, resulting in a substantial gene knockdown with a relatively low dose (47–49). Diverse surface modifications and conjugation of targeting agents attached to the vectors could render them desirable properties and enhance the therapeutic efficiency of nucleic acid therapy. Surface modification with high molecular weight hyaluronic acid which can mediate active CD44 targeting in tumors and increase circulation time of cationic siRNA lipoplexes improved the delivery efficiency and achieved supported reduction of the expression of luciferase mRNA in tumor due to the siRNA inhibition (52).

Administration Route and Formulation

Systemic administration of nucleic acids faces serious challenges, including rapid excretion, low bioavailability, and systemic toxicity. While local administration allows lower delivery doses and reduced side effects, making it an attractive route (53). Most of the FDA-approved nucleic acid-based drugs are locally delivered: Fomivirsen is delivered to the eyes by intraocular injection, Spinrazais by intrathecal injection, and Luxturnais by subretinal injection (7,8). For pulmonary disease, the target organ could be reached through systemic administration or pulmonary administration. The latter route could potentially enhance retention time of nucleic acids in the desired site of action, reduce systemic toxic effects, and provide a therapeutic

Table II. Non-viral Vectors Used in Nucleic Acid Delivery for Pulmonary Diseases

Class	Properties	Materials	References
Lipid-based system	Biocompatible Efficient delivery	Liposome/Lipoplex	(38,39)
Polymer-based system	Easy and cheap production	PEI	(3,40)
	Simple loading and complex formation with nucleic acids	Dendrimer	(41–43)
	Low toxicity Biocompatible Biodegradable	Chitosan	(44,45)
Hybrid system	Combine the unique strengths of both polymers and lipids	Lipid/PLGA	(46)
	Enhanced tolerance in the pulmonary tract	Pulmonary surfactant/Dextran	(47–49)
Inorganic material	Tunable size	Quantum dots	(50)
	Potential for diagnostic imaging Ease of chemical conjugation	Calcium phosphate	(51)

solution to a range of pulmonary disorders (54). Inhalation and intranasal route represent the most common way to deliver nucleic acid into the airways due to the ease of administration and non-invasive characteristic, and are the main administration routes in clinical trials. Biodistribution studies of aerosol inhalation of polyester-siRNA nanoparticles to mice bearing orthotopic lung tumors showed specific accumulation in the lungs (55).

Nucleic acids can be formulated into liquid aerosol generated by an inhaler or nebulizer, or dry powder aerosol for pulmonary delivery. Liquid aerosol formulations were almost exclusively adopted in clinical trials involving pulmonary delivery of nucleic acids. Among the three major types of inhalation devices consisting of pressurized metered dose inhalers (pMDIs), nebulizers, and dry powder inhalers (DPIs), pMDIs and DPIs are the most portable and commonly-used devices (56). pMDIs, in which the therapeutic agents are suspended in the hydrofluoroalkane (HFA) propellant, have been regarded as golden standard delivery system for asthma and chronic obstructive pulmonary disease therapies (56). A pMDI formulation containing mannitol microparticles which encapsulated siRNA polyplex nanoparticles showed good aerodynamic properties for deep lung deposition and significant gene knockdown efficiency in lung A549 cells (57). DPIs are usually thought as a better option to deliver therapeutic nucleic acids than pMDIs because their dry particle form enhances the stability of nucleic acids and decreases the risk of microbial contamination (58). Chow et al. first formulated naked siRNA into inhalable dry powders (at 2% *w*/*w*) using spray drying technology with the incorporation of mannitol and L-leucine; the latter acted as powder dispersibility enhancer, and the integrity of siRNA was well retained (59).

Although systemic administration does not provide the aforementioned advantages of local delivery, for some indications like lung metastasis and pulmonary hypertension, the desired target sites might locate on the interstitium and lung alveolar and endothelial cells rather than the airway epithelium. Lung metastases are expected to have an endothelial origin and therefore may be better accessible through blood vessel than through airways (60). Although intravenous injection is not direct delivery to the lung, this route is still able to achieve high levels of transgene expression in the lungs. A multifunctional lipid envelope-type nanodevice developed to target the lung endothelium was found to accumulate in the lung within 5 min after injection. This carrier did not quickly remove to other organs and remain in lungs for 6 h. Based on this carrier, systemic administration of anti-CD31 siRNA successfully suppressed the metastatic progression (61). Therefore, the administration route should be carefully chosen according to the therapeutic application.

APPLICATION OF NUCLEIC ACID DRUGS IN PULMONARY DISEASES

Since the discovery of nucleic acids, their association with multiple diseases and hence the therapeutic potential have been extensively demonstrated. In the last decades, many investigations have been successfully proved the therapeutic efficiency of nucleic acids on various lung diseases ranging from cancer to pulmonary inflammatory diseases. Some of the nucleic acid products have entered the clinical stage; recent clinical trials involving nucleic acid drugs for pulmonary diseases are summarized in Table III.

Lung Cancer

Lung cancer is the leading cause of cancer-related deaths in the USA and worldwide (62). According to the difference in histology, 87% cases of lung cancer are classified as non-small cell lung cancer (NSCLC) and 13% cases are small cell lung cancer (SCLC). In addition to SCLC and NSCLC, malignant pleural mesothelioma is a rare form of lethal cancer developing in the tissue lining of the lungs (63). Current treatments for lung cancers include surgical resection, chemotherapy, radiation therapy, and targeted drug therapy, but these existing therapeutics have limited efficacy, and survival rate of NSCLC patients has remained low (63). Therefore, studies on target treatment of lung cancers with selective nucleic acid against oncogenic pathways have drawn intensive interest and some of them have entered clinical practice. Custirsen (OGX-011) is a PS-ASO inhibitor of clusterin, an anti-apoptotic chaperone protein upregulated in cancer cells in response to chemotherapy and might mediate resistance (64). Preclinical data showed that custirsen

Table III. Summary of Nucleic Acid Drug Indicated for Pulmonary Diseases into Clinical Trials (Cited from http://www.clinicaltrials.gov/)

Drug	Year	Therapeutic agents	Target	Vehicle	Route	Condition	Phase	Status	Sponsors	Clinical trial ID
Custirsen (OGX-011)	2004–2010	ASO	Clusterin	None	Intravenous injection	NSCLC	I II	Completed	Achieve Life Sciences	NCT00138658
	2012–						III	Ongoing		NCT01630733
Imetelstat Sodium (GRN163L)	2007–2011	ASO	Telomerase	ASO-lipid conjugate	Intravenous injection	NSCLC	I	Completed	Geron Corporation	NCT00510445
	2010–2016						II	Completed		NCT01137968
MRX34	2013–2016	miRNA	miR-34a	Liposome	Intravenous injection	NSCLC	I	Terminated	Mirna Therapeutics, Inc.	NCT01829971
TargomiRs	2015–2017	ASO	miR-16	Nonliving bacterial minicells	Intravenous injection	NSCLC	I	Completed	Asbestos Diseases Research Foundation	NCT02369198
TPI-ASM8	2007–2007	ASO	βc subunit of the IL-3, IL-5, GM-CSF receptors(TOP004) CCR3(TOP005)	None	Inhalation	Asthma	II	Terminated	Pharmaxis	NCT00402948
	2008–2008						II	Completed		NCT00550797
	2009–2010						II	Completed		NCT00822861
	2010–2012						II	Completed		NCT01158898
AIR645	2008–2009	ASO	IL-4/IL-13 Receptor α chain	None	Inhalation	Asthma	I	Completed	Altair Therapeutics, Inc.	NCT00658749
	2009–2010						II	Completed		NCT00941577
SB010	2011–2012	ASO	GATA-3 mRNA	None	Inhalation	Asthma	I	Completed	Sterna Biologicals GmbH & Co. KG	NCT01470911
	2012–2012						I	Completed		NCT01554319
	2012–2014						I	Completed		NCT01577953
	2012–2015						II	Completed		NCT01743768
TPI 1100	2009–2010	ASO	PDE4/PDE7	None	Inhalation	COPD	I	Withdrawn	Pharmaxis	NCT00914433
QR-010	2015–2016	ASO	CFTR mRNA	None	Inhalation	Cystic fibrosis	I	Completed	ProQR Therapeutics	NCT02564354
	2015–				intranasal route		I b	Ongoing		NCT02532764
ALN-RSV01	2007 July–November	siRNA	RSV-N gene	None	Inhalation or intranasal route	Respiratory syncytial virus infections	II	Completed	Alnylam Pharmaceuticals	NCT00496821
	2008–2009						II	Completed		NCT00658086
	2010–2012						II	Completed		NCT01065935

Abbreviations: *ASO* antisense oligonucleotide, *NSCLC* non-small cell lung cancer, *siRNA* small interference RNA, *COPD* chronic obstructive pulmonary disease

significantly decreases clusterin production, increases the sensitivity of lung cancer cells to chemotherapies, and inhibits tumor growth in lung cancer models. In the phase 2 trial of custirsen in patients who were treated with a combination of a gemcitabine/platinum doublet, serum clusterin levels were notably reduced. A larger randomized phase 3 study is needed to demonstrate the potential survival benefit of custirsen in patients with NSCLC (65). Imetelstat (GRN163L) is a 13 base phosphoramidate oligonucleotide conjugated to a 5-palmitoyl lipid group against the RNA component of telomerase, an enzyme responsible for maintaining telomere length and crucial for the indefinite growth of tumor cells. Blocking telomerase with imetelstat leads to antineoplastic effects. In a phase 2 study, imetelstat failed to improve progress-free survival rates in advanced NSCLC patients with diverse telomere. But there was a trend toward survival improvement for patients with shorter telomeres. Further investigations on short telomeres as predictive biomarkers are warranted for clinical development of imetelstat (66).

A lot of siRNA-based therapeutics are being assessed in preclinical and clinical trials of pulmonary diseases. ALN-RSV01, a siRNA therapeutic directing against the mRNA encoding the N protein of the respiratory syncytial virus, has completed phase II clinical trials (67). siRNAs also hold great promise as therapeutic agents for cancer through RNAi silencing oncogene expression. siRNA for cancer therapies are beginning to be tested in human clinical trials, such as ALN-VSP(Alnylam Pharmaceuticals) for the treatment of liver cancer and CALAA-01(Calando Pharmaceuticals) as tumor inhibitor (68), and they have shown promising pharmacodynamics and tolerability. However, to extend small RNA therapy to other major cancer types, including lung cancer, delivery vehicles that target nonliver tissues and specific delivery route are needed. Lung cancer is an attractive cancer type for local or systemic small RNA delivery treatment. Various therapeutic target genes (*e.g.*, survivin, bcl2, HDM2) for lung cancer therapy have already been identified and become targets of siRNA therapy (33).

miRNAs play a central and complex role in cancer development and are generally classified as tumor suppressor miRNAs or tumor-promoting miRNAs (oncomiRNAs). Tumor suppressor miRNAs in lung cancer include let-7 family, miR-34/449 family, miR-15/16, miR-200 family, and miR-205; oncomiRNAs in lung cancers include miR-17~92 cluster, miR-21, and miR-221/222 (62). There are two approaches for miRNA modulators to act as cancer therapies: exploiting antisense-based inhibitors of oncogenic miRNAs or replacing downregulated tumor suppressor miRNAs with synthetic miRNA mimics (69). To date, there are two tumor suppressive miRNA mimics of miRNA-34 (MRX34; Mirna Therapeutics Inc.) and miRNA-16 (TargomiRs; EnGeneIC Ltd.) that have entered clinical trials. MRX34 is a synthetic version of miR-34a encapsulated in liposomes. miR-34a is a tumor suppressor often expressed at reduced levels in a broad range of cancer types, which functions to downregulate the expression of more than 30 different oncogenes across multiple oncogenic pathways (70). But immune-related serious side effects caused termination of the trial of MRX34. TargomiRs are double-strand synthetic miR-16-based microRNA mimics delivered by EnGeneIC Dream Vectors which are deprived from nonliving bacterial minicells with a targeting moiety (71,72). The miR-16 family has been implicated as tumor suppressor in a range of cancer types, and their primary targets are genes (*e.g.*, BCL2, CDK1, and JUN) involved in cancer progression. *In vitro* and *in vivo* studies showed that the restoration of expression of miR16 in malignant pleural mesothelioma induced the apoptosis of tumor cells and inhibited tumor growth. Long-term survival after a short treatment period was observed in the phase 1 study. However, the safety issue and early signs of activity of TargomiRs still warrant further clinical trials (71).

Inflammatory Diseases

Asthma is a kind of chronic inflammatory airway disease with high prevalence, which could induce airway hyperresponsiveness, infiltration of inflammatory cells, and airway remodeling. It has been estimated that about 300 million people suffer from this disease on a global scale (73).

The current therapeutics for asthma (including inhaled β2-adrenergic receptor agonists, inhaled corticosteroids, and monoclonal antibody against IgE) could effectively control the disease for most patients while there are still about 10% of the patients still out of control under the current treatments. (74). Besides, the current drugs fail to stop or reverse the airway remodeling and some of the drugs followed with concerns of long-term adverse effects, which means there are unmet needs for better drugs (75,76). Choi et al. developed a novel therapy combining traditional drugs with novel therapeutics. In the regimen, dexamethasone (DEXA) was attached to PEIs to act as a controller ingredient to control the airway inflammation. While siRNA against vitamin D binding protein, which is a responsible molecule of allergic asthma, was delivered by DEXA-PEI at the same time (77). This multi-target treatment effectively reduced the airway inflammation and secretion of inflammatory factors. Asthma is a complex disease associated with the interaction between genetic, epigenetic, and environmental parameters, involved with a plethora of cells and cellular factors (59). One direction for developing new drugs to treat asthma is to target central pathways to the pathogenesis of the disease, and nucleic acid-mediated therapies silencing the specific effector or the upstream regulator can be a potential approach. Ribosomal protein S3 (RPS3) was found to bind to the subunit of NF-κB complex and enhance the downstream inflammatory effect. Intratracheal delivery of RPS3 silencing siRNA effectively alleviate airway hyperresponsiveness (AHR) and immune cell infiltration, and decreased serum total IgE levels were also observed (78). SB010, a new class of ASO therapeutic sequence-specific targeting and cleaving GATA3 mRNA, has entered into phase 2a clinical trials. The overexpression of GATA3 was found in cells involved in allergic inflammation. The results of the trial showed that inhaled SB010 significantly attenuate both the early-phase and late-phase allergen-induced asthmatic responses (79). Another ASO drug TPI-ASM8, developed by Pharmaxis, contains two types of ASOs targeting the βc subunit of the IL-3, IL-5, GM-CSF receptors (TOP004), and human CCR3 (TOP005) respectively. TPI-ASM8 showed the protective effect against IgE-mediated early asthmatic response and reduced eosinophilic airway inflammation (80).

Chronic obstructive pulmonary disease (COPD) is one of the most common chronic respiratory diseases of the airways with an increasing morbidity and mortality; it has been forecasted that COPD will be ranked the fourth burden of disease worldwide by year 2030 (81,82). COPD is characterized by progressive airflow obstruction and airway inflammatory response. Current therapeutic strategies are through inhaled long-acting β2-agonists, long-acting muscarinic antagonists, and corticosteroids to dilate bronchus and suppress inflammatory, which is similar to the treatment of asthmas (81,83). Emerging drugs in COPD focus on the cellular and molecular components regulating airway inflammations (82). Phosphodiesterases (PDEs) are a group of 11 different isoenzymes (PDE1-11) hydrolyzing cAMP, increased levels of which promote airway smooth muscle relaxation and bronchodilation with anti-inflammatory responses. Among the big PDE family, PDE4 is present in many types of cells relating to COPD and thought to be a promising therapeutic target. TPI1100, a dual PDE inhibitor comprising two modified ASOs directing against PDE4B/4D and 7A, was designed to reduce the recruitment and activation of inflammatory cells in COPD and shown to reduce the neutrophil influx in bronchoalveolar lavage (BAL) and inflammation of smoke-exposure or LPS-challenge murine models (84). The phase I clinical trial of TPI1100 was initiated in 2009 but was withdrawn due to drug development suspension. The lungs of COPD patients show that the reduction of alveolar elastic fibers and self-healing ability is impaired due to chondroitin sulfate proteoglycan versican inhibiting tropoelastin assembling into fibers. Wu et al. employed a small interfering RNA (siRNA) against versicanin primary pulmonary fibroblasts from COPD patients and enhanced the deposition of tropoelastin, which offers a new direction to lung repairment in COPD therapy (85).

miRNA expression has been proposed as an accessible biomarker of COPD disease (86). Multiple miRNAs were found altered in COPD patients and murine models and could serve as potential biomarkers for the COPD detection and prognosis. For example, downregulation of miR-20a, 28-3p, 34c-5p, 100, and upregulation of miR-7 and 21 have intimate association with COPD development (30). MicroRNAs were also found to play an important role in COPD muscle dysfunction and mass loss (87). Elevated miR-424-5p expression in patients with muscle wasting might contribute to the inhibition of protein synthesis and loss of muscle mass (88). It was demonstrated that miR-422a, as a suppressor of TGF-β signaling by reducing the expression of SMAD4 protein, might attribute to the maintenance of muscle mass (89).

Cystic fibrosis (*CF*) is a genetic disorder giving rise to the functional failure of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which acts as an epithelial chloride channel. The interaction of CFTR and epithelial sodium channel (ENaC) is responsible for the homeostasis of the airways epithelial surface. The deficiency or flaw of CFTR leads to hyperactivity of epithelial sodium channel. Reduced chloride secretion and increased sodium absorption subsequently result in mucus dehydration, chronic infection, and airway inflammation (5,90). Using antisense oligonucleotides that correct the basic defect at the mRNA level could restore the crucial balance between ENaC and CFTR. A recent study exploited aerosol delivery of ASOs in *CF*-like mouse models to inhibit ENaC activity by triggering RNase H1-dependent degradation of Scnn1a mRNA, which encodes the ENaC α subunit. This strategy effectively reduced goblet cell hyperplasia and reversed *CF*-like symptoms, demonstrating that an ENaC antisense therapy may provide a potential therapy for *CF* (91). The drug QR-010 is a single-stranded antisense RNA-based oligonucleotide sequence designed to hybridize the sequences adjacent to the deleted F508 region in the CFTR mRNA to restore the full function of CFTR protein in patients with the F508del mutation. Preliminary studies in cell culture and mouse F508del model showed improved chloride efflux after QR-010 treatment (92). Data showed that topical administration of QR-010 to the nasal epithelium improved CFTR function by measuring the nasal potential difference of F508del *CF* subjects (93). A phase1b study to evaluate the safety, tolerability, and pharmacokinetics of QR-010 is ongoing in *CF* patients with homozygous F508del cystic fibrosis.

Acute respiratory distress syndrome (ARDS) is a type of acute diffuse lung injury with a high mortality rate, which is clinically characterized by pulmonary infiltrates, hypoxemic respiratory failure, and edema, (94). The mild form of ARDS is termed as acute lung injury (ALI). It is suggested that approximately 2~8 cases of ARDS per 100,000 population per year. ALI is more common, with rates up to 25 per 100,000 per year reported (95). The common risk factors conclude sepsis, trauma, pneumonia, and toxic inhalation (95). Current ARDS therapy is to improve impaired gas exchange and lung mechanics by anti-inflammatory drugs, bronchodilators, and mechanical ventilation, which show limitation in controlling the disease progression. As researchers digging into the mechanisms of ARDS, crucial regulatory agents participating in the initiation and progression of ARDS, like miRNAs and cytokines, have become appealing therapeutic targets. It was found that murine ALI models treated with ASOs against miR-155 gained the enhanced recovery of ALI as evidenced by the reduction of BAL protein and pro-inflammatory cytokines, and the number of BAL cells (96). NF-κB is a family of DNA binding proteins involved in the expression of pro-inflammatory factors and thus the development of ARDS. Depletion of NF-κB by specific siRNA targeted NF-κB p65 in lipopolysaccharide (LPS)-induced ALI rat models effectively reduced levels of the pro-inflammatory cytokines and ameliorated symptoms induced by LPS (97). *In vivo* administration of the siS1PLyase/HMGB1A/R3V6 complex reduced the S1PLyase level and weakened the inflammatory response and apoptosis in an LPS-induced ALI model, indicating that siS1PLyase and HMGB1A have a synergistic therapeutic effect for ALI (98).

CONCLUSION AND FUTURE PROSPECTS

Nucleic acid drugs hold great promises as new classes of therapeutic agents for pulmonary diseases, and some candidates have entered into clinical trials (Table III). The unique structures of lungs enable the delivery of nucleic acid to be implemented by intravenous and pulmonary routes. Inhalation and intranasal routes have been found to be ideal for effective delivery. For proper therapeutic use, researchers

have modified the chemical structure of nucleic acids to increase their ability against nuclease degradation and reduce immune responses. The transition from bench to bedside of nucleic acid-based therapy also depends heavily on the availability of a safe delivery system that can facilitate trafficking into site of action. The safety issue, especially the immunogenicity of nucleic acids and their vectors, is the biggest stumbling block before nucleic acid drugs for lung diseases become available in the clinic, and further work in this area need to be thoroughly investigated. It is still necessary to identify suitable carriers with the ability to successfully reach the action site in the lung and protect the activity of nucleic acids during the delivery. With the advances and ongoing clinical trials, the future of nucleic acid drugs for pulmonary diseases remains very promising.

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