“Pulmonary Delivery of Therapeutic siRNA For Cystic Fibrosis”

Introduction

Cystic Fibrosis (CF) is the most common, life threatening disease in the Caucasian population. It is estimated that there are between 100000 and 700000 people with CF worldwide but it is difficult to state an accurate figure, as people with CF in countries without developed healthcare may die before diagnosis. There are around 30000 people with CF in the USA, over 7500 in the UK and aprox. 30,000 in EU. It is estimate that over 80,000 people may be living with CF in India alone. Around 1 in 25 of the Caucasian population carries the faulty gene that causes CF. If both parents are carriers, there is a 1 in 4 chance that the baby will be born with CF(1). The frequency of common mutation ΔF508 in Indian children is between 19% and 34% (2). The average life expectancy is between 35 to 40 years.

Cystic fibrosis (CF) is one of the most common life-shortening, chronic hereditary diseases among the Caucasian population. Cystic fibrosis is autosomal recessive genetic disorder. It is caused by a defect in a single gene located on chromosome 7, and is characterized by defective chloride ion transport in airway epithelial cells (3). This defective gene results in a mutation of the cystic fibrosis transmembrane conductance regulator (CFTR), the most common mutation being the ΔF508 mutation, which occurs on the surface of nucleotide-binding domain 1 (NBD-1)4. The ΔF508, the most common mutation, indicating a missing phenylalanine molecule in position 508 of the 1480 amino acid protein.5 Following this mutation, lung pathology includes abnormal chloride ion transport, increased mucus secretion and viscosity, and decreased mucociliary clearance. These consequences lead to chronic inflammation by recurrent infection and obstructed airways (Figure 1).
Cystic fibrosis related complications are caused by a disturbed chloride transport in the body. Chloride channels are missing or malfunctioning which results in failure of the cell to transport chloride into the extracellular space. As a result, chloride and sodium ions accumulate within cells, inhibiting water transport across cell membranes and causing dehydration of the mucus that normally coats these surfaces, thereby affecting the respiratory systems (4). As a result of increased viscosity of the mucus in the respiratory tract, the clearance of microorganisms is reduced and chronic bacterial infections resulting in inflammation of lung tissue and fibrosis of the airways are a fact (5). Staphylococcus aureus (51.5%) and Pseudomonas aeruginosa (55.0%) infections play the greatest role in morbidity and mortality (6). Local application of aerosolized antibiotics in the lung to combat these microorganisms has been proven successful. The primary objective is to prevent the development of or to stabilize a chronic infection with Pseudomonas aeruginosa (7). Once a chronic infection has been established; daily use of aerosolized antibiotics to stabilize local inflammation and improve pulmonary function (FEV1) is common practice. This strategy has been shown improvement of lung function, or to slow down deterioration of lung function and in a reduction in hospital admissions (8).

**Current treatment available:**

In 1989, the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR) was cloned, (9) clarifying the genetic basis of CF. The CFTR protein functions as an apical Cl- channel in secretory epithelial cells, and loss of CFTR activity causes abnormal ion transport, with decreased secretion of Cl- ions and increased absorption of Na+ ions. The resultant thickened secretions in the...
airway lead to the formation of purulent mucus within organs. Consequently, CFTR defects induce recurrent bacterial infections in the lung and progressive pulmonary destruction. Until recently, few of the affected individuals survived childhood and, other than transplantation, there is no permanent therapy for CF lung disease. Drug therapy is the only supportive therapy for the treatment of cystic fibrosis. Gene therapy represents the best hope for these individuals. The ultimate goal for gene therapy for CF is gene transfer of the intact CFTR gene to appropriate target cells with resultant genotypic and phenotypic correction of the disease (10-11).

Drug therapy acts as a supportive therapy for the symptomatic treatment of the cystic fibrosis. Drug belongs to the class of antibiotics, anti-inflammatory medicines, bronchodilators, mucus thinning agents are mainly used to treat the cystic fibrosis. These medicines help treat or prevent lung infections, reduce swelling, open up the airways, and thin mucus.

Antibiotics (Oral, Inhaled, Intravenous) are the main treatment to prevent or treat lung infections. Eg. Ciprofloxacin, tobramycin, vancomycin, Amikacin, Levofloxacin, AmphoB (12-13). Anti-inflammatory medicines (Oral, Inhaled, Intravenous) can help reduce swelling in your airways that's caused by ongoing infections. Eg. Glucocorticoides, macrolides. Bronchodilator (Inhaled) help to open the airways by relaxing the muscles around them. These medicines are inhaled and often are taken just before CPT to help clear out mucus. Eg. Albuterol, Levalbuterol, Ipratropium, Theophylline (14). Mucus thinning agents (Inhaled) reduce the stickiness of your mucus and to loosen it up. These medicines can help clear out mucus, improve lung function, and prevent worsening lung symptoms e.g. DNase, Hypertonic saline, Mannitol (15-16).

Cystic fibrosis is the genetic disorder caused due to the mutation in the CFTR gene following the lung infection and inflammation. Use of the gene therapy is good option to cure the disease. For gene therapy to be effective in patients with cystic fibrosis, the cDNA encoding the cystic fibrosis transmembrane conductance regulator protein must be delivered effectively to the nucleus of the epithelial cells lining the bronchial tree within the lungs. Expression of the transgene must be maintained at adequate level throughout the life of the patient, either by repeat dosage of the vector (17).

**RNA interference (RNAi):**

RNA interference (RNAi) is the process of mRNA degradation that is induced by double-stranded RNA in a sequence-specific manner. RNAi has been observed in all eukaryotes, from yeast to mammals. The power and utility of RNAi for specifically silencing the expression of any gene for which sequence is available has driven its incredibly rapid adoption as a tool for reverse genetics in eukaryotic systems.

The cell has a specific enzyme (in Drosophila it is called Dicer) that recognizes the double stranded RNA and chops it up into small fragments between 21-25 base pairs in length. These short RNA fragments (called small interfering RNA or siRNA) bind to the RNA-induced silencing complex (RISC). The RISC is activated when the siRNA unwinds and the activated complex binds to the corresponding mRNA using the antisense RNA. The RISC contains an enzyme to cleave the bound mRNA (called Slicer in Drosophila) and therefore cause gene suppression. Once the mRNA has been cleaved, it can no longer be translated into functional protein. (18)
siRNA as a therapeutic agent:
The structure of siRNA is highly specific to prevent erroneous gene silencing. siRNA molecules are 21-23 nucleotide double stranded RNA (dsRNA) duplexes with symmetric 2-3 nucleotide 3’ overhangs and 5’ phosphate and 3’ hydroxyl groups (18).

RNA interference (RNAi) is a conserved cellular mechanism by which a small double stranded RNA (dsRNA) directs the degradation of complementary mRNA and therefore inhibits the expression of a specific gene (19). Since its discovery, RNAi has become a powerful tool to study gene functions in biological processes (20-22). The ability to induce RNAi in mammalian cells using synthetic small interfering RNA (siRNA) has stimulated great interest in therapeutic applications of RNAi (23-25). In numerous studies, siRNAs have shown promise for treating a variety of diseases, including influenza and HIV infection, cancer and genetic defects (26-28). The double stranded RNA-based molecule, siRNA, has a high potential as biopharmaceutical therapeutics. As RNAi interferes with translation, and not with DNA transcription, siRNA may not interact with chromosomal DNA. This lack of DNA interaction greatly reduces concerns about possible adverse gene alteration that might result from DNA-based gene therapy. The interaction of siRNA with mRNA, not protein, also makes it possible to reduce the production of harmful proteins before synthesis.

A key challenge of RNAi-based therapeutic application is the efficient delivery of siRNA into target cells. siRNA is usually 21 nucleotides in length and highly charged and therefore cannot cross the cytoplasmic membrane by free diffusion. In the circulation and interstitial space, siRNA is vulnerable to degradation by RNase (29). Although siRNA can be delivered directly and locally to the target sites in limited applications (30, 31) a carrier system is required in most applications to protect siRNA from degradation and to facilitate its uptake by target cells (32, 33). The proposed carrier system contains a key cationic component, such as a cationic lipid, a cationic polymer or a cationic peptide, in order to bind siRNA effectively along with other neutral lipids.

Delivery of therapeutic siRNA in Cystic Fibrosis:
The volume of the surface fluid covering the airways is maintained through a fine balance between ion and water secretion and absorption. This is obtained by exerting a tight control of the activity of ion channels and transporters localized on the apical and basolateral membranes of epithelial cells. In particular, Na⁺ absorption through the epithelial Na⁺ channel (ENaC), localized in the apical membrane, and the Na/KATPase, in the basolateral membrane, is in equilibrium with Cl⁻ secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) and other Cl⁻ channels in the apical membrane, and the NKCC cotransporter in the basolateral membrane (34). In cystic fibrosis
(CF), the equilibrium between absorption and secretion is disrupted by mutations in the CFTR Cl\(^{-}\) channel. Hence, Cl\(^{-}\) secretion is strongly reduced and Na\(^{+}\) absorption becomes predominant. Accordingly, airways of patients with CF are dehydrated, obstructed by thick mucus, inflamed, and frequently infected (35). This situation could last several years, but more often the lungs get colonized by opportunistic pathogens such as Pseudomonas aeruginosa that are responsible for the destruction of the lung and the development of respiratory insufficiency and death (36).

It has been suggested that down-regulation of ENaC may help to restore airway hydration and mucus clearance, and to reverse, at least partially, the airway phenotype in patients with CF. Earlier, double-blind clinical trials using the ENaC blocker amiloride were performed in patients with CF. Amiloride was given by topical administration in aerosol. However, while amiloride was reported to reversibly reduce the sodium reabsorption and increase the mucus clearance in subjects with CF (37, 38), the effects had short duration, probably because of a rapid amiloride removal from the epithelium surface (39). Short duration has also been reported by in vivo pharmacodynamic studies in sheep using the more potent ENaC blocker benzamil (40).

**Clinical trials for siRNA**

In fact, delivery of siRNA has more success than other RNAi molecules. In 2004, the first human clinical trial of RNAi therapy was initiated for the treatment of age-related macular degeneration (AMD) with siRNA targeting VEGF-receptor 1 delivered intravitreally (41). Since then a number of clinical trials of siRNA therapy are being conducted for different conditions (42), including solid tumor cancers (43) and respiratory syncytial virus (RSV) infection (44,45). Table 2 shows the summary of clinical trials of siRNA therapy.

<table>
<thead>
<tr>
<th>Latest stage of development</th>
<th>Target disease</th>
<th>Route of administration/delivery agent</th>
<th>Company</th>
<th>Product name</th>
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<tr>
<td>III (terminated- unlikely to meet primary endpoint)</td>
<td>AMD</td>
<td>Intravitreal injection/naked siRNA</td>
<td>Opko Health</td>
<td>Bevasiranib (formerly Cand5)</td>
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<tr>
<td>II</td>
<td>AMD</td>
<td>Intravitreal injection/naked siRNA</td>
<td>Allergen &amp; Sirna Therapeutics</td>
<td>AGN211745 (formerly Sirna-027)</td>
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<td>II</td>
<td>RSV infection</td>
<td>Nasal spray/naked siRNA</td>
<td>Alnylam Pharmaceuticals</td>
<td>ALN-RSV01</td>
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<tr>
<td>II</td>
<td>Acute kidney injury</td>
<td>Intravenous injection/naked siRNA</td>
<td>Quark Pharmaceuticals</td>
<td>QPI-1002 (formerly 15NP)</td>
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<tr>
<td>II</td>
<td>AMD</td>
<td>Intravitreal injection/siRNA</td>
<td>Quark Pharmaceuticals, Pfizer</td>
<td>PF-4523665 (formerly REDD14NP &amp; RTP8011)</td>
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<tr>
<td>I/II</td>
<td>Ocular hypertension &amp; glaucoma</td>
<td>Ophthalmic drops/naked siRNA</td>
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<tr>
<td>I</td>
<td>Solid state tumors</td>
<td>Intravenous injection/cyclodextrin nanoparticles</td>
<td>Calando Pharmaceuticals</td>
<td>CALAA01</td>
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Table-1: Clinical Trials for siRNA

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<th>Disease Description</th>
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<th>Delivery Vectors</th>
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<td>Solid cancers with liver involvement</td>
<td>Intravenous injection/lipid nanoparticles</td>
<td>Alnylam Pharmaceuticals</td>
<td>ALN-VSP02</td>
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<td>Transthyretin mediated amyloidosis (ATTR)</td>
<td>Intravenous injection/lipid nanoparticles</td>
<td>Alnylam Pharmaceuticals</td>
<td>ALN-TTR01</td>
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<td>Pachyonychia Congenita</td>
<td>Intradermal injection/naked siRNA</td>
<td>TransDerm</td>
<td>TD101</td>
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<tr>
<td>Chronic optic nerve atrophy &amp; recent onset NAION</td>
<td>Intravitreal injection/naked siRNA</td>
<td>Quark Pharmaceuticals</td>
<td>QPI-1007</td>
</tr>
<tr>
<td>Advanced solid cancer</td>
<td>Intravenous infusion/liposomal nanoparticles</td>
<td>Silence Therapeutics</td>
<td>Atu027</td>
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**siRNA delivery for Lung:**
A delivery vector is often required to facilitate the cellular delivery of siRNA as the highly charged, hydrophilic natures of the macromolecules make it unable to cross the biological membrane to reach its target sites. Due to the safety concerns with viral-vector, many siRNA delivery studies focus on the development of non-viral vectors. An ideal siRNA delivery vector should consist of the following criteria:

- siRNA into nanosize;
- Protection of siRNA from enzymatic degradation;
- Ease of cellular uptake;
- Endosomal escape;
- Release of siRNA into the cytoplasm to work with RISC and
- Reduced toxicity

Different non viral vectors can be used to deliver siRNA to the application site. Commonly used non-viral siRNA delivery vector includes lipids, polymers and peptides (46).

**Challenges of pulmonary delivery:**
The pulmonary delivery of therapeutic macromolecules such as proteins and peptides has been investigated for over thirty years (47). The challenges of siRNA delivery via the pulmonary route are similar to the delivery of other macromolecules. In order to develop an efficient siRNA pulmonary delivery system, it is important to understand the anatomical and physiological properties of the respiratory tract in the first place. The respiratory tract can be divided into two regions: (i) the conducting region which consists of nasal cavity, pharynx, trachea, bronchi and bronchioles; and (ii) the respiratory region which consists of the respiratory bronchioles and alveoli. The conducting region is responsible for air conductance and the respiratory region is where the gaseous exchange takes place. The most prominent feature of the respiratory tract is the high degree of branching. According to the Wiebel's model of lung, there are 24 generations in total. This highly branched structure comprises airways with varying length and diameter presents an early barrier to targeted pulmonary delivery. Many lung diseases affect the lower region of the lungs. For the therapeutic agents to reach the diseased area, they must follow the airstream around the bend along the branched airway to the deep lung area. The size of particles is an important factor in determining the site of deposition (48). In pulmonary delivery, size of particle is expressed in terms of aerodynamic diameter. Large particles...
(N6 μm aerodynamic diameter) carry high momentum and are more likely to be impacted on the airway wall at bifurcations instead of following the changing airstream. Therefore they are usually deposited higher up in the airway such as the back of the throat or pharynx. For small particles (b1 μm aerodynamic diameter), their movements are determined by Brownian motion. They are mostly exhaled during normal tidal breathing but pausing can enhance their deposition as the probability of the latter is proportional to the square root of time (49). The optimal particle size for efficient deposition at the lower respiratory tract is found to be between 1 and 5 μm (47,50). As the particle size further decreases towards the nanoscale, deposition in the lung increases again due to the increasing diffusional mobility (51). For nanoparticles that are less than 100 nm, they appear to settle effectively to the alveolar region with a fractional deposition of around 50%. However, these ultrafine particles usually enter the lungs as larger agglomerates which can be broken down relatively easily into smaller particles on deposition.

Major barriers to pulmonary delivery include the mucociliary clearance action of the ciliated epithelial cells, and the presence of mucus, alveolar fluid and macrophages along different parts of the airways (53,53). Particles that are deposited on the ciliated cells are rapidly removed by mucociliary clearance and are eventually being coughed up or swallowed. The mucus lines the respiratory epithelium from the nasal cavity to the terminal bronchioles (54). The major component of mucus is mucins which are glycosylated proteins. Mucus constitutes a physical barrier as it increases the viscosity of the moist surface of the lung epithelial cells, thereby reducing drug penetration and diffusion rate. The alveolar fluid is found on the surface of alveoli epithelium as a thin layer of pulmonary surfactant which comprises phospholipids and other surfactant proteins. It has been reported that the pulmonary surfactant severely impeded the transfection efficiency of lipid-based nucleic acids delivery system, but not polymer-based system (55–57). The alveolar macrophages located in the alveoli rapidly engulf the foreign particles by phagocytosis as a defense mechanism (58). The siRNA that is taken up into the macrophages are subsequently degraded inside the cells. At disease state, the physiological conditions of the airways might be altered and pose a huge impact on the efficiency of the pulmonary delivery system. During infection and inflammation, there is an increase in mucus secretion and the mucociliary clearance is impaired (58,59). The thickness, the viscosity and the composition of the mucus layer depend on the pathological condition and vary between individual (52). To overcome the anatomical and physiological barriers of the lungs, several delivery strategies can be incorporated. Besides using particles with small aerodynamic diameter suitable for deposition in the lower airways, it has been reported that the use of large porous particles can effectively avoid phagocytosis by the alveolar macrophages and prolong retention time in the lungs (60–62). Porous particles over 10 μm in geometric diameter usually have a smaller aerodynamic diameter so that they are within the ideal aerodynamic size range for effective lung deposition, but their actual geometric size is too large to be removed by macrophages. To overcome the mucus barrier, the use of mucolytic agents, such as nacystelyn which breaks down the three dimensional gel network of mucus, or the use of mucus inhibitor, such as glycopyrrolate which inhibit mucus secretion, could be considered (63). However their clinical benefits are limited (52,63). Inhaled mannitol has been clinically proven to increase the mucus clearance in patients with cystic fibrosis or bronchiectasis (64,65) and to improve the hydration and surface properties of sputum (66). Reducing the mucus barrier by mannitol inhalation prior to the delivery of siRNA may thus be beneficial. The use of ultrasound and magnetic field has also been reported to direct and control the site of deposition of nucleic acids delivery systems in the airways (67–69).

**siRNA as Dry Powder Inhalers:**
DPIs are aerosol systems in which drugs are inhaled as clouds of dry particles. The use of DPIs appears to be a promising way to deliver siRNA to the lungs as they demonstrated successful in vivo delivery of other therapeutic macromolecules including insulin (70), parathyroid hormone (71) and low molecular weight heparin (72,73). The formulation challenges and potential solutions for delivery of macromolecules such as proteins as powder aerosols have been reviewed (74). Formulating biological macromolecules as powders for aerosol delivery is a challenge as it requires not only flowability and dispersibility of the powders but also biochemical stability of the macromolecules. To satisfy the latter requirement, proteins are usually formulated in amorphous glasses which are, however, physically unstable and tend to crystallize with inter-particulate bond formation and loss of powder dispersibility. In addition, the biochemical stability requirement limits the manufacturing processes that can be used for protein powder production. Similar issues will be encountered for siRNA. Nevertheless, possible ways to tackle these challenges have been addressed (74). The inhaled dry powder form of insulin (Exubera, marketed by Pfizer) was approved in Europe and the US for the treatment of diabetes in 2006 (75). Although the product was withdrawn from the market in the subsequent year, Pfizer stressed that disappointing sales is the major reason for the withdrawal rather than safety or efficacy issues (76). The safety profile of inhaled insulin was indeed reassuring and the efficacy was not inferior to the conventional injection formulations (77–81). A second inhalable insulin product in dry powder form, Afresa, is currently awaiting for FDA approval. This encourages further development of dry powder form of macromolecules for inhalation. There are different designs of DPI device and their delivery performance may vary. The key advantages of DPIs are the improved stability and sterility of biomolecules over liquid aerosols, and the propellant-free formulation (82). Inhalable dry powder forms of proteins and peptides are commonly produced by spray-drying (83,84) and the same technique could be applied to siRNA. Size of the spray-dried product must be carefully optimized for efficient delivery to the desirable site along the respiratory tract. A suitable delivery agent or formulation is required to protect the nucleic acids from degradation caused by the shear force and raised temperature during the drying process. The major drawback of DPIs is that drug deposition could be dependent on the patient inspiration flow rate. Therefore a suitable DPI device must be carefully designed to minimize such variation. In addition, the problem associated with de-aggregation of dry powders must be overcome (82). Nebulizers are used to generate liquid aerosol and can be utilized to deliver large volumes of drug solutions or suspensions for inhalation. They are frequently used for drugs that are unsuitable to be formulated into MDIs or DPIs, and could be considered for siRNA delivery. During the process of nebulization, high shear stress is exerted on the siRNA which may lead to degradation of the nucleic acids. This is a particular problem as 99% of generated aerosol droplets are recycled back into the reservoir (47) and the shear stress could be repeatedly exerted to the nucleic acids. In addition, biomolecules tend to be less stable in liquid form then in dry powder form. Stability is the prime concern in delivering siRNA with nebulizers. A suitable delivery vector is therefore required to protect siRNA from both physical and chemical degradation. Although inhalation is a common way to deliver drug to the lungs, to our best knowledge, none of the in vivo study on siRNA therapy is intended for inhalation. Most of the in vivo studies use either intratracheal or intranasal route of delivery to the lungs. This could be due to the difficulty in formulating inhalable siRNA, especially in maintaining the stability and biological activity of siRNA during manufacturing and delivery process.

**Non-viral delivery of siRNA to the lung:**
Naked siRNA:
The term ‘naked siRNA’ or ‘unformulated siRNA’ refers to the delivery of siRNA without using any delivery agent. This includes the delivery of both unmodified siRNA and modified siRNA, formulated in saline or other simple excipients such as 5% dextrose. Since unmodified siRNA is susceptible to nuclease degradation, chemically modified siRNA was introduced initially to address this issue by increasing the nuclease resistance. The siRNA can also be chemically modified to improve potency, increase specificity, reduce immune response and reduce off-target effects.

Lipid-based delivery vectors:
Lipid-based delivery systems are commonly used to deliver siRNA both in vitro and in vivo (85). Typically cationic lipids or liposomes are used to form complexes with the negatively charged siRNA through spontaneous electrostatic interaction and the complexes are referred as lipoplexes. Different lipid based delivery vectors are listed below:

- Cationic lipoplexes and liposomes
- PEGylated lipids
- Neutral lipids
- Lipids particles
- Lipid-like molecules/Lipidoids

Polymer-based delivery vectors:
One of the attractive properties of polymer-based delivery vectors is their versatile nature that allows their physicochemical characteristics to be modified relatively easily to fit their purposes. In addition, it has been suggested that polymers generally do not evoke as strong an immune response as liposomes (86). In general, polymer-based vectors can be divided into two categories: polycations and polymeric nanoparticles. Synthetic polycations such as polyethylenimine (PEI) (87), polyamidoamine (PAMAM) dendrimers (88) and natural polycations such as chitosan (89) are used for delivering DNA for a long time. These polymers have high cationic charge density and form polypelexes spontaneously with the negatively charged nucleic acids through electrostatic interaction. The alternative way to deliver siRNA using polymer is to prepare polymeric nanoparticles which are usually solid nanoparticles made from hydrophobic polymers such as poly(D,L-lactide-co-glycolide) (PLGA) (84,89).

**Peptide-based delivery vectors:**
Since the discovery of TAT protein from HIV-1 which is responsible for the cellular uptake of the virus (90), a variety of cell-penetrating peptides (CPPs) have been derived or synthesized. CPPs are frequently employed to facilitate the transport of therapeutic macromolecules into the cells and this strategy has been extended to the delivery of siRNA (91,92). CPPs and derivatives that are investigated for siRNA delivery included TAT (93,94,95), penetratin (96–97), transportan (95), MPG (97,98), CADY (99,100) and LAH4 (101).

**References:**
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12. Thomas G O’Riordan, Inhaled Antimicrobial Therapy: From Cystic Fibrosis to the Flu, Respiratory Care, 2000, 45(7).


77. T. Quattrin, A. Belanger, N.J.V. Bohannon, S.L. Schwartz, I.I.I.S.G. Exubera Phase, Efficacy and safety of inhaled insulin (Exubera) compared with subcutaneous insulin therapy in patients


Title: Pulmonary Delivery of Therapeutic siRNA For Cystic Fibrosis

Subject/Area: Life science

Contact details of the Institution (Collaborator): (Name, Address, Phone, Mobile Number, Fax, email, etc.)
The Maharaja Sayajirao University of Baroda
Baroda - Gujarat -390002
Investigators:

Project Title: Pulmonary Delivery of Therapeutic siRNA For Cystic Fibrosis

General Objectives: Development of siRNA Dry Powder Inhalers (DPI) for Effective Treatment of Cystic Fibrosis

Specific Aims:
I. Selection of specific siRNA for silencing of ENaC (Epithelial Sodium Channel).
II. Development, optimization, and characterization of DPI encapsulating siRNA.
III. Stabilization of siRNA formulation by lyophilization, spraydrying or supercritical fluid technology for local pulmonary delivery.
IV. In vitro and in vivo evaluation in suitable human lung cell lines and athymic nude mice animal models.

Hypothesis:
It is hypothesized that specific siRNA DPI targeted towards ENaC will maintain the phenotype of CFTR protein.

Research Design and Method:
A. Selection and design/customization of specific siRNA for targeting ENaC gene.
B. Formulation and development of Nano-construct encapsulating siRNA using nonviral vector delivery like Lipid particle, lipoplex, polyplex, block co-polymeric micelle etc.
C. Stabilization of nano-construct using Lyophilization, Spray drying, super critical fluid technology etc.
D. Characterization and evaluation of siRNA nano-construct:
   Particle size, zeta potential, % siRNA loaded and encapsulated, stability, ligand attachment efficiency, In-vitro release study, Serum stability,
E. In-vitro cell line studies:
   Transfection study, Gene silencing, MTT assay
F. In-vivo studies
   Pharmacodynamic study
   Pharmacokinetic study
   Organ distribution study
G. Patent application: National/International

Expected results:
Silencing of ENaC gene using specific siRNA nano-construction may lead to improvement in therapeutic benefits in treatment of cystic fibrosis.
Gene silencing study will provide rock proof for the inhibition of the gene of interest. Also animal studies will also effectiveness and reduced toxicity profile as well as lung deposition of the targeted nano-construct.
## TECHNICAL DETAILS
### Part IV – Timelines for Quantifiable Outputs

| Period of study | Achievable targets (Physical and Technical) | Department involved | Required financial input (Rs. in Lakhs) *
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<td>• Design/customization of siRNA sequencing</td>
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<td>• Ordering and Confirmation of siRNA sequencing</td>
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