REVIEW ARTICLE

siRNA Drug Delivery for Cystic Fibrosis: A Review

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ABSTRACT

Cystic Fibrosis (CF) is a genetic condition that impacts life expectancy, caused by mutations in CFTR gene, which interferes with ion transport across epithelial membranes. This condition primes to manufacture of thick, dehydrated mucus that can block airways &ducts, especially in the lungs &pancreas, resulting in ongoing infections and gradual tissue harm. Even with the progress made in pharmaceutical treatments like CFTR modulators, finding a definitive cure continues to be a challenge. Nonetheless, the clinical promise of siRNA faces obstacles such as swift degradation by nucleases, inadequate membrane permeability, restricted mucus penetration, and challenges in effective intracellular delivery. Improved delivery methods are essential to protect siRNA and ensure accurate targeting to the areas in need. This review discoversprobable of siRNA-based DDS in treatment of cystic fibrosis, highlighting the role of non-viral carriers like lipid-based nanoparticles, cationic polymers, dendrimers&peptide-mediated vectors. There is a strong focus on bio-inspired methodologies, including exosomes and mucus-penetrating particles. Special attention is given to tackling the unique challenges posed by cystic fibrosis, including changes in mucus composition and the presence of inflammation. Recent advancements in preclinical research, innovative inhalation formulations, and initial findings from clinical trials are examined to offer a comprehensive understanding of the current developments. The research also explores how siRNA therapies can be combined with existing CFTR modulators to enhance their effectiveness. Ultimately, it explores future paths focused on personalized delivery methods, cutting-edge nanotechnology, and navigating regulatory challenges. Continuous progress in siRNA delivery presents a promising opportunity for a transformative therapy that could change the course of cystic fibrosis.

Keywords: Gene silencing, nanocarriers, hindrances, paradigmatic, exosomes, pathophysiological.

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INTRODUCTION

The main organs involved in this life-limiting autosomal recessive genetic condition called cystic fibrosis, or CF, are the respiratory, digestive, and reproductive systems. The mutation is CFTR gene that codes for a protein required to regulate the flow of sodium and chloride ions through epithelial membranes. When this protein is absent or defective, thick, sticky mucus accumulates in the pancreas, airways, and other organs, causing inflammation, damage to the organs, and chronic respiratory infections. Even with the dramatic advances in CF treatment in recent decades, the disease is still a major health threat, with sufferers typically having a worse superiority of life & reduced life expectancy.

The major aims of traditional CF treatment methods have been the alleviation of symptomatology and problem management. Symptoms' response and problem management for many have been augmented by therapies such as airway clearance therapy, antibiotics, and more currently, CFTR modulators. Potentiators, correctors, and amplifiers are classified under CFTR modulators that target the remediation of select CFTR mutations by rescuing some level of protein function. Nonetheless, varied treatments, effective to varying degrees on varying individuals, fail to successfully treat all the variations found in CFTR mutations. So, novel and relevant therapeutic options dealing with the intrinsic pathology of CF by tackling diverse and diverse genetic alterations are long overdue.[1]

One of the potential therapies for gene disorders like cystic fibrosis is RNA interference (RNAi) technology. It is small interfering RNA (siRNA) that is behind the technique, consisting of short double-stranded RNA molecules that selectively silence particular sections of messenger RNA (mRNA). SiRNAs are able to reduce the production of abnormal or deleterious CFTR protein by degrading mutant CFTR mRNA through normal cellular RNA interference. Treatments based on the principle of siRNA are much sought after as it possesses an efficiency of exact targeting of specific genetic sequences in patients harboring resistance mutations to current pharmaceutical treatments.

Despite the vast potential, numerous challenges lie ahead for development of siRNA-based therapies for cystic fibrosis. The primary challenges are the limitation of immunological responses and off-target effects, overcoming inherent instability of RNA molecules in an extracellular environment, and efficient delivery of siRNAs to the lungs. Through innovative ways to develop targeted delivery systems, chemical modification of siRNA

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molecules, and the growth of nanotechnology, researchers have been successful in overcoming these issues since the past. Among them, lipid nanoparticles, polymer-based carriers, and viral vectors are some promising ones that have proved to be efficient for delivery of siRNA to the lung.[2]

The intent of this report is to present a critical assessment of the promise of siRNA technology as a revolutionary treatment modality for CF. It will review the molecular pathways underlying RNA interference (RNAi), highlight innovative delivery systems that are being explored to bypass existing hurdles. The review shall also encompass the limitations of siRNA therapies, including regulatory hurdles, immunogenic responses, and off-target activities. The article aims to highlight the way this therapy may transform the treatment of CF and the care of patients by discussing the status of ongoing research and the future directions in this field of therapy. To translate this technology into practical therapeutic applications, we would like to highlight both the potential that siRNA has to offer and the essential gaps that must be bridged. Therapeutics based on siRNA have the potential to become an integral part of CF therapy as the science unfolds, with potential for a more integrated and effective solution to this multifaceted inherited disease.[3]

Mechanism of siRNA Action

By RNAi, a highly evolved natural process, siRNA plays a pivotal role in the control of genes. Biological process that serves as a gene silencing tool, known as RNA interference,

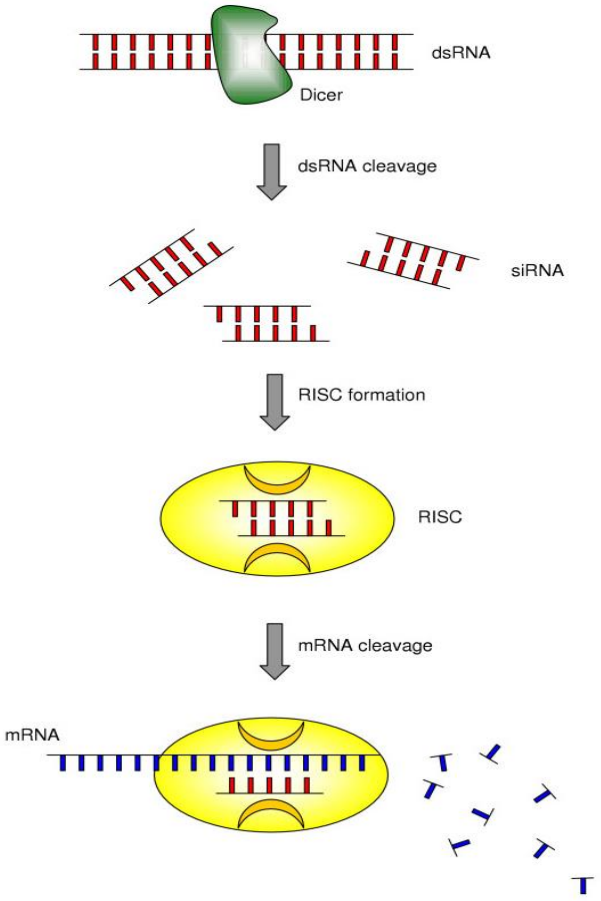

Figure 1: Mechanisms of RNA interference[5]

focuses on specific messenger RNA (mRNA) molecules and tags them for degradation and thereby allows cells to regulate gene expression specifically. This procedure assists in maintaining cellular equilibrium and holds the promise of therapeutic potential by restricting the synthesis of particular proteins. Because of its highly selective mechanism, RNA interference is well suited.

Cancer, viral infection, and genetic conditions like cystic fibrosis are some of the examples. To do this, siRNA utilizes several key molecular actors. Collectively, in a series of precisely coordinated steps, these components provide efficient, targeted gene silencing.[4]

RNA Interference Pathway

RNA interference (RNAi) is a conserved biological mechanism that cells use to protect themselves against foreign nucleic acids, such as transposons and viral RNA, and to regulate gene expression after transcription has taken place (Figure 1). Silencing genes is made possible by tiny RNA molecules, primarily miRNAs and small interfering RNAs (siRNAs), which tell the RNA-induced silencing complex (RISC) to impede or destroy the translation of complementary messenger RNA (mRNA) molecules.[6,7] The RNAi pathway comprises the following major steps:

1. Formation of Double-Stranded RNA (ds RNA)- RNAi is either of endogenous origin (e.g., pri-miRNA transcripts) or exogenously introduced (e.g., synthetic si RNAs). In therapeutics, chemically synthesized si RNAs are engineered to be approximately 21–23 nucleotides in length with 2-nucleotide 3' overhangs, similar to natural Dicer products.
2. Cutting of dsRNA by Dicer- The enzyme Dicer, which is an RNase III endonuclease, identifies and cleaves long precursors of dsRNA into short double-stranded fragments of siRNAs. Dicer works with the assistance of cofactors like transactivation response RNA-binding protein (TRBP) and protein activator of PKR (PACT). The resultant siRNAs have characteristic structural features required for effective identification by the subsequent silencing machinery.
3. 3. Loading into the RNA-Induced Silencing Complex (RISC): The siRNA duplex is integrated into the multiprotein complex, primarily composed of Argonaute proteins, particularly Argonaute 2 (AGO2). The duplex is unraveled, removing the "passenger strand" (sense strand) and keeping the "guide strand" (antisense strand) in RISC.
4. Target Recognition- Following base-pair complementarity, the guide strand in RISC scans and binds to complementary sequences of target mRNAs. In the case of siRNA, perfect or nearly perfect complementarity is typically required to ensure efficient cleavage of the target mRNA.
5. When the AGO2 protein in RISC attaches, it enables endonucleolytic cleavage of the target mRNA in the midst of the base-paired region with the siRNA guide strand. Cleavage usually occurs between the guide strand's 10th and 11th nucleotides from the 5' end.
6. Degradation and Gene Silencing- After cleavage, the broken-down mRNA is then quickly degraded by cellular exonucleases, and target protein expression is

decreased. Therefore, RNAi is effective in silencing the gene in a sequence-dependent manner and regulating gene expression as well as presenting therapeutic prospects for genetic defects disorders of known disease.[8,9]

Mechanism of Gene Silencing

Gene silencing is the control of gene expression so that a gene product, like RNA or a protein, is not produced. It can happen at several stages — either by blocking transcription (transcriptional gene silencing, TGS) or by breaking down the messenger RNA. Both of these mechanisms are important in development, genome stability, and virus defense.[10]

Transcriptional Gene Silencing (TGS)

TGS is the repression of gene expression at the transcriptional level.

DNA Methylation: Cytosine residues, especially in CpG islands upstream of gene promoters, are methylated by DNA methyltransferases (DNMTs), resulting in condensation of the chromatin and silencing of transcription. Methylated DNA may attract proteins such as MeCP2, binding to methylated loci and also drawing histone deacetylases (HDACs) to further condense chromatin and suppress transcription initiation.

Histone Modifications

Histones may be chemically modified by methylation, acetylation, ubiquitination, and phosphorylation. It is linked with gene repression through the creation of heterochromatin regions that are not accessible to transcription machinery.

SiRNAs can guide sequence-specific DNA methylation and chromatin remodeling in plants and some mammals. SiRNAs drive Argonaute (AGO) proteins to homologous DNA sequences, which bring in methyl transferases via RNA-directed DNA methylation (RdDM) pathways.[11]

Post-Transcriptional Gene Silencing (PTGS)

PTGS primarily takes place by mechanisms that destroy mRNA or block its translation once it has been synthesized.

RNA Interference (RNAi)

One of the most well-studied PTGS mechanisms is RNA interference. Double-stranded RNA (dsRNA) is cleaved by the enzyme Dicer into short pieces (~21-25 nucleotides) known as siRNAs or microRNAs (miRNAs).

Mechanism:

- SiRNA or miRNA is received by the RNA-induced silencing complex (RISC).
- The RISC complex, guided by complementary siRNA or miRNA, targets the target mRNA.
- Complete complementarity (e.g., siRNA routes) leads to mRNA cleavage and destruction.
- If there is partial complementarity (more common of miRNAs), the translation of the mRNA is inhibited without destruction.

Endogenous vs. Exogenous Sources: PTGS can be initiated by endogenous sources such as microRNAs, or by exogenous sources such as viral infection or experimentally added dsRNA.[12]

Challenges in siRNA Delivery for Cystic Fibrosis

Use of siRNA in treatment of cystic fibrosis (CF) is very promising, largely through silencing expression of the defective CFTR gene. Effective delivery however, faces various challenging barriers. These challenges can be broadly classified as biological barriers, specificity problems of delivery, and immune system responses.[13]

Biological Barriers

One of the major challenges facing siRNA-based CF therapies is penetrating the lung's intricate biological milieu:

1. Thickened Mucus in CF Airways: One of the most characteristic features of CF is the formation of a dense, tacky mucus layer in the airways. The defective mucus blocks the diffusion of therapeutic agents, such as siRNA, limiting their entry into epithelial cells. In contrast to normal mucus, which is permeable for relatively free diffusion, CF mucus is extremely viscous and dehydrated and creates an effective physical barrier to nanoparticle or carrier entry. This requires the creation of delivery systems that can penetrate or circumvent the mucus layer.
2. Cell Membrane Permeability: A second challenge is the inherent problem of siRNA delivery to cells. siRNA molecules are negatively charged, hydrophilic, and fairly large molecules, properties that bar passive diffusion through the lipid membrane of cells. Specialized delivery systems, usually lipid nanoparticle- or polymeric carrier-based, are needed to enable cellular uptake.
3. Endosomal Entrapment: After endocytosis-mediated internalization, siRNA molecules are usually captured inside endosomes. If they cannot escape into the cytoplasm ahead of endosomal maturation, the siRNA will be destroyed inside lysosomes, making it therapeutically inactive. Effective endosomal escape is still a significant drawback in siRNA delivery technology.
4. Degradation by Nucleases: Extracellular nucleases within the lung microenvironment can easily degrade unprotected siRNA.[14]

Delivery Specificity and Immune Response

Aiding in precise delivery of siRNA to the appropriate lung cells without interfering with non-target tissues is yet another essential challenge:

Targeting Diseased Epithelial Cells: The therapeutic siRNA needs to find and target airway epithelial cells, where CFTR gene expression needs to occur. Selective targeting of the siRNA to these cells is extremely challenging.

Prevention of Off-Target Effects: Even slight sequence homology between the therapeutic siRNA and non-target mRNAs can lead to off-target effects, i.e., unintended gene silencing. These unwanted interactions may cause unwanted cellular dysfunction or toxicity. Therefore, siRNA design has to be extremely specific, with meticulous sequence selection and modification approaches to reduce off-target activity.

Designing Effective Carriers: Not only should such carriers protect the siRNA but also ensure specific uptake by the target cells. Yet, varying expression levels of possible surface receptors for targeted delivery among patients and stages of the disease make the development of uniformly effective targeting mechanisms challenging.

The innate and adaptive immune responses present major challenges to the repeated and safe administration of siRNA therapies:

Activation of Innate Immunity: siRNA molecules, particularly those possessing specific sequence motifs or double-stranded conformations, are detectable by pattern recognition receptors. This can trigger pro-inflammatory cytokine production and other immune responses with unforeseen side effects

Immunogenicity of Delivery Vehicles: The delivery vehicles utilized to transport siRNA, in this case cationic lipids and certain viral vectors, are themselves potent immune stimulants. This not only restricts repeated dosing but may induce inflammation within the already inflamed environment of the CF lung.

Risk of Increased Inflammation: Cystic fibrosis is linked with chronic airway inflammation, which already impairs lung function. Immune activation caused by siRNA could exacerbate this inflammation, potentially overriding the therapeutic value unless delivery is tightly regulated and immune-stimulating activity of siRNA and carriers is reduced.[15,16]

Stratergies for siRNA Drug Delivery

The effective use of small interfering RNA (siRNA) in clinical medicine relies heavily on design of efficient delivery systems. These are naturally unstable, subject to enzymatic degradation, and not very efficient at entering target cells. Therefore, sophisticated delivery systems must be implemented to shield siRNA from enzymatic degradation, facilitate its internalization by cells, enhance endosomal escape, and deliver it to its desired site of action.[17]

Nanoparticle Based Delivery System

Nanoparticle-based systems are among the most extensively studied systems for conveyance of siRNA because they have the ability to improve stability, cellular uptake, and target specificity (Figure 2). Since naked siRNA has inherent limitations, including poor membrane permeability, nuclease susceptibility, and rapid renal clearance, nanoparticles offer a strong platform for overcoming these issues.[18]

Polymeric Nanoparticles

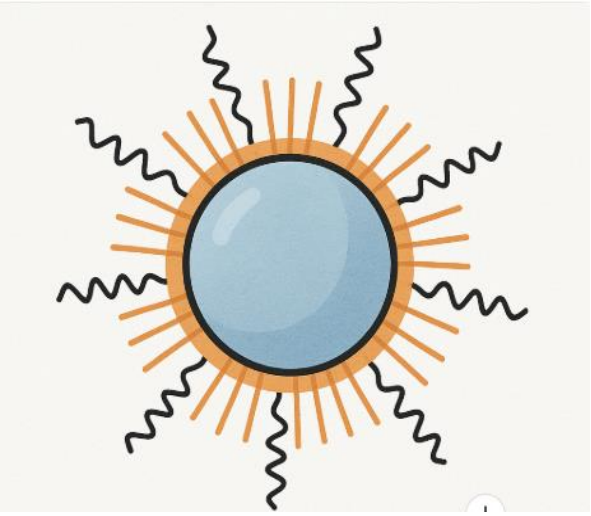

Figure 2: Structure of a typical SI RNA loaded particle

Polymer carriers are the key to effective delivery of siRNA, which is mainly based on their tunable structure, biodegradable nature, and capacity to evade enzymatic digestion. Polymer carriers facilitate siRNA to cross barriers in the living organism, enhance cellular uptake, and facilitate intracellular release inside the cytoplasm where silencing of genes takes place. Different polymers have been examined for their capacity to self-assemble into nanoparticles that can deliver siRNA safely and effectively to target tissues, especially in conditions including cancer, viral diseases, and genetic diseases like cystic fibrosis.

One of the most extensively researched synthetic polymers used in siRNA delivery is polyethyleneimine (PEI). One of advantages of PEI is its "proton sponge" effect, which allows the nanoparticles to buffer endosomal pH changes, causing osmotic swelling and endosomal disruption. This allows the release of siRNA into the cytoplasm. Nonetheless, PEI's high molecular weight forms, though efficient in transfection, are cytotoxic and non-biodegradable. To overcome these drawbacks, modifications like PEGylation (polyethylene glycol attachment) or the incorporation of low-molecular-weight PEI with degradable linkers have been used to improve its biocompatibility without affecting delivery efficiency.

Another widely utilized polymer is PLGA, a biodegradable & FDA-approved polymer that has found widespread use in controlled drug delivery systems. PLGA nanoparticles are generally neutral or slightly charged and usually need surface modifications with cationic agents such as chitosan or PEI to load and deliver siRNA effectively. Its primary strength is its controlled degradation that enables long-term sustained release of siRNA. In spite of these strengths, hydrophobic nature of PLGA may restrict encapsulation of hydrophilic siRNA molecules, and hence additional formulation strategies need to be developed for better loading and targeting.

Chitosan, a naturally occurring polysaccharide that is obtained from chitin, is another well-known polymer used for gene delivery. It is biocompatible and biodegradable and has a natural positive charge under slightly acidic conditions, which allow it to bind with siRNA to produce stable complexes. Chitosan is specifically good for mucosal delivery pathways like pulmonary and nasal delivery because of its mucoadhesive character. This makes it a strong contender for siRNA delivery in diseases such as cystic fibrosis, which has major barriers of mucus delivery. Still, chitosan's poor solubility at neutral pH and relatively less transfection efficiency than synthetic polymers have made derivatives such as trimethyl chitosan an attempt to optimize its performance.

Finally, polycaprolactone (PCL), another synthetic biodegradable polymer, has a slow degradation rate and is also used for long-term therapeutic purposes. PCL is a semi-crystalline material with hydrophobic properties, which favors encapsulating siRNA in some formulations, particularly in the presence of surfactants or cationic agents. While nanoparticles derived from PCL provide extended siRNA release, its modest solubility and slow degradation can postpone the onset of gene silencing activity. Hence,

they are usually more suitable for long-term diseases where longer dosing intervals are needed.[19,20]

Inorganic Nanoparticle

Inorganic nanoparticles have also risen as a compelling group of carriers for siRNA because of their physicochemical characteristics such as structural stability, functionalizability, and surface tunability. In contrast to organic carriers, inorganic nanoparticles tend to be more stable in physiological conditions and can be made responsive to diverse internal or external stimuli, rendering them desirable for targeted and controlled siRNA release.

Gold nanoparticles (AuNPs) are widely investigated due to their biocompatibility, facile surface modification, and potential for functionalization with thiol-modified nucleic acids. AuNPs can be conjugated on their surface with siRNA by either covalent attachment or electrostatic attraction, enabling high loading and protection from nucleases. In addition, their small size makes them easy to be taken up by cells, and when they are conjugated with targeting ligands like folic acid or peptides, they show improved cell-specific delivery. Additionally, AuNPs have inherent photothermal properties that can be utilized for simultaneous siRNA delivery and photothermal therapy, particularly in cancer therapy. Nonetheless, issues of long-term tissue retention and possible cytotoxicity at high concentrations need to be thoroughly weighed.[21]

Another crucial class comprises mesoporous silica nanoparticles (MSNs) that provide high pore volume and surface area, perfectly suited for encapsulating and stabilizing siRNA molecules. MSNs are easily surface-modified using cationic polymers such as chitosan or polyethyleneimine (PEI) to increase siRNA affinity and induce endosomal release. Their porous nature allows for the co-delivery of therapeutic payloads, including small-molecule drugs and siRNA, for synergistic action. In addition, MSNs have been engineered to be stimuli-responsive (e.g., pH or redox-sensitive) to trigger on-demand release in the acidic tumor or inflamed tissue microenvironment. While promising, the biocompatibility and long-term safety profile of silica-based systems are still under investigation.[22]

Iron oxide nanoparticles (IONPs) constitute another group of inorganic carriers used to deliver siRNA, mainly due to their magnetism. The nanoparticles are capable of being directed to targeted tissues by an external magnetic field, which would allow for targeted delivery. Additionally, IONPs may be functionalized with siRNA and coated with biocompatible polymers to enhance circulation and minimize immune clearance. They show particular utility in theranostic systems where they facilitate one-step imaging (e.g., MRI) alongside gene silencing. However, the issue of aggregation in in-vivo biological fluids and any risk of oxidative stress caused by release of the iron ion have to be counteracted for appropriate clinical safety.[23]

Quantum dots (QDs), semiconductor nanocrystals with excellent fluorescence characteristics, have also been investigated for siRNA delivery and imaging. QDs can be designed to have siRNA on their surface while also acting as imaging agents. This dual use enables scientists to visualize the biodistribution and cellular uptake of siRNA therapeutics in real time. Yet, their application is constrained by issues regarding heavy metal toxicity and long-term biocompatibility, which demand extensive surface modification and encapsulation in biocompatible coatings.

Inorganic nanoparticles provide versatile benefits for siRNA delivery, such as structural stability, multifunctionality, and responsiveness to stimuli. With considerable potential to enhance the specificity and efficiency of gene therapy, especially in cancer and respiratory diseases, more research needs to be conducted to adequately resolve their toxicity, biodegradability, and clinical applicability. Controlled surface engineering and the inclusion of targeting moieties can increase their safety and specificity, positioning them as potential candidates for future therapeutics.[24]

Lipid Based Vectors

Lipid-based vectors are delivery vehicles composed mainly of lipids and are widely applied in biotechnology and medicine to deliver genetic material (such as DNA or RNA), drugs, or other therapeutic agents into cells. They are particularly significant in gene therapy and mRNA vaccine technologies.[25]

Liposomes and Lipid Nanoparticle (LNPs)

Liposomes and lipid nanoparticles (LNPs) are two lipid-based delivery systems commonly applied in pharmaceutical and biomedical fields, especially for delivering therapeutic molecules into target cells. Though both share the same lipid nature, they differ remarkably in structure, functionality, and application. A case in point is Doxil, a liposomal preparation of doxorubicin employed in cancer treatment. Nonetheless, liposomes are prone to stability problems like fusion, leakage of the entrapped material, and fast clearance from the circulation unless chemically modified—e.g., by PEGylation, which serves to increase their circulation time.

Liposomes and lipid nanoparticles (LNPs) are two lipid-based delivery systems widely used in pharmaceutical and biomedical applications, particularly for the delivery of therapeutic molecules into target cells. Although both have the same lipid character, they differ significantly in structure, function, and use. Their amphiphilic nature allows them to encapsulate hydrophilic drugs (within the aqueous lumen) as well as hydrophobic drugs (within the lipid bilayer), hence prove to be potent carriers for drugs of a broad range. Due to their biocompatibility and structural similarity with cell membranes, liposomes have been extensively researched in drug delivery, particularly in the delivery of chemotherapeutic drugs. An example is Doxil, a liposomal formulation of doxorubicin used in oncology. However, liposomes are susceptible to stability issues such as fusion, leakage of the entrapped substance, and rapid clearance from the circulation unless chemically altered—e.g., through PEGylation, which acts to prolong their circulation time.

While both liposomes and LNPs consist of lipid constituents and share the aim of effective cargo delivery, their principles of design, modes of action, and applications in the clinic are different. Liposomes are preferred for drug delivery of small molecules, particularly in oncology,

whereas LNPs have emerged as the go-to platform for genetic therapeutics because of their enhanced capacity for encapsulation and protection of nucleic acids. The evolution of both systems has greatly progressed drug delivery and gene therapy, presenting optimistic solutions to formerly difficult-to-treat diseases.[26]

Advantages and Challenges

Liposomes and lipid nanoparticles (LNPs) are two sophisticated lipid-based drug delivery systems that have transformed the areas of pharmaceutical sciences and nanomedicine. Both systems possess unique strengths in therapeutic delivery, as well as particular limitations that need to be overcome to achieve their maximum clinical benefits. Liposomes can be chemically modified by the addition of polyethylene glycol (PEG) or targeting ligands, which increases their circulation time, decreases immune system recognition, and enables targeted delivery to particular tissues or tumor sites. These features have resulted in successful liposomal products like Doxil, a PEGylated liposomal formulation of doxorubicin for the treatment of cancer.

But liposomes are not without their problems. Their physical instability is a major concern: they are prone to aggregation, fusion, and leakage of the entrapped drug, particularly under storage or upon interaction with biological fluids. In addition, in the absence of suitable surface modification, they are cleared from the blood rapidly by the mononuclear phagocyte system (MPS) with considerable depletion of their bioavailability. Mass production of liposomes and maintaining consistency in particle size, encapsulation efficiency, and drug release profiles also pose technical and economic challenges.[27]

Conversely, lipid nanoparticles (LNPs) have appeared relatively more recently, generally to meet the challenge of the delivery of nucleic acid-based therapeutics, including mRNA, siRNA, and DNA. Structurally, LNPs are solid or semi-solid particles comprising ionizable lipids, which engage negatively charged nucleic acids under low pH to enable stable complexes. Upon entry into the body, these ionizable lipids assist in endosomal escape so that the therapeutic nucleic acids can gain access to the cytoplasm where they can perform their desired biological functions. In addition to ionizable lipids, LNPs also include helper lipids like cholesterol (for membrane stability), phospholipids (for structural integrity), and PEG-lipids (to increase circulation time and decrease immune clearance). One of the breaking points for LNP technology was the COVID-19 pandemic, in which the mRNA was delivered using the Pfizer-BioNTech and Moderna vaccines. These vaccines showed not just high efficacy and safety but also established LNPs as an efficient and scalable platform for nucleic acid delivery.

However, LNPs are also subject to various challenges. One major practical concern is the need for cold-chain logistics since many LNP formulations, especially those that contain mRNA, need to be kept very cold in order to maintain stability and functionality. This has caused distribution challenges, particularly in low-resource or hard-to-reach regions. Moreover, certain studies have pointed towards the possibility of immune responses, particularly against PEG-lipids, that can result in hypersensitivity or decreased efficacy after repeated dosing. The ionizable lipids employed in LNPs must also be optimally optimized since some formulations can result in cytotoxicity or initiate inflammatory reactions. Lastly, the intricate formulation procedure and challenge in fully characterizing LNPs owing to their dynamic and multi-component nature create regulatory and quality control issues during scale-up and development.

Whereas both liposomes and LNPs have enhanced the functionality of drug and gene delivery, they are appropriate for various forms of therapeutics and offer distinct sets of opportunities and challenges. Liposomes continue to be a great option for delivering small molecule drugs and some biologics, particularly where controlled release and biocompatibility are top concerns. In contrast, LNPs are the platform of choice for nucleic acid therapeutic delivery because of their enhanced stability, endosomal escape capability, and proven success in mass vaccination campaigns. Ongoing innovation in lipid chemistry, formulation science, and delivery modalities is needed to overcome their respective shortcomings and realize the full potential of these potent delivery technologies.[28]

Polymer Based Carriers

Polymer-based carriers are an extremely flexible family of drug and gene delivery vehicles that use natural or synthetic polymers to carry therapeutic drugs to the targeted tissue. The carriers have drawn wide interest based on their structural plasticity, tailorable properties, and capability for controlled release. They are engineered to undergo physiological responses toward pH, temperature, or redox potential as a means for site-specific delivery of drugs so that therapeutic effectiveness is increased but side effects minimized. Some such commonly used synthetic polymers include PLGA, polyethyleneimine (PEI), and poly(N-isopropylacrylamide) (PNIPAAm), whereas naturally occurring polymers like chitosan, alginate, and dextran are popular among them. These carriers are able to encapsulate a large variety of molecules, ranging from small therapeutic drugs to nucleic acids such as siRNA and plasmid DNA, and can be further targeted with ligands or PEG for increased targeting and circulation time. Perhaps the most well-known polymeric system is PLGA, which has been FDA-approved owing to its biodegradability and biocompatibility.[29,30]

Chitosan PEI Based Systems

Chitosan and polyethyleneimine (PEI) are the two leading non-viral vectors for drug and gene delivery based on their specific physicochemical characteristics. Chitosan, being a naturally occurring polysaccharide resulting from deacetylating chitin, provides superior biocompatibility, biodegradability, and mucoadhesiveness. Its cationic character allows it to create stable complexes with negatively charged biomolecules like DNA, RNA, and proteins, which shield them from enzymatic breakdown and allow them to be taken up by cells. In addition, chitosan's capability to open up tight junctions between epithelial cells makes it highly desirable for mucosal delivery routes like nasal, oral, and pulmonary administration. Nonetheless, its restricted solubility at physiological pH and relatively lower

transfection efficiency compared to synthetic polymers have limited its use, thus leading to extensive research on chemical modifications like quaternization, PEGylation, or thiolation in order to enhance its performance.

Conversely, PEI, a branched or linear cationic synthetic polymer, is generally considered to be among the most effective non-viral vectors of gene delivery owing to its excellent proton-buffering capacity—a phenomenon referred to as the "proton sponge effect." This allows PEI-DNA complexes to escape from the endosome following cellular uptake, resulting in increased transfection efficiency. Nonetheless, this efficacy tends to come at the expense of considerable cytotoxicity, particularly for highly branched or high-molecular-weight PEI variants, which can cause membrane disruption and apoptosis. Attempts to mitigate PEI toxicity without compromising transfection efficiency have resulted in the creation of PEGylated, biodegradable, or low molecular weight PEI derivatives, which better balance safety and efficacy. In spite of their shortcomings, both PEI and chitosan-based systems remain widely investigated in preclinical models and are used as reference platforms to establish next-generation non-viral delivery vectors for gene therapy, vaccines, and cancer chemotherapy drugs.[31]

Biodegradibility and Toxicity

Biodegradability and toxicity profiles of polymeric carriers like chitosan and polyethyleneimine (PEI) are key determinants of their potential for clinical use. Chitosan, a natural polysaccharide, is biodegradable by design, degrading to non-toxic, biocompatible oligomers and monomers (predominantly glucosamine) through enzyme-mediated hydrolysis by lysozymes and other hydrolases in the human body. Its degradation products are generally well tolerated, and chitosan is low in immunogenicity, thus being very favorable to be used in long-term biomedical applications, especially for mucosal and injectable systems. In addition, its biodegradability may be finely regulated through the adjustment of the extent of deacetylation and molecular weight, so that precise release kinetics and bioresorption rates may be controlled. By contrast, PEI, particularly high molecular weight or branched PEI, poses critical biocompatibility issues. Although PEI's high cationic charge density is advantageous for nucleic acid condensation and endosomal escape, it is also the primary cause of cytotoxicity because it can destabilize cellular membranes, produce reactive oxygen species (ROS), and cause mitochondrial damage resulting in cell apoptosis.

The polymer is non-biodegradable in its native state, which can lead to tissue accumulation and chronic toxicity if it is not cleared from the body. To mitigate these issues, recent studies have centered on the synthesis of biodegradable PEI derivatives, usually by the incorporation of ester, disulfide, or amide linkages into the polymer backbone. These alterations are intended to maintain the gene delivery efficiency with decreased toxicity by making the polymer degrade into smaller, less toxic fragments after delivery. Despite its drawbacks, PEI continues to be a gold standard for non-viral gene delivery, and efforts are still being made to balance efficacy and biocompatibility. On the other hand, chitosan's positive biodegradability profile and low toxicity still underpin its extensive application, although further improvements are still required to enhance its gene transfection efficiency to levels comparable to PEI.[32]

Viral Vectors

Viral vectors, particularly adenoviruses and lentiviruses, have long been studied for delivering therapeutic nucleic acids, like siRNA. These vectors provide important benefits because of their natural capacity to effectively transduce host cells, express high levels, and enable intracellular delivery of genetic material, such as siRNA that can be used to repress aberrant gene expression or regulate inflammatory processes in CF lungs. This is particularly valuable in the targeting of airway epithelial cells, which are normally non-dividing. Their application in CF has been hampered by high immunogenicity, causing inflammation and rapid clearance, particularly troublesome in CF patients already experiencing chronic airway inflammation. Furthermore, multiple dosing of adenoviral vectors is challenging due to the formation of neutralizing antibodies.[33]

Conversely, lentiviral vectors, which are HIV-derived, or siRNA, even in non-dividing cells like airway epithelia. This aspect is especially encouraging for CF, as it would allow sustained silencing of disease-promoting genes or increased CFTR expression. Additionally, lentiviruses have lower innate immunogenicity than adenoviruses and are thus more suitable for repeated administration. Nonetheless, their genomic integration is problematic in terms of insertional mutagenesis and possible oncogenesis, particularly for high-dose or non-targeted delivery. Moreover, lentiviral production and vector packaging are technically challenging and expensive and prove burdensome for large-scale clinical translation.

In general, although both viral vectors are therapeutic candidates for siRNA delivery in CF, both have unique trade-offs. Adenoviruses are highly efficient but induce robust immune reactions, while lentiviruses provide longer-lasting outcomes but present safety and scalability issues. Improved vector engineering, such as the creation of gutless adenoviruses and self-inactivating lentiviral systems, seeks to counteract these limitations and move viral vector-based gene therapy closer to clinical use in cystic fibrosis therapy.[34]

Exosomes Mediated Delivery

Exosomes, a form of extracellular vesicles (EVs) 30 to 150 nanometers in diameter, are an encouraging natural delivery system for RNA therapeutics, such as small interfering RNA (siRNA), microRNA (miRNA), and messenger RNA (mRNA). Secreted by most cell types, exosomes play a natural role in intercellular communication, carrying proteins, lipids, and nucleic acids from cell to cell. Their endogenous nature and biocompatibility provide them with a great advantage over man-made delivery systems, including decreased immunogenicity, increased stability in the bloodstream. Exosomes have intrinsic ability to target, as the surface proteins on their membrane can mirror the identity of their originating cells, possibly allowing tissue-specific delivery without heavy modification. To deliver RNA, exosomes can be modified to have exogenous RNA cargo either via electroporation, donor cell transfection, or

endogenous packaging pathways. This renders them good candidates to deliver therapeutic RNA to inaccessible tissues such as tumors, inflamed tissues, or neural cells in neurodegenerative disorders.[35]

Nevertheless, although exosome-mediated RNA delivery is very promising, there are a number of challenges to be overcome. One of the most significant constraints is that there is low yield and heterogeneity of exosome production, making standardization and scale-up for therapeutic use challenging. Additionally, loading therapeutic RNA into exosomes efficiently and reproducibly is technologically challenging, with available strategies sometimes leading to degradation or aggregation of the RNA. Moreover, while exosomes are less immunogenic than viral vectors, they remain capable of eliciting immune responses based on their cellular source and surface markers. Off-target effects and uncertain pharmacokinetics are also issues of concern. In spite of such challenges, investigation is progressing to optimize exosome engineering, purification, and targeting, positioning them as one of the most biologically advanced and promising RNA delivery tools in current nanomedicine.[36]

Targeting Stratergies for CF- Specific siRNA Therapy

Optimal siRNA drug delivery for cystic fibrosis (CF) involves passage through multiple physiological barriers, more significantly delivery to the airway epithelial cells that form the locus of CFTR gene pathophysiology. Amongst the most promising of approaches, ligand-receptor-mediated delivery achieves maximum specificity and receptor-bearing, siRNA-containing carrier accumulation by using overexpressed or disease-specific receptors in lung epithelium. For example, transferrin—a physiological iron-transport protein—has been used widely as a targeting ligand due to the overexpression of transferrin receptors (TfR) on damaged or proliferating epithelial tissues. By conjugating transferrin onto the particle surface of nanoparticles or liposomes, researchers can facilitate receptor-mediated endocytosis, which increases siRNA delivery and reduces off-target effects (Figure 3).[37]

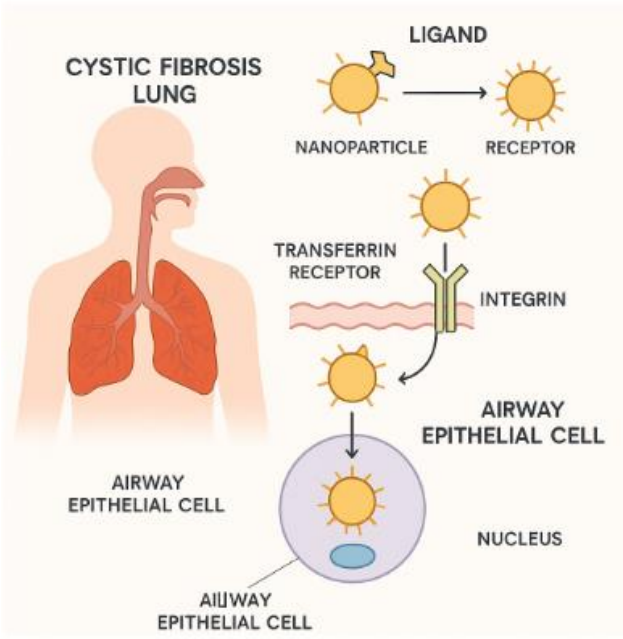

Figure 3: Targeted delivery mechanisms for CF lungs

Ligand- receptor Targeting

Ligand-receptor targeting is a highly selective and advanced method of improving the delivery of siRNA therapeutics, especially for conditions like cystic fibrosis (CF), where delivery to particular cell types—i.e., airway epithelial cells—is essential for therapeutic efficacy. This approach is based on surface decorating nanocarriers with molecular ligands capable of binding to overexpressed or disease-related receptors on the target cell surface, thus promoting active targeting and receptor-mediated endocytosis. Some of the most well-studied ligands include transferrin, a natural iron-binding glycoprotein, which binds to transferrin receptors (TfR). These receptors are present in higher numbers on most epithelial and immune cells in regenerating or inflamed tissues, states that commonly exist in CF-diseased lungs. Through the conjugation of transferrin to the surface of liposomes or polymeric nanoparticles, delivery systems can be targeted to TfR-rich cells, facilitating increased cellular uptake of siRNA via a natural endocytic pathway, while at the same time minimizing uptake by non-target tissues. This enhances the therapeutic index and reduces systemic exposure and off-target effects.

Aside from transferrin, integrin-binding ligands—particularly peptides with the RGD (Arg-Gly-Asp) motif—have been increasingly used in targeted delivery. Such peptides bind selectively to αvβ3 and αvβ5 integrins, which are often overexpressed on stressed, inflamed, or remodeling cells—frequent pathological characteristics of CF lungs. As presented on the surface of siRNA-loaded carriers, RGD motifs can facilitate preferential targeting and cellular uptake into lung epithelial cells or immune cells in inflamed tissues.

This targeted strategy not only increases cellular uptake but also facilitates navigation through physiological barriers, like the dense mucus layer that defines CF airways, by localizing delivery vehicles at or near their desired site of action. In addition, pairing these ligands with stealth modifications such as PEGylation can optimize mucus penetration with active targeting, enhancing both bioavailability and specificity. In general, ligand-receptor targeting—specifically through transferrin and integrin interactions—is a very customizable and potent platform for siRNA therapeutic delivery in CF, with the potential to overcome much of the difficulty that constrains traditional delivery methods.[38]

Surface Modification for Lung Epithelial Cell Targeting

Surface modification of nanocarriers has become a key strategy in improving the delivery of siRNA therapies to the lung epithelium, particularly in the treatment of diseases such as cystic fibrosis (CF). Due to the multifaceted microenvironment of CF lungs—thick mucus, chronic inflammation, and damage to epithelial cells—unmodified delivery systems tend to encounter major obstacles to effective cellular uptake and therapeutic impact. To overcome this, researchers have functionalized the surface of nanoparticles with functional groups or molecules that aid penetration of mucus, cell-specific adhesion, and

endosomal escape. PEGylation is one popular method of doing this by appending polyethylene glycol (PEG) onto the surface to enhance solubility and prevent premature clearance of the nanoparticle. Although PEG enhances permeability through the thick mucus barrier by decreasing mucoadhesion, it can be detrimental to cellular uptake as well. To counter this, cleavable PEG linkers have been developed which dissociate at the target tissue so that the carrier can better engage with the epithelial cell membrane.[39]

Aside from PEGylation, cell-penetrating moieties and targeted peptides have been employed to facilitate interaction with lung epithelial cells. Lung-homing peptides that have been identified using phage display libraries, for example, can be attached to nanoparticles to facilitate binding to receptors specifically expressed or upregulated on respiratory epithelial cells. Furthermore, cationic surface modifications (such as chitosan or polyethylenimine derivatives) can enhance interaction with negatively charged cell membranes, although these should be optimized with great care to prevent cytotoxicity. In recent advances, bioinspired coatings from lung extracellular matrix proteins or exosome membranes have also been investigated to replicate natural cellular interaction and enhance biocompatibility. Combined, these surface engineering approaches not only improve targeted delivery and transfection efficiency, but also provide a route toward reducing off-target effects, enhancing safety profiles, and ultimately enhancing the clinical viability of siRNA therapeutics in CF and other pulmonary disorders.[40]

Preclinical and Clinical Studies

The discovery of small interfering RNA (siRNA) drugs as potential therapeutics for the cure of cystic fibrosis (CF) and other respiratory disease conditions has seen remarkable advances during the past two decades, buoyed by expectations of very precise gene silencing and disease control. Much of this advance has been facilitated through massive preclinical work in models of disease-relevant lung biology and pathology, established to reproduce key features of human lung pathophysiology and disease pathologies. Notably, CFTR-knockout mice have been crucial in illustrating the potential of siRNA-loaded nanoparticles—particularly lipid-based carriers and cationic polymers—to effectively deliver genetic material to airway epithelial cells. These experiments have indicated decreased pro-inflammatory cytokines like IL-8 and TNF-α, enhanced chloride ion channel function, and diminished airway mucus blockage upon siRNA delivery. Nonetheless, although such murine models yield initial proof-of-concept information, their anatomical and physiological divergence from humans constrains their predictive power. Attention has therefore focused on larger and more physiologically relevant models like CFTR-deficient pigs, which display human-like airway formation, submucosal gland morphology, and mucus viscosity. In these models, aerosolized or intratracheally delivered siRNA has shown improved delivery to distal airways, bacterial load reduction, and ion transport mechanism restoration—providing strong clinical viability evidence.[41]

These studies showed that intranasal administration of siRNA was tolerable and effective in decreasing RSV replication and related pathology in the upper airway. Furthermore, siRNA candidates against fibrotic markers have been tested in idiopathic pulmonary fibrosis (IPF), again indicating the viability of siRNA-based pulmonary therapies. These initial clinical achievements emphasize the therapeutic value of RNA interference in respiratory disease settings, but they also call attention to the challenge of moving from preclinical promise to clinical use, especially for diseases such as CF that pose specific delivery challenges.[42]

One of the main challenges in transferring these therapies to CF patients is the unique pathophysiological environment of the human CF lung, which is very different from that of animal models. In CF, the airway surface liquid is dehydrated, and mucus is hyperviscous and adhesive, creating a strong barrier that restricts the diffusion and uniform deposition of inhaled nanoparticles. Additionally, long-term bacterial colonization and recurrent inflammation reconfigure the epithelial cell surface and immune environment, which could compromise nanoparticle-cell interactions and promote off-target effects or immune activation. Human lungs are also much larger and more complex than rodent or pig lungs, making it difficult to attain efficient, homogeneous delivery of siRNA throughout the airway tree. A second challenge is interpatient heterogeneity; CF patients have heterogeneous genotypes, severities of disease, and airway microbiota profiles, and thus it will be challenging to develop a single delivery system to fit all. Additionally, immune reactions to delivery vehicles such as cytokine release and complement activation need to be tightly regulated since materials that are inert in mice can cause reactions in human tissue. These issues are especially critical in CF, where the immune system is already pre-conditioned by chronic inflammation and infection.

In spite of these obstacles, continued research is continually optimizing siRNA formulations to improve mucus penetration, cellular uptake, and target specificity. Approaches like PEGylation with cleavable linkers, ligand-mediated targeting, and biodegradable polymers have been promising in preclinical models and are now being translated to human-compatible systems. New developments in aerosol delivery technologies—like vibrating mesh nebulizers and dry powder inhalers—also provide enhanced deposition in the lower airways while maintaining siRNA integrity. The inclusion of patient-specific disease modeling through the use of organoids or primary airway epithelial cultures further enhanced predictive reliability, contributing to the construction of personalized therapeutic plans. Even with no siRNA therapy for CF having advanced as far as late-stage clinical trials, however, the intersection of strong preclinical data, preliminary human trial information, and advancements in technology appears to indicate a promising avenue to pursue. The crucial element for successful translation is sustained interdisciplinary cooperation among molecular biologists, clinicians, material scientists, and regulatory

Table 1: Summary of clinical and preclinical siRNA-based studies in CF

Study type	Objective	Sirna target	Delivery system	Key findings
Preclinical	Correct CFTR function in CF models	CFTR mutant	Lipid- based nanoparticles	Partial restoration of CFTR function in CF airway epithelial cells
Preclinical	Suppress ENaC overactivation	Epithelial sodium channel	Polymer- based nanoparticles	Reduced sodium ion absorption, improved airway hydration
Preclinical	Target inflammatory pathways	IL-8 or NLRP3 inflammasome	Peptide based nanoparticles	Decreased inflammation and enhanced mucocilliary clearance
Preclinical	Inhibit bacterial biofilm formation	Quorum sensing pathways	Liposomal Sirna	Reduced biofilm density of pseudomonas aeruginousa
Clinical	Phase I safety evaluation of si RNA therapy	CFTR or ENaC	Inhalable nanoparticle- base si RNA	Demonstrated safety and tolerability ongoing efficacy evaluations
Clinical	Reduction of airway inflammation in CF	TNF-a	Antibody-conjugated siRNA	Initial results indicate reduced inflammatory markers in sputum
Clinical	Si RNA for polymicrobial lung infection	Pseudomonas aerginosa biofilm	Ph- sensitive nanocarriers	Reduced bacterial load and inflammation in CF patients sputum

specialists to overcome the complexities that now impede clinical advances (Table 1).[43]

Current Limitations and Future Perspective

Although siRNA therapies show considerable promise for treating cystic fibrosis, their translation from laboratories to regular clinical practice remains stalled by a number of sophisticated challenges. One of the biggest obstacles is the effective delivery of siRNA molecules into the lungs, especially in the case of the CF airway, where thickened mucus secretions, chronic inflammation, and bacterial colonization create an impenetrable barrier to drug delivery. Most delivery vehicles—like lipid nanoparticles or polymeric carriers—tend to be entrapped in this mucus or are unable to reach the target epithelial cells in adequate numbers. Next-generation drug delivery systems will need to address mucus penetration, specificity of targeting, and cellular uptake, perhaps by employing muco-inert coatings, biodegradable carriers, and inhalable formulations specific to diseased lungs. In addition, optimization of aerosol dispersion and deposition in the lungs will be critical, particularly with the variable anatomy and levels of obstruction within CF patients.[44]

Emerging personalized medicine approaches also form a strong trajectory for improving the efficacy of siRNA-based therapy. These technologies focus on designing treatments according to the patient's specific genetic make-up and presentation of disease. As an instance, the use of siRNA together with CRISPR-mediated gene editing might permit temporary suppression as well as long-term fixing of faulty genes such as CFTR. Similarly, hybrid delivery systems capable of delivering both mRNA to restore CFTR function and siRNA to reduce inflammation may provide a synergistic therapeutic effect. Platforms like patient-derived organoids, airway-on-chip devices, and high-throughput genetic screens are already utilized to forecast individual response to RNA therapies and inform treatment tailoring. Still, the sophistication of such systems creates further hurdles in terms of formulation design, dosing accuracy, and regulatory clearance.[45]

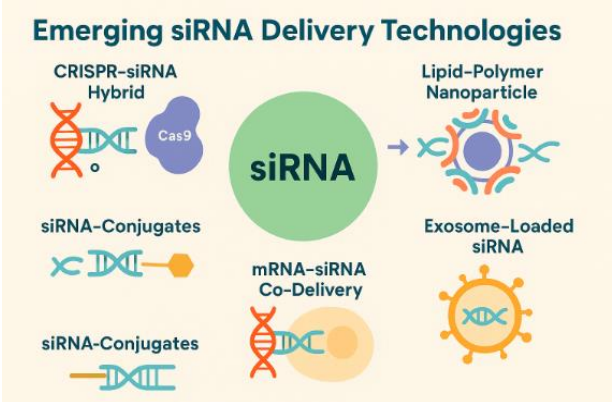

Figure 4: Emerging siRNA delivery technologies

Generally speaking, regulatory and ethical issues are the most pressing problems that have to be met with as the therapies evolve. Application of drugs based on RNA and gene editors provokes such concerns as long-term safety, off-target outcomes, and even risks of adverse unintended immune response. These worries are especially meaningful for pediatric patient groups, to which the vast majority of CF patients belong, as the long-term consequences of genetic treatments remain uncertain. Regulatory systems are continuing to adapt to the inclusion of such advanced therapies, and explicit guidance is required to facilitate clinical trials, maintain patient safety, and enable eventual licensure. Ethical issues regarding access, cost, and equity must also be considered, particularly to avoid disparities in who gains from these new technologies. Ultimately, the future of siRNA treatment for CF will depend not only on scientific and technological advancements but also on careful clinical translation and policy-making (Figure 4).[46]

CONCLUSION

Cystic fibrosis (CF) continues to be a life-shortening genetic disease due to mutations in the CFTR gene, with progressive respiratory compromise and complications elsewhere. Conventional treatments have improved survival but do not touch the underlying cause for most patients. The discovery of small interfering RNA (siRNA) presents a new and highly targeted approach to knockdown

disease-producing genes and alter the course of disease at the molecular level.

But the effective use of siRNA therapy in CF depends vitally on overcoming significant biological hurdles—namely, the dense pulmonary mucus layer, enzymatic breakdown, and cellular uptake constraints. Recent developments in delivery systems such as lipid nanoparticles, polymer-based vectors, viral and exosome-based carriers have demonstrated potential in preclinical models for enhancing siRNA stability, targeting, and efficacy.

Although early-phase clinical trials have shown safety and promise in analogous respiratory disorders, no siRNA-based CF therapy has reached regulatory approval to date. Continuing research into targeted delivery, personalized vectors, and combinational strategies (e.g., siRNA-CRISPR or mRNA co-delivery) provides a promising way forward.siRNA treatments are a revolutionary strategy for treating CF, yet their clinical adoption will depend upon sustained innovation in delivery technologies, extensive testing within CF-specific model systems, and overcoming regulatory obstacles. If the challenges can be overcome, the delivery systems of siRNAs could soon form part of the next generation, gene-targeted therapies for cystic fibrosis.

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