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Gene Therapy Avenues for Chronic Obstructive Pulmonary Disease

By: Jacob Calendine, Sesha Barta, Jude Cherfils, Omar Said and Vanloan Nguyen

Correspondence:
jakoboc21@gmail.com

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Abstract

Gene therapy is a growing field in research and development that may offer a long-lasting solution to several complex diseases, including chronic obstructive pulmonary disease (COPD). COPD is characterized by chronic inflammation in the lungs and the airways, leading to respiratory problems. COPD includes chronic bronchitis and emphysema. Optimizing treatments for gene therapy in COPD is of paramount importance given COPD's prominence as the fourth leading cause of disease-related death in the United States. We reviewed delivery methods in the current research, including liposomes, nanoparticles, electroporation, adeno-viruses, and adeno-associated viruses (AAV). The broad customizability in the diagnostic and treatment methods is evident in the recent studies. In this paper we explore each method and/or biomarker and evaluate several gene therapy avenues for COPD.

Background

Chronic obstructive pulmonary disease, also known as COPD, is characterized as a chronic inflammatory lung disease that causes obstructed airflow from the lungs. It includes multiple progressive lung diseases that affect millions of people yearly. In 2018, COPD was the fourth leading disease related cause of death in the United States, 6.4% of Americans were diagnosed with the disease, unfortunately, this number is likely higher due to undiagnosed cases[1].

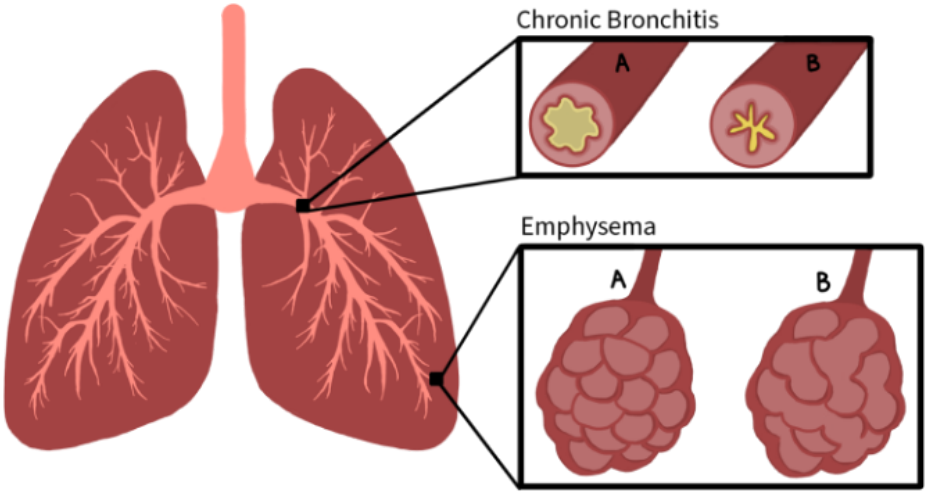

***Figure 1**: Diagram of the lungs and the effect chronic bronchitis and emphysema has on them. Boxed under chronic bronchitis are images of healthy (A) and unhealthy (B) bronchial tubes, (B) shows the inflammation and increased mucus in the unhealthy bronchial tubes. Boxed under emphysema are images of the alveoli, healthy (A) and unhealthy (B). In the unhealthy (B) image there is a membrane breakdown due to inhaled pollutants that causes larger sacs with a decreased surface area.*

COPD is not caused by a single gene or factor, this makes a single treatment for all patients less effective, and makes an appeal towards a more personalized treatment[1]. Two of its major contributors include emphysema and chronic bronchitis. Chronic bronchitis is characterized by the inflammation of the bronchial tube lining and excess mucus production, the bronchial tubes are responsible for carrying air to and from the air sacs (alveoli) within the lungs. Emphysema is a condition in which the alveoli membranes are destroyed due to chronically inhaled pollutants. The membrane breakdown creates larger air sacs rather than smaller ones,

this decreases the surface area and the amount of oxygen that can diffuse into the blood.

There are many factors that cause the development and progression of COPD, the main factors include cigarette smoke and work-related pollutants[2]. COPD is also considered to be inheritable, as there are genetic components that can play a role in disease development. The first gene identified to be associated with COPD was SEROINA, which encodes alpha1-antitrypsin (A1AT). Deficiencies in A1AT can lead to emphysema, but only 1-3% of COPD patients have the deficiency. Additionally, it has been recently discovered that altered miRNA expression in the lungs might also play a role in the COPD mechanism. The characteristic symptoms that define COPD include the following: breathing difficulty, coughing, wheezing, oxidant/antioxidant imbalance, emphysema (alveolar wall destruction), mucus hypersecretion, enhanced cytokines, chemokines, protease, and inflammation[3].

Current conventional therapeutic strategies of COPD utilize antioxidant and anti-inflammatory drugs[3]. These drugs tend to be bronchodilators, such as β-agonists and muscarinic antagonists, and inhaled corticosteroids, both of which are only used for short-term management. These treatments only target the patient's symptoms, and do not stop or reverse damage to the lungs[2]. Bronchodilators, such as β-agonists and muscarinic antagonists work to relax the muscles in the lungs to alleviate coughing and make breathing easier, whereas inhaled steroids reduce airway inflammation and help prevent exacerbations. Working towards a treatment for COPD that could prevent or reverse lung damage, would impact the lives of millions. Gene therapy is an attractive alternative to current treatments, it is able to deliver medications and therapeutics to specific target sites within the lungs. However, it's important to note that there are limitations due to various biological barriers. These limitations can include; off-target vector effects, genetic material, and delivery efficacy[1].

Due to the variety of infections causing diseases in the respiratory system, there are only 17 FDA approved treatments available for patients, none of which treat lung diseases[1]. ABECMA is an example of an approved gene

therapy that treats multiple myeloma, a disease where the body's white blood cells proliferate and cause harmful build ups throughout the body.

This specific gene therapy modifies the patient's T-cells to attack the cancerous white blood cells[4]. Another example includes Luxturna, a viral vector gene therapy form that treats congenital blindness. Most patients with congenital blindness contain a mutation in the RPE65 gene, which produces the chromophore 11-cis retinal protein that is vital for eye function. Luxturna works by delivering functional copies of the RPE65 gene to the retinal epithelial cells[5].

Cystic Fibrosis (CF) is a common candidate for gene therapy research and can offer some insight in the treatment of COPD as well. Due to defective sodium/potassium ion channels in the ciliated cells, the airway becomes dehydrated and starts secreting more mucus which is a breeding ground for infection-causing bacteria. Because of the fatality of the airway obstruction, doctors are researching to replace the CFTR gene during the neonatal period to maximize the success of the therapy and the patients' quality of life[6]. To deliver the CFTR gene into the lung, scientists have experimented with the oral inhalation of aerosolized vectors using a nebulizer rather than a liquid filled nasal tube. This method of delivery reduces the risk of aspiration and could be useful when treating other lung diseases, including COPD[6]. Modulator therapies are also burgeoning methods to treat CF, and each therapy targets a specific mutation in the CFTR gene. The success of the modulator therapy was shown in a research study when 55% of the subjects experienced significant decrease in *P.aeruginosa*[7].

Genes that contribute to COPD such as SERPINA1 that causes an α-1 antitrypsin deficiency would be a good candidate gene for potential modulator therapies with certain mutations in the gene[8]. Recent genetic, biochemical and histological evidence also suggests altered transforming growth factor beta (TGF-β) signaling is associated with COPD development and progression. TGF-β regulates the respiratory system and can lead to diseases if it is mutated. Subduing altered TGF-β signaling in the airways and alveolar sacs via gene silencing technologies may provide similar

therapeutic outcomes to modulator therapies and other treatment options[9].

Deliveries

The development in gene therapy for COPD hinges on several factors, one of the most prominent of which is delivery method to ensure efficacy and precision in use of the particular therapy. Delivery methods must optimize performance and transfection as well as safety both for the patient and protection of genetic material. Even in common inhalable drug delivery, limitations are present due to the complex defense mechanisms of the respiratory system. These factors are limited in efficacy and primarily focus on airway obstruction via anticholinergics and dual β2-dopamine 2 receptor antagonists[10].

Techniques used for gene therapy can be separated into 3 major categories: chemical, physical, and viral. Chemical techniques entail the non-viral methods for creating materials and particles used to transfect or insert genetic material into the target cells. This can be achieved in several ways whether it be to weaken the cell function or to create new vectors that take advantage of common cellular functions and mechanisms, such as liposomes and nanoparticles (NPs). Next, physical techniques revolve around mechanical methods to typically achieve new access points or alter the function of the cell slightly without introducing a chemical alteration or manipulation to the equation. Increasing the permeability and uptake of genetic material of the cell or direct injection is the most common mechanism in which these methods are able to insert the genetic material. The more prominent of these is a shock treatment to open the cell membrane for a brief period of time known as electroporation (EP). Thirdly, viral delivery methods are created by modifying viral genomes to take advantage of viral mechanisms of inserting genetic material. The most common vectors that have extended into the realm of gene therapy for pulmonary diseases are EP, liposomes, viral vectors, and NPs. Each of the aforementioned methods give way to their own advantages and problems; seen in *Figure* 2, thus validating the need for further research on gene therapy for symptoms of COPD. [JG1] Longevity and finding the ideal carrier to inhibit the effects of COPD using these treatments is necessary to

counteract the lifelong effects traditionally associated with it and other commonplace pulmonary diseases.

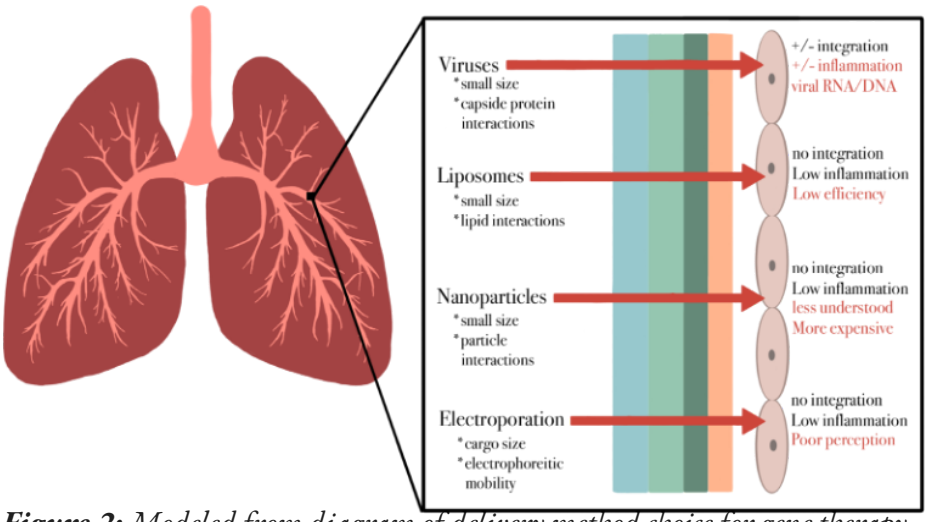

Figure 2: *Modeled from diagram of delivery method choice for gene therapy in the lungs [1]. The more generally observed shortcomings and advantages of each key delivery method to be discussed in detail. Individual modifications, especially in the case of liposomes and NPs, can be made to better these such as particle coating and more effective targeting mechanisms.*

Non-Viral Methods

Liposomes

Taking advantage of natural lipid bilayers to employ liposomes with genetic material, lipoplexes, for delivery cargo to cells utilizing endogenous functions of the cell membrane is one of the primary strategies in gene therapy for COPD. The most glaring functional advantage of lipofection as a technique is the lack of immune response and cytotoxicity upon transfection that has been observed[11,12]. In addition to this, low cost and ease of use make liposome based gene therapy a preferred treatment. Morphology which mimics that of cell membranes' lipid bilayer can be modified with targeting molecules to adhere to different cells with more precision and limited collateral damage. The accepted but not completely understood mechanism of action takes advantage of random Brownian motion of the liposomes upon entrance into the cell to effectively disperse the material[13]. In generic drug delivery this allows for seamless infiltration and dosing of the cell. However, the specificity of gene delivery

requires nuclear transport of the contained genetic material; these shortcomings can be observed through endocytosis inhibitors and endosome trackers to visualize delivery sites[14].

The endogenous nature of liposomes is massively important for pulmonary diseases and specifically COPD where constant inflammation and exacerbations take place regularly. A well observed phenomena since the 1970s, liposomal immunogenicity, can be built upon even further to trigger minimal immune responses. For example, liposomes for drug delivery that showed an inverse correlation with immunoglobulin (IgM) in the brain were modified to enhance absorption of IgM and thus the immune response and overall effectiveness of the treatment was improved[15]. Relative to the lungs, clinical trials have utilized lipoplexes in tandem with a plasmid carrying human CFTR in inhalable doses to limit mucus problems in patients with CF, a common comorbidity of COPD[16].

The benefits of these native characteristics of liposomes are significant but can present their own pitfalls as well which must be monitored. Natural mechanisms can act as impediments to treatments such as the bovine pulmonary surfactant Alveofact have been shown to weaken

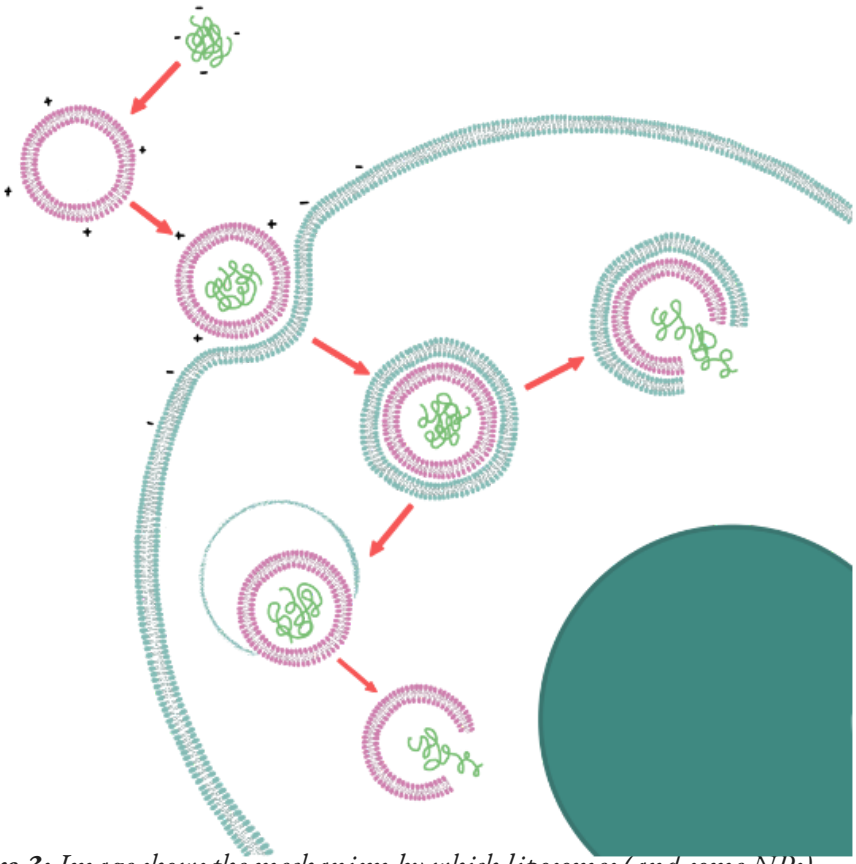

Figure 3: *Image shows the mechanism by which liposomes (and some NPs) can introduce genetic information into a cell by taking advantage of endocytosis, a natural cell mechanism, and limiting the immune response– thanks to the endogenous nature of the liposomal structure and makeup.*

several types of lipoplexes[17]. Overcoming these innate obstacles while maintaining the key benefits of lipoplex usage is of paramount importance and needs more research to fully understand the functionality in different cell types within the pulmonary system. Specific work with cells and the mechanisms of the pulmonary system will yield a better understanding of how lipofection can be used as a widespread treatment.

Nanoparticles

NPs (nanoparticles) are a massive and growing field of research for a number of possibilities including gene therapy for the lungs. Nanoparticles for gene therapy can be characterized by submicron sized particles that

optimize surface to volume ratios while maintaining good performance of biocompatibility and biodegradation[3]. The customizability of NPs for different cell types, degrees of accumulation, and degradation offer several advantages as carriers for gene therapy treatment in the pulmonary system. Taking advantage of endocytosis and permeability factors of cells in similar manner as lipoplex-based techniques, NPs can utilize similar lipid based qualities but are ultimately defined by size and their diverse makeup which extends beyond liposomes.

Targeting cells and material within the airways limits the abilities to penetrate into the tissue for many delivery methods. As mentioned, liposomes can encounter problems with penetrating surfactant and mucosal layers due to degradation and dissociation. Modifications can be made to NPs to resist mucoadhesion and trapping or degradation that may otherwise occur. One of the most common methods for this is a coating of polyethylene glycol (PEG) polymer to provide a hydrophilic and neutral coating to counteract traditional charge based dynamics used in synthesis[18]. It has been found that these PEG coatings provide varying levels of improvement in penetration of the thick mucus that exacerbates the issue of access to airway epithelial cells in patients with advanced COPD; degraded structures such as neutrophils and dense meshes of highly negative charged structures create a less permeable mucus layer[19,20].

These findings further the ability to overcome airway mucus, one of the primary issues in inhalable gene therapy to treat COPD and prevent significant worsening of symptoms.

PLGA

A candidate in gene therapy using NPs is that of poly ((d,l-lactide-co-glycolide) (PLGA)) based nanoparticles. PLGA-NPs are FDA and European Medicine Agency approved for drug delivery systems, not gene delivery. Good results have been demonstrated regarding biocompatibility that exceeds that of common liposome based treatments in transfection of HepG2 cells for gene delivery[21,22]. Surface modifications can be made to PLGA-NPs to enhance efficiency in performance such as the addition of polyethyleneimine (PEI) made by Bivas-Benita et al. to enhance performance in the pulmonary system given its merits as a gene delivery

vector; the particles were seen to escape the endolysosomal envelope and subsequent damage to continue release of material in some instances as well[23].

Dendrimers

A relatively unique NP structure that has gained exposure in gene therapy treatment given success in diagnostics is dendrimers. These NPs are based on the dendrimer polymers that exhibit a radially symmetric pattern of branching chains. This allows for many exposed ends at the surface that can be modified much like other NPs to optimize their function as a delivery method for genetic material. Hypotheses revolve around the potential for these large amounts of ionizable branches to be modified, most commonly utilizing poly-(amidoamine) and poly-(propyleneimine) as a base, or immediately paired with genetic material for testing[29]. To help in structure stability and formation the aforementioned PEG coating is applied to dendrimer NPs prior to material attachment[1]. Given the plentiful terminals, rapid absorption and dissipation of the NPs is another problem solved via these PEG coatings by enhancing retention by the lung tissue. The majority of the published work on dendrimer NPs in lung tissue gene therapy presents them in the context of this 'PEGylation" given the ability to control uptake with a much higher degree of precision than without[30,31]. Dendrimers share in the success of NPs as a whole in their continued research and have significant potential for delivery of gene therapy to inhibit COPD symptoms provided the state of knowledge continues to advance.

Electroporation

Electroporation (EP) differs from previously discussed delivery methods in its physical based mechanism of action rather than the chemical basis on which NPs and lipoplex therapies rely. It has emerged as one of the few physical methods researched for gene delivery given its safety and efficacy compared to others. EP takes advantage of an electric field applied across cells to alter permeability and allow previously injected genetic material to be more readily endocytosed. Transfection efficiency in tumors using EP has provided the foundation of knowledge for its usage in lung tissues. Intense

tissue analysis and/or electric field testing is required in most cases to ensure optimal settings given the natural variation in biological tissues[32]. Traditional chemical based approaches make up the vast majority of research due to customizability. However, as these fields move forward experiments have validated EP as a comparably effective method when physiological responses are considered in tandem with pure gene expression amongst cells[33].

Viral Methods

Viral vectors have been researched as avenues for gene therapy for years now given their development in the early 1970s and 80s and ability to take advantage of mechanisms native to the viruses themselves. The primary vector types found in modern gene therapy for COPD and lung tissue oriented studies are adenovirus and adeno-associated virus (AAV) based approaches. The differences in viral vectors and efficacy is contingent on the exact protein capsid and tissue tropism, the gene of interest for therapeutic purposes, and the alterations that control the gene expression for the vector[34]. These work together and can be chosen or altered to perfect one's vector for its desired purpose. Common problems brought about in viral vector usage despite modifications is the innate immune response. T-cell responses and toxicity within tissue is a cause of viruses that has developed to help the body respond. However, redesign and manipulation of these viral genomes and bodies can still carry markers to inhibit their expression when used as vehicles for gene therapy.

Adenoviruses

With regard to the aforementioned characteristics adenoviruses are a family of icosahedral nucleocapsid viruses. They are unenveloped and hence carry their genetic material, double stranded DNA, within this characteristic icosahedral chamber. Adenoviruses account for several acute respiratory illnesses and human adenoviruses (HAdVs) have a wide range of categorized species based on key characteristics that number over 80 types[35]. Work in gene therapy for COPD stems from HAdVs' versatile tissue tropism and prevalence in lung-related diseases and replication[36]. Imaging techniques

have revealed significantly higher carrying capacity for genetic material in adenoviruses than alternative viral vector platforms at around 36 kb pairs while delivering roughly 8 kb pairs. A capacity that can be increased using a newer generation of helper-dependent adenoviruses (HD-AdVs) by deleting the viral coding sequences[1,37-38]. Such delivery methods have been used in gene delivery to pig airway epithelia to demonstrate delivery of the CFTR protein in hopes to reverse mucus production and inflammation in patients with cystic fibrosis and associated lung diseases[39].

As prominent viruses in everyday life a common phenomena observed in usage of AdVs for clinical trials is pre-existing immunity [40] which presents the need for research of multiple HAdV serotypes to allow for versatility in practical usage as gene vectors. AdV types that rely on a singular method of cell entry have limited access points and see more pre-existing immunity. As previously mentioned, HAdVs types are plentiful and subcategorized into 7 grand species A-G, the majority of which belong to the HAdV-D family[40]. HAdV-D type 49 is one of many HAdV types that utilize varying surface molecules to enter the cell and as a result is a prime candidate for research in AdV vectors to solve this issue of immunity. The viability of HAdV-D49 is compounded by its observed transduction in lung and spleen tissues while showing reduced targeting in liver and other less desirable tissues when looking at in vivo biodistribution[41]. Immune response has also been mitigated by using HD-AdV vectors as their stripped down genome removes key markers for the immune system to identify and target. Airway basal cells of mice and pigs have been targeted successfully using these HD-AdVs following intranasal delivery[42,43]. Limiting immune response while maintaining a focus on the efficacy of AdV based gene therapy is the current objective that needs fine tuning in research for AdV vectors to obtain significant clinical success.

Adeno-associated viruses

To build upon the problem of immune response many relatively distant derivatives of AdV virus vectors have been created known as adeno-associated virus (AAV) vectors. These have more desirable immunogenicity. They evoke a weak inflammatory response compared to

AdV vectors by lacking a staple viral coding sequence and contain a linear single-stranded DNA. Behavioral differences in host integration led to its prevalence in research as an option for gene therapy. Carrying capacity in AAV vectors is limited in comparison to AdVs at typically less than 5 kb though reports have seen up to ~9 kb of genome efficiently incorporated into AAV vectors for therapeutics[44,45].

Increased packaging capacity typically comes at a cost of transduction rate and overall efficacy in AAV vectors though research continues to be done to improve the most pressing drawback of the delivery method. Researchers at Stanford have altered charges in the lumen of AAV variants known as AAV-DJ to transduce cells derived from human kidneys better than wildtypes when packed at a higher rate of up to 6.2 kb[46]. More akin to lung therapies for COPD, viral/AAV chimeric gene therapy has seen significant research in navigating human epithelial cells. Chimeric viral vectors look at combinations of multiple vector methods in hopes to take advantage of favorable characteristics in each and pose grounds for significant advancement in gene therapy for respiratory diseases given local cell selectivity. A type of chimera vector packaging plasmids using AAV and human bocaviruses (HBoV) been shown to work well in tandem with human airway epithelia, primary human hepatocytes, skeletal muscle cells, and T cells; in addition to good performance in extensive studies in ferret airway models more recently[47,48]. These AAV/HBoV combination vectors offer a novel approach to creating vectors for gene therapy in treatment of COPD.

Potential Treatment

There are many limitations to modern gene therapies used today caused by many biological barriers. [JG1] Most lung-related therapies must pass through multiple barriers such as the mucus, pulmonary surfactant, and local inflammation[24]. This could cause a problem since patients with diseases such as asthma and cystic fibrosis could also cause a biological barrier due to the airway mucus hypersecretion, which also plays a role in COPD, since around 50% of COPD patients have airway mucus hypersecretion[25].

Another limitation is when targeting JG3 cells other than the epithelium cells will cause the epithelium cells itself to become a major barrier[24].

Although there are several limitations to current gene therapies, there are other alternatives to target cells that could be considered in treatments. For example, epithelial cells, alveolar cells, and macrophages all can be accessed through inhalation of nucleic acid containing nanoparticles (NANs)[26]. This method of treatment helps target the lung and not target other cells that could cause biological barriers. Although inhalation of nanoparticles seems like a great treatment for lung disorders, it is actually limited in some COPD treatments. The inhalation of BIBW 2948, which is used for treating COPD, helped reduce the internalization of EFGR, which plays an important role in epithelial changes in COPD, but does not reduce the mucin storage[27,28].

BAMBI, which is the bone morphogenic protein and activin membrane-bound inhibitor, plays an important role in indicating if a patient has COPD. BAMBI are expressed stronger in COPD patients [28]. Also there is a correlation between the levels of BAMBI and plasma TGF -β1 levels. Therefore, the inhibition of TGF-β1 signaling might provide an alternative therapeutic strategy for treating COPD[28].

Inhale Gene Therapy

Another potential therapeutic treatment for COPD is through Inhaled Gene Therapy. This treatment provides direct access to the target of gene therapy for obstructive lung diseases via inhalation. There are limitations that surround inhale gene therapy. In a study conducted by Dr. Magdalena Humenberger from the Kepler University Hospital in Austria concluded that complete adherence to inhaled therapy was only seen in 33.6% and was higher among those with more severe COPD, based on these results[47]. It was shown that mucus poses a barrier for this specific treatment. Although this is a current problem, other studies have mentioned a different type of gene vector that could be inhaled that would penetrate that mucus barrier. A type of nanoparticle that was introduced by

Dr. Jung Soo Suk, from the The Center for Nanomedicine in the Johns Hopkins University School of Medicine, was the mucus-penetrating DNA nanoparticles (DNA-MPP), which possess non-adhesive coatings that allow them to rapidly penetrate mucus layers[48]. The PEG coating helps with the DNA-MPP treatment since it does not have a dense surface, helping the nanoparticle to penetrate other mucin-based meshwork[48].

Stem Cell Therapy

Another potential way of treating COPD is through stem cell therapy. This treatment is considered one of the newer treatments that still has a lot of questions floating around it. Although stem cell therapy is not considered safe, it still has potential when it comes to treating disorders such as COPD. Recently there have been several studies and clinical trials that have focused on stem cell treatment. The studies on COPD focused on a specific stem cell treatment called Mesenchymal Stem Cells (MSCs), which is important for making and repairing skeletal tissues[49]. In a clinical study, they were infused with expanded allogeneic umbilical cord tissue derived mesenchymal stem cells (MSCs) to 20 COPD patients and then were monitored for 6 months[49]. This study concluded a significant improvement in some important outcomes of COPD, including mMCR, CAT, number of symptoms, and the downregulation of inflammation[49]. Although this study is considered a pilot study, a small scale clinical study, it provides a unique perspective when it comes to treating COPD via stem cell therapy.

Alpha1-antitrypsin

Alpha1-antitrypsin (A1AT) is a protease inhibitor whose deficiency is most commonly associated with the ZZ mutation which causes abnormal folding in the ER of hepatocytes during biogenesis, causing its retention within the ER. The retention of 90% of the normal A1AT levels greatly increases the risk for early onset COPD[50]. In a large Lithuanian cohort, out of 1167 patients who had COPD ranging from moderate to severe, 3.4% had the MZ mutation, 3.3% had the MS mutation, 0.3% had the SZ mutation, and 0.7% had the ZZ mutations. Results from the screen indicated that there

was a significant increase in MZ, SZ and ZZ genotypes in COPD patients; it also supported the concept of using a targeted screen for A1AT deficiency when diagnosing COPD[51]. Another study conducted in Brazil on 926 COPD patients found that 2.8% had A1AT deficiencies and 0.8% had the ZZ mutation. These results also supported the importance of screening for A1AT levels in all COPD patients[52].

miRNA

miRNAs play an important role in lung development, homeostasis, and pathogenesis. They also play a role in the regulation of cellular response to inhaled toxins and in regulation of inflammatory and anti-inflammatory processes. miRNAs are one of the primary epigenetic modifiers that can affect gene expression through post-translational gene silencing and mRNA degradation. Their improper regulation can lead to chronic infections and inflammation. A study was done that obtained lung tissue samples from 15 COPD patients and 11 subjects with normal lung function. 12 differentially expressed miRNA in COPD patients compared to subjects with normal lung function were identified which showed to mostly target the nuclear lumen and transcription. Of the 12 differentially expressed miRNAs, miR-28-3p was most significantly down-regulated and miR-212-5p was most significantly up-regulated [53]. More recently, a study showed that two constructed miRNA-mRNA pathways; miR-126-5p and miR-130-5p-FOXO1 could be potential biomarkers for the diagnosis and treatment of COPD[54]. This year, a study showed that miR-126 was higher in COPD patients with acute exacerbation compared to stable COPD patients and healthy non-COPD patients and distinguished the groups. This led to the conclusion that the dysregulation of miR-126 relates to COPD susceptibility and acute exacerbation risk, but also is linked to severity and inflammatory cytokines in COPD patients[55].

Discussion

Chronic obstructive pulmonary disease provides unique grounds for exploration in new therapies. The compounding factors of several potential diseases or developmental miscues on a cellular level and beyond make it an issue to solve. However, this promotes much more *de novo* methods and research into the usage of several therapeutic methods in hopes to prevent such a prominent issue that 6.4% of Americans were diagnosed with it in 2018. Focus on lung tissue dynamics and genetic development can be accomplished in a number of ways through gene therapy is one of the most promising and has great potential in future endeavors[JG5] . As an overarching field, gene therapy is on the forefront of pulmonary medicine given its success in diagnosis, treatment, and identification in epidemiology of cystic fibrosis, emphysema, and other common issues which all act as compounding factors categorized as COPD[5-9].

Gene therapy works as a two pronged tool in lung therapy given the vast possibilities in the field between the potential delivery methods and actual genetic and cellular targets. The combination effect of these two issues leads to near endless possibilities in potential layouts for therapeutic strategies. In non-viral delivery methods, liposomes, nanoparticles, and electroporation are the more heavily researched aspects and provide a basis for most clinical studies[1,3,15]. Liposome based approaches and many potential nanoparticles limit immune response thanks to their endogenous nature and customizable size to desired scale yet the key difference comes when one looks at the efficiency. Liposomes and liposomal NPs sacrifice ease of use and optimal immunogenicity for efficiency in many cases due to the body naturally developing barriers to them such as nuclear transport of their genetic material and significant degradation before they can have a significant effect on the target[15,17]. Non-liposomal NPs are the most wide-open area of research given the plethora of potential formulations.

PLGA and dendrimer nanoparticles for lung therapy have seen success given their idealized interaction. Yet, price points become an issue when one looks at the extensive research and time that must be put into each individual iteration of a given treatment method[22,30]. For example,

taking a simple PLGA coated nanoparticle with targeting sites to deliver to lung epithelia for production in A1AT vs. the exact same base particle complemented to target ciliated cells will require entirely different sets of trials and FDA compliance before any significant levels of usage can occur. This is obviously necessary for safety and ethical concerns but presents a significant holdup in advancement of NP as a therapy for COPD. Electroporation uses the manipulated electric fields in cells to increase permeability significantly. Targeting specificity and overall quality of research eludes the field but it presents a purely physical method for gene therapy delivery and even assisting other methods should cell access present a significant issue[1,33]. Viral methods for delivering gene therapy are composed of mostly adeno and adeno-associated viruses given the broad spectrum they offer for behavior and ease of modification[35]. Both offer one of the more sought after qualities in gene therapy strategies in their integration abilities. The inherent function of viruses makes them ideal vectors that just need to be altered for the desired function. Immune response is an issue that can be improved upon but presents one of the only significant issues in their usage as gene vectors[44].

Targets for the readily available delivery methods is where the specificity of COPD comes to the forefront of treatment. Inhalable therapies provide a unique avenue for treating pulmonary issues that might not be possible with other groups of diseases, thus it has been explored heavily with iterations of liposomal NP and viral vector bodies optimized for breaking through the heavy mucus and surfactant layers in lung tissues[17,42-43,48]. A variety of cells are still accessible through this minimally invasive gene therapy[26]. New stem cell therapy for COPD focus primarily on mesenchymal stem cells given their limited exhibition of pluripotent properties. One of the most well researched causes and potential targets for COPD is that of A1AT where gene alteration to the common ZZ mutation or to improve production in A1AT deficient individuals has been seen to improve both physiological qualities and provided long term expression[50-52]. The final but potentially most broad method for focusing on the issues presented in COPD is that of working with miRNA using the aforementioned delivery methods. As a primary regulator for gene expression, in depth studies have found disparities in miRNA quality in

COPD patients[53]. Insertion of NPs to change miRNA expression for issues like lung irritation have also seen success[56]. Looking at these delivery methods in tandem with targeting goal is the only way to effectively evaluate the viability of a therapeutic strategy for COPD and look to improve the current state of understanding in treatment for it.

Conclusion

Although there are many potential treatment avenues, there needs to be a large focus on personalized medicine as the source of patients' COPD can vary. Severity and associated symptoms are plentiful in COPD which only serves to necessitate more research into the field. Making strides towards being able to identify the best suited treatment for an individual will result in the most effective outcomes and treatments. The demonstration of effectiveness in both specific cell targets in vitro as well as delivery methods must come together to formulate idealized gene therapy treatments. Issues present themselves in all areas discussed though they remain the primary modes of treatment in modern medicine. Understanding the subject and dynamics of both the disease and methods used is necessary to maintain good quality of care and building upon these to improve treatment of COPD.

References

1. Baliga UK, Dean DA. Pulmonary gene delivery-Realities and possibilities. *Exp Biol Med (Maywood)*. 2021;246(3):260-274. doi:10.1177/1535370220965985

2. Kim N, Duncan GA, Hanes J, Suk JS. Barriers to inhaled gene therapy of obstructive lung diseases: A review. *J Control Release*. 2016;240:465-488.

3. Passi M, Shahid S, Chockalingam S, Sundar IK, Packirisamy G. Conventional and Nanotechnology Based Approaches to Combat Chronic Obstructive Pulmonary Disease: Implications for Chronic Airway Diseases. *Int J Nanomedicine*. 2020;15:3803-3826. Published 2020 May 28. doi:10.2147/IJN.S242516

4. Voelker R. Cell-Based Gene Therapy Is a New Option for Multiple Myeloma. *JAMA*. 2021;325(17):1713. doi:10.1001/jama.2021.6401

5. Narfström, K., Vaegan, Katz, M. *et al.* Assessment of Structure and Function Over a 3-year Period after Gene Transfer in RPE65−/− dogs. *Doc Ophthalmol* 111, 39–48 (2005). https://doi.org/10.1007/s10633-005-3159-0

6. Flotte, T., Laube, B. Gene Therapy in Cystic Fibrosis. *CHEST*. 2001;3;124S-131S. https://doi.org/10.1378/chest.120.3_suppl.124S

7. Saiman L, Cacalano G, Prince A. Pseudomonas cepacia adherence to respiratory epithelial cells is enhanced by Pseudomonas aeruginosa. *Infect Immun*. 1990;58(8):2578-2584. doi:10.1128/iai.58.8.2578-2584.1990

8. Kim WJ, Lee SD. Candidate genes for COPD: current evidence and research. *Int J Chron Obstruct Pulmon Dis*. 2015;10:2249-2255. Published 2015 Oct 19. doi:10.2147/COPD.S80227

9. Afzal S, Ramzan K, Waqar AB (2020). Alternative approaches for the treatment of Asthma and COPD: Focus on Cell-based therapies, Epigenetics, and Gene silencing approaches. *Adv. Life Sci.* 7(3): 181-189.

10. Liu et al., Cationic liposome-mediated intravenous gene delivery. *J. Biol. Chem.*, 270 (1995), pp. 24864-24870

11. Balazs DA, Godbey W. Liposomes for use in gene delivery. *J Drug Deliv*. 2011;2011:326497. doi:10.1155/2011/326497

12. Cardarelli F, Digiacomo L, Marchini C, et al. The intracellular trafficking mechanism of Lipofectamine-based transfection reagents and its implication for gene delivery. *Sci Rep*. 2016;6:25879. Published 2016 May 11. doi:10.1038/srep25879

13. Yang J, Bahreman A, Daudey G, Bussmann J, Olsthoorn RC, Kros A. Drug Delivery via Cell Membrane Fusion Using Lipopeptide Modified Liposomes. *ACS Cent Sci*. 2016;2(9):621-630. doi:10.1021/acscentsci.6b00172

14. Hao L, Wu Y, Zhang Y, et al. Combinational PRR Agonists in Liposomal Adjuvant Enhances Immunogenicity and Protective Efficacy in a Tuberculosis Subunit Vaccine. *Frontiers in Immunology*. 2020;11. https://www.frontiersin.org/article/10.3389/fimmu.2020.575504

15. Griesenbach U, Pytel KM, Alton EW. Cystic Fibrosis Gene Therapy in the UK and Elsewhere. *Hum Gene Ther*. 2015;26(5):266-275. doi:10.1089/hum.2015.027

16. Ernst, N., Ulrichskötter, S., Schmalix, W.A., Rädler, J., Galneder, R., Mayer, E., Gersting, S., Plank, C., Reinhardt, D. and Rosenecker, J. (1999), Interaction of liposomal and polycationic transfection complexes with pulmonary surfactant. *J. Gene Med.*, 1: 331-340. https://doi.org/10.1002/(SICI)1521-2254(19 9909/10)1:5<331::AID-JGM60>3.0.CO;2-8

17. Chen D, Liu J, Wu J, Suk JS. Enhancing nanoparticle penetration through airway mucus to improve drug delivery efficacy in the lung. *Expert Opin Drug Deliv.* 2021;18(5):595-606. doi:10.1080/17425247.2021.1854222

18. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev*. 2009;61(2):75-85. doi:10.1016/j.addr.2008.09.008

19. Tosi G., Bortot B., Ruozi B., Dolcetta D., Vandelli M.A., Forni F. and Severini G.M., Potential Use of Polymeric Nanoparticles for Drug Delivery Across the Blood-Brain Barrier, *Curr Med Chem.* 2013; 20(17). https://dx.doi.org/10.2174/0929867311320170006

20. Liang, G.F., Zhu, Y.L., Sun, B. et al. PLGA-based gene delivering nanoparticle enhance suppression effect of miRNA in HePG2 cells. *Nanoscale Res Lett.* 6, 447 (2011). https://doi.org/10.1186/1556-276X-6-447

21. Bivas-Benita M, Romeijn S, Junginger HE, Borchard G. PLGA–PEI nanoparticles for gene delivery to pulmonary epithelium. *Euro J Pharma Biopharm*. 2004;58(1):1-6. doi:https://doi.org/10.1016/j.ejpb.2004.03.008

22. Baliga UK, Dean DA. Pulmonary gene delivery-Realities and possibilities. *Exp Biol Med* (Maywood). 2021;246(3):260-274. doi:10.1177/1535370220965985

23. Shen Y, Huang S, Kang J, et al. Management of airway mucus hypersecretion in chronic airway inflammatory disease: Chinese expert consensus (English edition). *Int J Chron Obstruct Pulmon Dis.* 2018;13:399-407. Published 2018 Jan 30. doi:10.2147/COPD.S144312

24. Sanders N, Rudolph C, Braeckmans K, De Smedt SC, Demeester J. Extracellular barriers in respiratory gene therapy. *Adv Drug Deliv Rev.* 2009;61(2):115-127. doi:10.1016/j.addr.2008.09.011

25. de Boer, W. I., Hau, C. M., van Schadewijk, A., Stolk, J., van Krieken, J. H., & Hiemstra, P. S. (2006). Expression of epidermal growth factors and their receptors in the bronchial epithelium of subjects with chronic obstructive pulmonary disease. *Am J Clin Path*. 125(2), 184–192. https://doi.org/10.1309/W1AX-KGT7-UA37-X257

26. Wang, C., Zhou, J., Wang, J. et al. Progress in the mechanism and targeted drug therapy for COPD. *Sig Transduct Target Ther.* 5, 248 (2020). https://doi.org/10.1038/s41392-020-00345-x

27. Khan OF, Zaia EW, Jhunjhunwala S, et al. Dendrimer-Inspired Nanomaterials for the in Vivo Delivery of siRNA to Lung Vasculature. *Nano Lett.* 2015;15(5):3008-3016. doi:10.1021/nl5048972

28. Ryan GM, Kaminskas LM, Kelly BD, Owen DJ, McIntosh MP, Porter CJ. Pulmonary administration of PEGylated polylysine dendrimers: absorption from the lung versus retention within the lung is highly size-dependent. *Mol Pharm.* 2013;10(8):2986-2995.

doi:10.1021/mp400091n

29. Bharatwaj B, Mohammad AK, Dimovski R, et al. Dendrimer nanocarriers for transport modulation across models of the pulmonary epithelium. *Mol Pharm.* 2015;12(3):826-838. doi:10.1021/mp500662z

30. Cemazar M, Golzio M, Sersa G, et al. Control by pulse parameters of DNA electrotransfer into solid tumors in mice. *Gene Ther*. 2009;16(5):635-644. doi:10.1038/gt.2009.10

31. Young JL, Dean DA. Chapter Three - Electroporation-Mediated Gene Delivery. In: Huang L, Liu D, Wagner E, eds. *Adv Gen*. Vol 89. Academic Press; 2015:49-88. doi:10.1016/bs.adgen.2014.10.003

32. Bulcha, J.T., Wang, Y., Ma, H. et al. Viral vector platforms within the gene therapy landscape. *Sig Transduct Target Ther.* 6, 53 (2021). https://doi.org/10.1038/s41392-021-00487-6

33. Hage E., Gerd Liebert U., Bergs S., Ganzenmueller T., Heim A. (2015). Human mastadenovirus type 70: a novel, multiple recombinant species D mastadenovirus isolated from diarrhoeal feces of a haematopoietic stem cell transplantation recipient. *J. Gen. Virol.* 96, 2734–2742. 10.1099/vir.0.000196

34. Campos SK, Barry MA. Current advances and future challenges in Adenoviral vector biology and targeting. *Curr Gene Ther.* 2007;7(3):189-204. doi:10.2174/156652307780859062

35. San Martín C. Latest insights on adenovirus structure and assembly [published correction appears in Viruses. 2012;4(12):3952]. *Viruses.* 2012;4(5):847-877. doi:10.3390/v4050847

36. Lee D, Liu J, Junn HJ et al. No more helper adenovirus: production of gutless adenovirus (GLAd) free of adenovirus and replication-competent adenovirus (RCA) contaminants. *Exp Mol Med.* 2019;51:1–18. https://doi.org/10.1038/s12276-019-0334-z

37. Cao H, Machuca TN, Yeung JC, et al. Efficient gene delivery to pig airway epithelia and submucosal glands using helper-dependent adenoviral vectors. *Mol Ther Nucleic Acids.* 2013;2(10):e127. Published 2013 Oct 8. doi:10.1038/mtna.2013.55

38. Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important?. *Hum Vaccin Immunother.* 2014;10(10):2875-2884. doi:10.4161/hv.29594

39. Bates EA, Counsell JR, Alizert S, Baker AT, Suff N, Boyle A, Bradshaw AC, Waddington SN, Nicklin SA, Baker AH, Parker AL. In Vitro and In Vivo Evaluation of Human Adenovirus Type 49 as a Vector for Therapeutic Applications. *Viruses*. 2021;13(8):1483. https://doi.org/10.3390/v13081483

40. Bandar, RA, Chen ZR & Hu J. Potential of helper-dependent Adenoviral vectors in CRISPR-cas9-mediated lung gene therapy. *Cell Biosci.* 2021;11(145). https://doi.org/10.1186/s13578-021-00662-w

41. Cao H, Ouyang H, Grasemann H, et al. Transducing Airway Basal Cells with a Helper-Dependent Adenoviral Vector for Lung Gene Therapy. *Hum Gene Ther.* 2018;29(6):643-652. doi:10.1089/hum.2017.201

42. Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. *Mol Ther.* 2010;18(1):80-86. doi:10.1038/mt.2009.255

43. Allocca M, Doria M, Petrillo M, et al. Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice. *J Clin Invest.* 2008;118(5):1955-1964. doi:10.1172/JCI34316

44. Tiffany M, Kay MA. 253. Expanded Packaging Capacity of AAV by Lumenal Charge Alteration. *Mol Ther.* 2016;24:S99-S100. doi:10.1016/S1525-0016(16)33062-3

45. Fakhiri J, Schneider MA, Puschhof J, et al. Novel Chimeric Gene Therapy Vectors Based on Adeno-Associated Virus and Four Different Mammalian Bocaviruses. *Mol Ther Methods Clin Dev*. 2019;12:202-222. Published 2019 Jan 18. doi:10.1016/j.omtm.2019.01.003

46. Humenberger, M., Horner, A., Labek, A. *et al.* Adherence to inhaled therapy and its impact on chronic obstructive pulmonary disease (COPD). *BMC Pulm Med.* 2018;18(163). https://doi.org/10.1186/s12890-018-0724-3

47. Ensign LM, Schneider C, Suk JS, Cone R, Hanes J. Mucus penetrating nanoparticles: biophysical tool and method of drug and gene delivery. *Adv Mater.* 2012;24(28):3887-3894. doi:10.1002/adma.201201800

48. Broekman W, Khedoe PPSJ, Schepers K, Roelofs H, Stolk J, Hiemstra PS. Mesenchymal stromal cells: a novel therapy for the treatment of chronic obstructive pulmonary disease?. *Thorax.* 2018;73(6):565-574. doi:10.1136/thoraxjnl-2017-210672

49. Broekman W, Khedoe PPSJ, Schepers K, et al. Mesenchymal stromal cells: a novel therapy for the treatment of chronic obstructive pulmonary disease? *Thorax.* 2018;73:565-574. https://doi.org/10.1136/thoraxjnl-2017-210672

50. Sitkauskiene B, et al. Screening for alpha1-Antitrypsin Deficiency in Lithuanian Patients with COPD. Resp Med. 2008;102(11):1654–1658. https://doi.org/10.1016/j.rmed.2008.07.003.

51. Russo R, Zillmer LR, Nascimento OA, et al. Prevalence of alpha-1 antitrypsin deficiency and allele frequency in patients with COPD in Brazil. *J Bras Pneumol*. 2016;42(5):311-316. doi:10.1590/S1806-37562015000000180

52. Kim WJ, et al. Altered MIRNA Expression in Lung Tissues of Patients with Chronic Obstructive Pulmonary Disease. *Mol & Cell Tox*. 2017;13(2):207–212. https://doi.org/10.1007/s13273-017-0022-9

53. Zhu M, Ye M, Wang J, Ye L, Jin M. Construction of Potential miRNA–mRNA Regulatory Network in COPD Plasma by Bioinformatics Analysis. *Int J Chron Obstruct Pulmon Dis.* 2020;15:2135-2145. https://doi.org/10.2147/COPD.S255262

54. Wang C, et al. Dysregulated Circulating Microrna-126 in Chronic Obstructive Pulmonary Disease: Linkage with Acute Exacerbation Risk, Severity Degree, and Inflammatory Cytokines. *J Clin Lab Ana*. 2002;36(3). https://doi.org/10.1002/jcla.24204

55. Bobba CM, Fei Q, Shukla V, et al. Nanoparticle delivery of microRNA-146a regulates mechanotransduction in lung macrophages and mitigates injury during mechanical ventilation. *Nat Commun*. 2021;12(289). https://doi.org/10.1038/s41467-020-20449-w