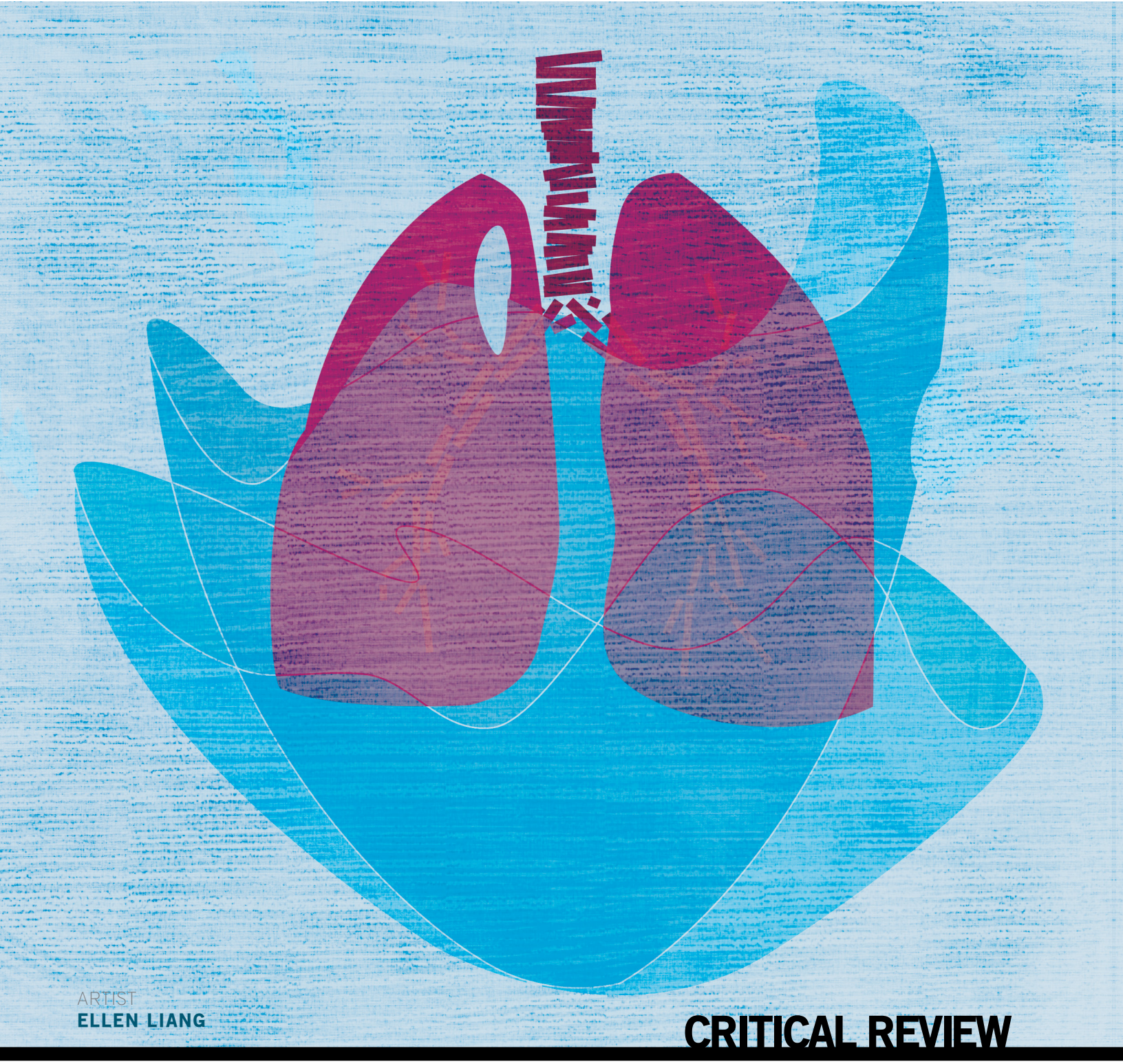

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CRITICAL REVIEW

Potential Therapies for Cystic Fibrosis

A LOOK AT CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR CORRECTORS AND POTENTIATORS

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ABSTRACT

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that is caused by a mutation of the anion channel termed the cystic fibrosis transmembrane conductance regulator (CFTR). Treatment for CF has improved over time; however, many therapies focus only on alleviating symptoms as they occur. There have been recent advances in therapies that repair mutant CFTR, including the administration of small molecules called CFTR modulators. CFTR modulators fall under two categories: correctors that prevent early degradation of CFTR and potentiators that increase conductance of existing CFTR. This review discusses the advantages of repairing mutant CFTR, some recent advances in treatment development, and the future of CFTR modulator therapy.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that was first documented in the 1930s by Dorothy Hansine Andersen.[1] CF is most prominent in Caucasians, with other ethnic groups being affected at lower incidence rates. In Europe, the incidence of CF is one in every 2000-3000 neonates, and in Canada nearly 4000 individuals are affected by the disorder.[2,3] CF is caused by mutation to the cystic fibrosis transmembrane conductance regulator (CFTR) gene which is located on chromosome 7 and encodes the anion channel, CFTR.[2] This channel is found in secretory epithelial cells of various organs including the lung, pancreas, and reproductive tract.[4] CFTR utilizes the energy of adenosine triphosphate (ATP) to allow for anions such as chloride and bicarbonate to flow down their electrochemical gradient.[5] Clinical symptoms of cystic fibrosis occur when a mutation alters the function of CFTR, preventing proper anion movement.

The classic clinical manifestation of CF is reduced airway mucociliary clearance, resulting in chronic sinopulmonary infection by pathogens including *Pseudomonas aeruginosa* and *Burkholderia cepacia*. These infections cause chronic coughing and sputum production resulting in airway obstruction.[6] CF mutations also affect most exocrine glands, leading to impaired function of the pancreas, intestine, liver and bile duct.[4]

In the past 50 years, there have been important developments in the treatments that improve the survival and quality of life of CF patients. These treatments have increased the median survival age in the United States from ~28 in 1991 to 36.8 in 2011, and the hope is that it will be common for children born with CF in the 21st century to live beyond 50 years of age.[7] However, the purpose of many current CF treatments is only to alleviate symptoms. To improve survival of CF patients, future treatments must address the root cause of the disease: mutant CFTR. This review will explore CFTR modulators, specifically correctors and potentiators, which are molecules that can "repair" mutant CFTR.

GENETICS OF CFTR

The CFTR gene, which spans approximately 230 kb, was first identified in 1989. Since then, approximately 2000 variants have been identified.[2,8] CFTR mutations can have differing effects on the CFTR protein and create varying phenotypes; however, these mutations usually fall in one of six classes (Table 1).[9] The most common variant of CFTR is ΔF508, a Class II mutation which accounts for 66-70% of mutations.[4] ΔF508 is a deletion of the amino acid phenylalanine at position 508. This deletion causes CFTR to become mislocalized in the endoplasmic reticulum, preventing the protein from attaining a mature conformation. Without this conformation, the ΔF508 CFTR protein rapidly degrades through an ubiquitin-proteasome pathway.[10]

The product of the CFTR gene is a 1480-amino-

TABLE 1: Classification system for CFTR mutations

CLASS OF MUTATION	EFFECT ON PROTEIN	CF PHENOTYPE
I	Defective protein synthesis	Severe phenotype from reduced functional CFTR
II	Abnormal trafficking from improper conformation	Severe phenotype from reduced functional CFTR
III	Defective regulation; prevention of ATP binding	Severe phenotype from a normal amount of non-functional CFTR
IV	Decreased conductance	Mild phenotype from a normal amount of reduced functioning CFTR
V	Reduced synthesis and trafficking	Mild phenotype from a reduced amount of normal functioning CFTR
VI	Decreased stability	Severe phenotype from minimal functional CFTR

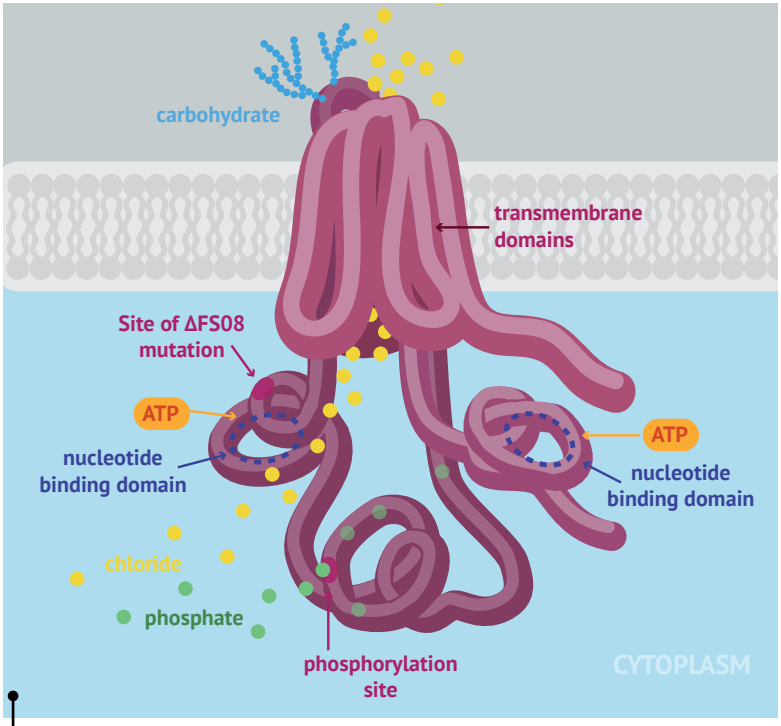

FIGURE 1: A diagram displaying the current understanding of the structure of CFTR protein.[4]

acid-long polypeptide chain.[11] The CFTR structure consists of two transmembrane domains (TMD1/2), two nucleotide binding domains (NBD1/2), and a regulatory domain with multiple phosphorylation sites (Figure 1). Channel opening is thought to occur when the regulatory domain of CFTR is phosphorylated by protein kinase A. After phosphorylation, ATP is recruited to NBD1 and NBD2. These domains dimerise (NBD1:NBD2) to open the channel pore. Upon subsequent ATP hydrolysis the NBDs dissociate, thereby forcing CFTR into a closed conformation. This entire process is called "gating."[2,11] Knowledge regarding the structure of CFTR is important as it allows researchers to develop molecules that can be targeted to fix specific mutations.

THE IMPORTANCE OF REPAIRING MUTANT CFTR

Treatments that can directly correct mutant CFTR and restore anion channel function are important as they directly prevent manifestations of CF. For example, a treatment that repairs CFTR would theoretically restore airway ciliary functioning, thereby effectively reducing or eliminating the probability of developing a pathogenic infection, a primary cause of respiratory failure in CF patients.[11] When pathogenic infections occur, the immune system releases neutrophils in an attempt to remove the infection. However, these neutrophils also indiscriminately destroy lung tissue such as the muscular and elastic portions of the bronchi, which creates potential for further infection and loss of lung function.[12] Treatments that deal solely with the symptoms of pathogenic infection, like antibiotics, may not be able to prevent some of the initial lung damage. Additionally, these treatments do little to prevent symptoms from recurring and, in the case of infection, further reduction in lung function. Thus, it is important that future therapies directly target mutant CFTR to prevent CF symptoms from initially occurring.

THE IDEAL CFTR-REPAIRING DRUG

When repairing mutant CFTR, two important questions must be asked: How much CFTR needs to be repaired in order to see therapeutic benefits and how will such a treatment be administered? To address the former question, Zhang et al. looked to restore CFTR function in ΔF508 cells by delivering a normal copy of CFTR through use of a human parainfluenza virus vector. They demonstrated that to restore near-normal mucus transport, non-mutant CFTR must be delivered to approximately 25% of surface epithelial cells.[13] This figure is debated as some early trials of CFTR-repairing drugs have shown that *in vitro* potency does not necessarily correlate with therapeutic benefits.[14] Thus, results from the Zhang et al. study should, at most, be regarded as a benchmark.

To answer the latter question about treatment administration, one must look at the ideal profile of a pharmacological agent. The ideal drug can be orally administered, has high potency, and displays minimal side effects.[4] CFTR-repairing drugs or CFTR modulators have been shown to display this profile and, therefore, could have strong therapeutic potential.

CFTR MODULATORS

CFTR modulators can be broadly defined as small molecules that target and repair specific defects caused by mutations to the CFTR gene. CFTR modulator drugs can either have corrector or potentiator capabilities.[2,11]

CFTR correctors allow for mutated CFTR proteins, which would normally be degraded prior to embedding in the cell membrane, to be trafficked to the cell surface. The mechanism of CFTR correctors is unique to the specific mutant. For example, CFTR correctors that are designed to target ΔF508 are thought to inhibit deglycosylation, thus reducing CFTR interaction with calnexin, a protein that induces early degradation.[15] By definition, correctors could be used for therapy on CF patients with mutations in Class I, II, V and VI.[2,11]

Conversely, CFTR potentiators interact with mutated CFTR channels that are able to embed in the cell membrane, but display reduced conductance or altered gating. These potentiators

act to either enhance anion movement or repair the gating mechanism of the mutant CFTR. It is thought that potentiators work by altering NBD dimerization. Specifically, these potentiators might be binding at the interface of the NBD dimer, lowering the free energy of the transition state and accelerating channel opening. Also, it is thought that potentiators may slow down the rate of channel closure by stabilizing the dimer conformation.[11, 16] Potentiators could be used as therapy for CF patients with mutations in Class III and IV. It should also be noted that some small molecule CFTR modulators have been shown to have both corrector and potentiator properties.

As ΔF508 is the most prevalent mutation of CFTR, it will be the focus of the discussion of treatments. Currently, the most advanced chemical corrector of ΔF508 is an efficacious and selective ΔF508 corrector, VX-809, as demonstrated *in vitro* by Van Goor et al. This was confirmed by measuring the fractional conversion of endoplasmic reticulum-associated, immature CFTR to the mature glycosylated form. Van Goor et al. also showed that VX-809 was able to restore chloride transport in cultured human bronchial epithelial (HBE) cells to approximately 14% of that measured in non-CF HBE cells.[17] As these early results of VX-809 have shown the potential for clinical benefits, Phase II trials have begun. One early trial by Clancy et al. has shown that VX-809 has a good safety and adverse events profile. Additionally, VX-809 was shown to reduce sweat chloride levels in a dose-dependent manner. However, when using forced expiratory volume in one second (FEV_1) as a measure of clinical efficacy, no significant differences were seen in patients taking VX-809 in comparison to placebo.[18] Thus, it is currently thought that a CFTR corrector alone is not sufficient to be clinically significant and needs to be administered adjunctively with a CFTR potentiator.

One important CFTR potentiator that has been developed is VX-770.[5] It has been tested on patients carrying the CFTR mutation G551D, which causes altered CFTR gating (Class III mutation). Like VX-809, VX-770 has shown to be well-tolerated without notable side-effects. Additionally, in one randomized, double-blind, placebo-controlled trial of VX-770, positive clinical effects have been shown. In comparison to the placebo, patients with the G551D mutation taking VX-770 showed an FEV_1 change of more than 10%. Additionally, these subjects had 55% fewer pulmonary exacerbations.[19] Due to the positive clinical effects of VX-770, it is thought that this potentiator could have synergistic effects with VX-809 in patients with the ΔF508 mutation. Combining these two therapies in a dual therapy is sensible, as a corrector drug like VX-809 may save CFTR from degradation, but once embedded in the apical membrane it still may display minimal function. By utilizing both a corrector and a potentiator drug, CFTR could be fully "repaired" by the combination of corrector-induced degradation prevention and potentiator-induced conductance increase.

The combination of VX-809 and VX-770 has been tested in two Phase I trials.[20,21] The purpose of these trials was to test for drug-drug interactions and safety. Although the results of these trials were not published, it can be assumed that there were no serious safety issues, as a Phase II study is currently in progress.[22] The primary outcome measures of this trial are to look at safety and tolerability assessments, change in sweat chloride, and the relative change in percent predicted FEV_1. Results of this trial could be important in determining if a combination of CFTR potentiators and correctors could be useful in a clinical setting. Although trials are still occurring for VX-809 and VX-707, they have been designated as "breakthrough therapies" by the US Food and Drug Administration. This designation gives patients access to these drugs, which aid in reducing mutant CFTR.[23]

CONCLUSION

In summary, CF is a disorder that has benefited greatly from advancements in treatment, as evidenced by the increased survival age of CF patients. However, it is clear that to see further improvement, treatments need to be developed to directly target the root cause of CF: mutant CFTR. CFTR modulators, small molecules that repair CFTR, have shown great potential to improve CFTR function *in vitro*, though results in the clinical setting are varying. Due to these varied results, the potency of some CFTR modulators has been questioned. Currently, the use of multiple CFTR modulators is being tested for synergy in the clinical setting. If these CFTR modulators prove to be clinically beneficial, it is likely that we will see further improvement in the quality of life and survival age of CF patients. ■

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