Modulating the cystic fibrosis transmembrane regulator – breaking the basic defects

Abstract
The defective allele responsible for cystic fibrosis (CF) is found in one in 19 people in Ireland. Historically, treatment of CF comprised ameliorating patient symptoms, but recently there has been much progress in CF therapy, not least of which are the CFTR gene modulators. These target specific basic defects in the disease: that of deficient or abnormal synthesis; and, processing of the CFTR channel, thus treating the disease, for the first time, systemically. These compounds have implications for targeted CF therapy and mark a large step forward in the field. So far, three targets have been overcome, with varying degrees of success. There are six mutation classes in CF; Class I is most severe, but patient presentation depends on the specific combination of alleles. Kalydeco was developed for the class III G551D mutation, and demonstrated efficacy in homozygous patients, but the high cost of the drug remains an issue. Lumacaftor and VX-661 were developed to address the ΔF508 Class II mutation; lumacaftor was effective when used in conjunction with Kalydeco, and VX-661 is as yet undergoing trials. Lastly, investigations are ongoing to assess Ataluren as a modulator of the effects of the premature termination codons caused by Class I CF mutations; two of three phase II studies showed varied improvement in patient symptoms following treatment with Ataluren and a phase III trial is currently underway. This article discusses the progress in CF-modulating therapy hitherto.

Introduction
Cystic fibrosis (CF) is a disorder that results from mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. It causes a build-up of mucus with resulting cycles of inflammation and infection, and primarily affects the lungs and digestive system. The disease was so named due to the cyst formation in the pancreas after pancreatic ducts became blocked in the course of the disease’s pathology. The disorder is also termed mucoviscidosis as a result of the thick mucus that plugs the airways in its pulmonary pathology. It has even been dubbed ‘65 roses’ by those patients too young to pronounce its name or understand its significance (Figure 1).

CF is a multisystemic hereditary disease with protean manifestations and an autosomal recessive pattern of inheritance. Since the discovery of the CFTR gene in 1989, hopes of clinicians and researchers have been focused towards the time when knowledge of its effects could be built upon to cure CF, but until just three years ago there had been no significant progress.

CF has particular importance in Ireland, where 2.98 in 10,000 individuals have CF and one in 19 of the population is a carrier of a defective CFTR allele. The incidence of CF in Ireland is the highest in the world – nearly four times the rate in other European countries and in the USA. Reflecting this, much work has been done in Ireland directed towards individuals with the disease, ranging from the RCSI Department of Medicine’s significant participation in the CF therapy trial of VX-770 to the building of dedicated CF centres in hospitals nationwide. To date, the majority of treatment for CF has been symptomatic, not curative, involving the use of hypertonic saline, dornase alfa, pancreatic enzymes, albuterol and inhaled antibiotics. These are given to improve airway surface liquid volume, break down mucus build-up, aid nutrient absorption in the gastrointestinal tract, relax bronchial muscle, and prevent and combat infection. Life expectancy for CF patients has increased and is now approaching 40 years,
exciting new developments are underway as more discoveries are being made with regard to the gene and its effects. Mutation-specific CFTR-modulating therapy is among these advances along with gene therapy and newer, more effective antimicrobials. This article discusses the upcoming therapeutic CFTR modulators.

**CFTR channel and function**

The CFTR gene encodes for a chloride channel found in the apical membrane of secretory epithelia in respiratory, gastrointestinal, reproductive and other exocrine glands. Its structure consists of two transmembrane domains (of six α-helices each), two cytoplasmic nucleotide binding domains (NBDs) and a regulatory domain (R domain) containing protein kinase A and P phosphorylation sites. The channel through which the chloride molecules pass is formed by the two transmembrane domains. The CFTR protein is a member of the ATP-binding cassette (ABC) family, a group of proteins that bind ATP in order to transport molecules across cell membranes.

**The importance of CFTR – ion transport regulation**

One of CFTR’s main functions is to allow the exit of Cl⁻ ions from epithelial cells into the lumen. However, it is a multifunctional protein and many of its additional functions have only recently been described. In addition to facilitating chloride transport, CFTR also regulates transport of ions such as Na⁺ and CO₃⁻ predominantly through interactions involving its NBD. Thus, normal CFTR function is essential on several levels to prevent duct blockage, mucous build-up, infection and inflammation.

**Mutation classes**

Many of CF’s clinical presentations result from the varied effects on ion conductance. The severity of the disease, in turn, depends upon the extent to which CFTR function is impaired. There are six classes of CFTR mutation based on their effects on the CFTR protein. The CFTR modulators are an attempt at treatment specific to mutation class, and thus understanding these mutations is key to understanding many of the ongoing therapeutic advances in the disease.

The presentations of CFTR mutation classes I-VI (Figure 2) range from most severe to sub-clinical, respectively:

- **Class I mutations** result in a premature termination codon (PTC). This causes the production of a truncated form of CFTR that is degraded before it reaches the plasma membrane – thus it results in a complete absence of CFTR on the membrane.
- **Class II mutations** include the most common ΔF508 mutation. This class causes abnormal post-translational modifications to the complete protein – in this class, too, the mutated protein is degraded before it can reach the cell membrane.
- **Class III mutations** result in defective regulation of CFTR channel function. This occurs due to impaired ATP binding and hydrolysis, which stops energy-dependent channels from opening. In Class III mutations there is a normal amount of CFTR on the cell membrane but it is non-functional. A prominent example of this class is G551D (incidence of 4% worldwide, 10% in Ireland). It is the second most prevalent mutation in Ireland. The G551D mutation has recently received much media attention as its respective modulator treatment, Kalydeco, was, as of January 2012, approved by the FDA for use after an accelerated Phase III trial.
- **Class IV mutations** lead to a defective CFTR channel pore. These mutations result in decreased protein function, and include the relatively common R117H mutation.
- **Class V mutations** cause a reduced amount of functional CFTR protein to be present at the membrane.
- **Class VI mutations** cause altered regulation of channels other than the chloride channel. There is overlap of this class with other classes, such as for the ΔF508 mutation, which is both a Class II and Class VI mutation (Figure 2).

The phenotypic presentation of these mutations depends upon the combination of an individual’s alleles. If both the alleles are of the more severe genotypes (classes I-III), the individual is likely to present with the classic CF phenotype, i.e., pancreatic insufficiency, gastrointestinal symptoms and sinopulmonary infections.
Correcting and potentiating CFTR

Drugs that correct the problem of early degradation (as in Class I) are called CFTR ‘correctors’. Those that correct aberrant trafficking or gating of the channels once at the membrane (as in Classes II and III) are known as CFTR ‘potentiators’ (Figure 3).

Three different mutation targets are currently being addressed by two pharmaceutical companies:

1. PTC124 (Ataluren) by PTC Therapeutics, to overcome Class I mutations that result in PTCs.
2. VX-809 (Lumacaftor) by Vertex Pharmaceuticals, to correct CFTR trafficking defects in Class II mutations.
3. VX-770 (Kalydeco/ivacaftor), also being developed by Vertex Pharmaceuticals, which potentiates CFTR at the membrane in Class III mutations.

PTC suppression and correction of aberrant CFTR trafficking are both more difficult objectives to reach as they involve modulation issues on several levels:

- PTCs can result from several mutations, which need to be targeted individually;
- restoration of translation is also required; and,
- the functional protein must then be trafficked to the cell membrane.

The three upcoming treatments are discussed here in order of discovery, which also (understandably) reflected the targeting of the least to most severe class.

Kalydeco – potentiating at the plasma membrane

The story of the first CFTR-modulating drug to be approved in the therapy of CF, Kalydeco (also known as VX-770 and ivacaftor), is an interesting one. This compound was a result of a commitment on the part of the Cystic Fibrosis Foundation to, based on the knowledge of CFTR accumulated to date, treat CF as a multisystem disorder and to invest in the search for new therapies targeting basic CFTR defects. A collaboration with Vertex Pharmaceuticals led to high-throughput screening with CF lines to evaluate 228,000 molecules, from which VX-770 was discovered. It demonstrated ability to potentiate CFTR gating once at the membrane (as in Classes II and III).

The exact mechanism by which Kalydeco exerts its effects is as yet unknown, but it has demonstrated positive results in all its pivotal studies to date: one Phase II and two Phase III trials. It resulted in chloride transport. However, it would be valuable to measure quantitative chest imaging and lung clearance index in future investigations as they have been noted to be more sensitive as measures of pulmonary disease in CF.

Accurso et al. conducted a two-part Phase II trial evaluating Kalydeco’s safety. The adverse events profile of Kalydeco was similar to placebo, with serious adverse events being fewer in the treatment group (24% vs. 42%). Ramsey et al.’s Phase III STRIVE study of 2011 was conducted in patients over 12 years old. It demonstrated a significant increase in FEV1, a decrease in sweat chloride levels (some out of the threshold of CF diagnostic), a decrease in pulmonary exacerbation rate and an increase in weight from baseline. This study was followed by the two-year-long PERSIST open-label study, in which the long-term safety and efficacy of Kalydeco were examined; the benefits of the drug remained significant. The ENVISION study in six- to 11-year-olds demonstrated that Kalydeco was also safe and effective for this age group. The weight gain in these trials’ results remains unexplained and warrants further investigation – postulations include that improvement in CFTR function in the digestive tract may augment nutritional absorption.

Although Kalydeco is a potentiator, it has its use in patients with the ΔF508 mutation. On its own it has no efficacy in ΔF508 homozygous patients but trials are ongoing to investigate its efficacy when combined with other CFTR modulators. Further research gaps include the appropriateness of its use for other Class III (gating) mutations, for patients two to five years old, and for patients with one or more copies of the Class IV R117H mutation. Kalydeco, approved by the FDA in January 2012, costs $294,000 per year for its twice-daily regimen, though Vertex Pharmaceuticals has programmes that allow patients with no insurance and a household income of less than $150,000 per year to obtain the drug for free. Those with insurance can receive up to 30% of the drug’s list price. After being approved by the European Medicines Agency in July of this year for individuals over the age of six years with at least one copy of the G551D mutation, Kalydeco is currently being reviewed for use in Ireland by the drug regulatory authorities. Kalydeco was approved by the Irish Minister for Health, Dr James Reilly, in February 2013.

Correcting ΔF508 – Lumacaftor and VX-661

Class II mutations result in abnormal CFTR folding and degradation of the protein before it reaches the cell membrane. The ΔF508 mutation, the most common mutation in Ireland (found in approximately 66% of CF patients) belongs to this class. There is no CFTR present on the cell membrane as a consequence of homozygous ΔF508
mutations. Class II mutations therefore require more regulation – they require a correctly formed protein to be produced as well as translocation of the CFTR channel to the cell surface. Due to the hypothesis that there may be residual defective ΔF508 CFTR at the membrane, Kalydeco was trialled in isolation for patients homozygous for the ΔF508 mutation but, as discussed, did not prove clinically effective. A Phase II randomised controlled trial (RCT) in a similar group was conducted for VX-809, also known as Lumacaftor, and it demonstrated safety and modest efficacy in reducing sweat chloride, and concluded that Lumacaftor is a viable candidate for further investigation. 

A Phase II RCT investigating the combined effect of Lumacaftor and Kalydeco was conducted in 2011. In combination, the two drugs work together to remedy the defect: Lumacaftor corrects the folding and processing of CFTR and thus aids in transporting the protein to the membrane, and Kalydeco then supports its function. The 62-participant study concluded that the combination improved CFTR function significantly in patients homozygous for the ΔF508 mutation and recommended further clinical investigation. In June 2012, the results of a second part of that combination trial were announced, enrolling 109 people over 18 years of age. Improvement in pulmonary function was dose dependent, with those who received the greatest dose of VX-809 demonstrating most improvement, and homozygous individuals benefiting more than heterozygotes. A pivotal trial of the combination improved CFTR function significantly in patients homozygous for the ΔF508 mutation. 

Another drug is being investigated called VX-661, which is postulated to increase CFTR trafficking to the membrane in the ΔF508 mutation. An exploratory Phase II clinical trial is in progress to evaluate the safety and effects of VX-661 alone and in combination with Kalydeco. The study is due to be completed in August 2013. 

Terminating PTCs – Ataluren 

Class I comprises the most severe forms of mutation and represents 10% of CF genotypes. It consists of nonsense mutations that cause the production of a truncated or unstable form of CFTR, which is detected by chaperone proteins at the ER and degraded before it reaches the cell surface. This class is associated with complete lack of CFTR protein at the apical membrane of epithelial cells. G542X is the most common of these mutations. Gene therapy and aminoglycosides such as gentamicin are currently being investigated for a therapeutic approach to Class I mutations. Aminoglycosides allow the attachment of a near-cognate tRNA to the defective template, allowing a full-length protein to be produced. This has been shown to be efficacious in vitro, in animal studies of CF and muscular dystrophy, and in small numbers of CF patients.

Of more relevance to this discussion of CFTR modulators, an orally bioavailable non-glycoside compound specifically developed to promote ribosomal read-through of nonsense mutations is under review, designated PTC124 and also known as Ataluren. Ataluren binds to eukaryotic ribosomes and suppresses PTCs in cell- and animal-based disease models. There have been three randomised open-label dose-ascending Phase II trials in CF. The results of all showed tolerability to Ataluren in the short term. Improved function of CFTR (measured by NPD) was seen in two. One study showed that CFTR localisation to the nasal cell membrane was improved, and another’s results demonstrated a reduction in cough over three months. However, the third study did not demonstrate an improvement in NPD and limitations of all studies included the small sample size and lack of placebo controls. Concerns about Ataluren include its potential to permit oncotic change, as its read-through capabilities could potentially allow native mutations to go undetected.

Adherence may also be an issue, as the medication needs to be taken thrice daily. A Phase III RCT is in progress to determine the drug’s safety, efficacy and tolerability for the duration of 48 weeks, to be completed in October 2012.

Conclusion 

The advances made in CF therapeutics are many and encouraging, but several questions remain to be answered. The long-term efficacy of these medications is yet to be elucidated and more than 1,600 disease-associated mutations have to be tackled before CF can be known as a curable disease. These drugs also provide the hope that the damage CF can cause can be stopped in its early stages. Use of Kalydeco has improved quality of life, for those who can afford it, with decreased cough and sputum production and a reduced burden from conventional treatment. Progressive lung disease and premature death do not seem as imminent with the advent of such potentially curative therapies, though longer-term studies remain to be conducted. The issue also arises as to whether investment should focus solely on the G551D patients by subsidising the drug or whether interventions catering for all CF patients should be allocated the limited healthcare budget – the ever-present ‘distribution problem’.

It remains to be seen whether PTC- and ΔF508-targeting modulators are potent enough to have clinical effectiveness. Despite, or even because of, these uncertainties, CFTR modulators, coupled with the introduction of newborn screening in Ireland in July 2011, highlight the area of CF therapy as one to watch in the near future.

References 


