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Re-*Imagining* Cystic Fibrosis *Care*: Next Generation *Thinking*

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Summary

Cystic fibrosis care has advanced dramatically over the last decade, with CFTR modulator therapy a game charger for some patients. With its increasing use, unexpected benefits and side-effects are being unmasked and must be managed accordingly.

Abstract

Cystic fibrosis is a common multi-system genetically inherited condition, predominately found in individuals of Caucasian decent. Since the identification of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in 1989, and the subsequent improvement in understanding of CF pathophysiology, significant increases in life-expectancy have followed. Initially this was related to improvements in the management and systems of care for treating the various affected organ systems. These cornerstone treatments are still essential for CF patients born today. However, over the last decade, the major advance has been in therapies that target the resultant genetic defect - the dysfunctional CFTR protein. Small molecule agents that target this dysfunctional protein via a variety of mechanisms have led to lung function improvements, reductions in pulmonary exacerbation rates and increases in weight and quality of life indices. As more patients receive these agents earlier and earlier in life, it is likely that general CF care will increasingly pivot around these specific therapies, although it is also likely that effects other than those identified in the initial trials will be discovered and need to be managed. Despite great excitement for modulator therapies, they are unlikely to be suitable or available for all: whether this is due to a lack of availability for specific CFTR mutations, drug-reactions or the health economic set-up in certain countries. Nevertheless, the CF community must be applauded for its ongoing focus on research and development for this life-limiting disease. With time, personalised individualised therapy would ideally be the mainstay of CF care.

They are ill discoverers that think there is no land, when they can see nothing but sea

Francis Bacon

The big picture is all in the details

Charles Darwin

Overview

I. Introduction

Cystic fibrosis (CF) is an autosomal recessive monogenetic condition with considerable phenotypic variability. It is the commonest genetically inherited lethal disorder in Caucasian populations with over 90 000 people with CF worldwide. The incidence is 1 in 3000 live births in individuals of northern European descent with a carrier rate of 1 in 25 individuals(1, 2). As a multi-system disorder, it is characterised by chronic airway infection, pancreatic insufficiency leading to gastrointestinal malabsorption and malnutrition, diabetes, and premature death.

CF results from mutations in the cystic fibrosis transmembrane conductor regulator (CFTR) gene located on chromosome 7(1). The gene was identified in 1989 and to date, more than 2000 CFTR mutations have been classified with over 300 known to be disease causing. Despite this, only five mutations have a frequency rate greater than

1%(2). The commonest and most well characterised genetic abnormality is a deletion of three base pairs encoding a phenylalanine residue at position 508 on chromosome 7 (Phe508; Δ508). Between 80-90% of individuals with CF have at least one copy of the Phe508del-CFTR mutation and approximately 50% of Caucasian individuals with CF are homozygotes(3-5).

CFTR codes for an ABC-transporter class C ATPase protein, which facilitates chloride and bicarbonate transport at the apical membrane of epithelial cells, primarily in glandular epithelia. This apical anion channel is highly expressed in the bronchial epithelium, pancreas, gastrointestinal tract, vas deferens and sweat glands with lower expression in other tissues(6-11). Loss of functional apical CFTR proteins within the bronchial epithelium causes a reduction in chloride and bicarbonate secretion from the cell and as a consequence, the airway surface is fluid depleted and has an altered pH. It also regulates the activity of other ion channels including the epithelial sodium channel (ENaC; the amiloride-sensitive sodium channel). Attenuated CFTR activity is postulated to cause unopposed ENaC-dependent sodium and water absorption, further dehydrating the cell surface. Fluid depletion together with abnormally adherent mucus leads to delayed mucociliary clearance. In addition, reduced or absent CFTR results in abnormal innate immunity and dysregulated inflammation (1, 12). Thus, a favourable environment is produced within the within the airways that is susceptible to unchecked inflammation and chronic bacterial infection.

Six different functional classes of CFTR mutations have been identified. These groups are classified according to a combination of amount of cell surface protein, channel stability and function(1) (Figure 1). More recently proposals have been made to subdivide class I mutations into two groups depending upon whether the mutation leads to no function CFTR protein or no messenger RNA (mRNA)(13). Classifying the different mutation groups based upon the resultant functional defects assists with identifying targeted corrective therapies. However, it is unlikely that one classification system will be able to encompass all facets of importance; such as clinical disease features, therapeutic strategies and CFTR defects(14). Classically it has been thought that each class confers a differing disease severity, relating to the degree of CFTR dysfunction and thus has prognostic implications(15, 16). However, a large registry

review did not identify any difference between individuals with a stop codon mutation, Phe508del homozygotes or those with a class III mutation in terms of lung function decline. Nevertheless, it is clear that class IV and V mutations confer a milder phenotype, relating to the presence of some 'residual' CFTR channel function, and thus are termed residual function (RF) mutations(17).

It has been widely accepted that inadequate functional CFTR protein and therefore chloride transport at the cell surface is the major driver of pathophysiological disease. However, it is by no means clear whether some of the pathophysiology observed in individuals with CF is not also secondary to other factors for which altered chloride transport at the cell surface may only be an indirect marker. CFTR related pathophysiology may also involve: an altered extracellular environment with a reduced cell surface pH; changes in phospholipid raft protein assembly and subsequent abnormal transmembrane and intracellular signalling processes; the heavy burden of misfolded CFTR protein on the proteasome; and metabolic disturbances within subcellular structures, including the mitochondria, due to the indirect effects of dysfunctional electrolyte movement or protein trafficking processes (Figure 2a & 2b). Although, chloride channel functional changes are likely to represent an easily accessible surrogate marker for all these processes, individual heterogeneity in any of these areas may significantly contribute to the phenotypic heterogeneity seen in CF patients with the same genotype.

In vivo studies have demonstrated that approximately 10% of residual CFTR function is all that is required to restore near-normal chloride transporting properties when compared with 100% corrected cell sheets(18). CF individuals with approximately 10% CFTR expression per cell generally do not develop classical CF related disease features (19, 20). Currently, it is unknown whether full correction of CFTR in just 10% of cells is comparable to 10% of expression in all cells(20). Furthermore, we speculate that even a single CFTR mutation may be associated with specific organ dysfunction in the setting of a "gross" second hit phenomenon, for example, lung disease in setting of severe early infection, allergic disease or immunodeficiency; and exocrine pancreatic insufficiency with ageing especially in the setting of life-long accumulated environmental stressors such as smoking(21-23).

II. General Cystic Fibrosis Care: ***Basic Standards***

When CFTR was discovered, the median age of death was approximately 20 years(24). Since then, life expectancy has improved considerably with the median age of death now approximately 30 years with the median predicted survival of an individual born today with CF in the mid-40s(4, 5). The development of various therapies and systems of care, which have predominately focused on improving salt and fluid balance, nutritional status and reducing airway inflammation with its corresponding lung parenchymal destruction have been instrumental in this(25, 26).

Despite survival advances, CF lung disease is still the major cause of morbidity and mortality and thus pulmonary exacerbations should be treated expediently to reduce the risk of further airway damage. Clinical outcomes are improved with eradication attempts of initial bacterial isolates, especially in the case of *P. aeruginosa*(27). Once colonised, nebulised antipseudomonal antibiotic treatments result in an improvement in forced expiratory volume in 1 second (FEV_1) and a reduction in pulmonary exacerbation rates(28, 29). Azithromycin usage has been shown to reduce exacerbation frequency and to improve a patient's health status, related to its antibiotic, anti-inflammatory and immune modulatory properties (30, 31).

Antimicrobial therapies are used in conjunction with therapies to address the impaired mucociliary clearance and mucus accumulation. Nebulised treatments, such as dornase alpha (32) and hypertonic saline (33), aid the delayed mucociliary clearance. These must run in parallel with good quality modern airway clearance techniques that try to avoid provocation of gastro-oesophageal reflux(34).

Fat maldigestion and malnutrition are also characteristic of CF and are predominantly secondary to pancreatic insufficiency with a variable contribution from chronic gastrointestinal inflammation in individual patients. Being underweight negatively

impacts long-term survival in CF and dietetic support is essential to ensure weight is maintained for both general well-being and to prevent lung function decline (12, 35, 36). Furthermore, fluid and salt replacement are often under managed, especially given the blunting of the thirst response and general tolerance to chronic dehydration that is clinically observed with this condition. This is compounded by the difficulties individuals face with the increased amounts of calories and salt/fluid that are required to maintain neutral balances and which is further exacerbated under conditions of growth, illness, exercise or exposure to extreme heat environments.

Although these strategies paved the way for substantial advancements in patient quality of life and survival, they do not target the underlying genetic mutations or the activity of the CFTR protein. Current research has thus focused on identifying mechanisms by which the underlying CFTR protein dysfunction could be reversed.

III. Cystic Fibrosis Gene Specific Therapy*: <u>Recent Advances</u>*

Genetic Therapies

Gene therapy was first trialled in the 1990s in an attempt to reverse the CFTR dysfunction. The initial murine studies found that adenoviral vectors could successfully transfer the human CFTR gene, leading to human CFTR protein being expressed in murine epithelial cells(37). It is hypothesised that CFTR gene replacement during the neonatal period, prior to any parenchymal lung damage or bacterial colonisation, has the potential to significantly alter morbidity and mortality in CF (36). Hence, gene therapy research focussed on gene-addition therapy to the airways whereby wild-type CFTR gene is inserted into an individual's cells resulting in functional CFTR channel production. As the respiratory epithelium is comprised of terminally-differentiated cells, any form of gene therapy must either be able to be repeatedly delivered to the airway surface or be able to alter the stem/progenitor cells(38).

Gene transfer via aerosolised means to the respiratory epithelia is possible with the use of both viral and non-viral vectors. However, the initial gene delivery approaches were too inefficient for it to be a viable therapeutic option(39). Also, repeated administrations of viral vectors or DNA can result in immune reactions developing in the individual receiving the treatment(20, 40). More recently Phase IIb trials in patients 12 years and older have demonstrated that gene therapy can alter the progression of CF lung disease, albeit only by modest amounts. The repeated nebulisation of non-viral CFTR gene therapy each month for a year lead to stabilisation of lung function over that time period when compared with placebo, the percentage change in FEV_1 was -0.4% versus -4.0% in the placebo arm(41). Change in lung function decline does impact morbidity and mortality in CF but notably this study did not lead to an increase in lung function or result in any quality of life improvements.

Other methods have focussed on **mRNA therapy** and **mRNA repair**, whereby the correct nucleotide sequence code for CFTR is delivered to the cytoplasm resulting in normal CFTR protein production or repair of the CFTR mRNA. The benefit of these strategies is that translocation across the nuclear membrane barrier is not necessary. Delivery methods include liposomal or polymeric non-viral vector formulations that can

be administered via several routes (42). Haque et al (43) found that nanoparticle-chemically modified mRNA led to lung function improvements without any immune reactions following repeated applications in mouse models. However, as these studies are in the preclinical stages of development additional work is required before such treatments will be readily available.

A further genetic therapy strategy is **gene editing**. This approach attempts to repair the mutant CFTR DNA resulting in normal CFTR proteins being produced. The current technique involves the use of CRISPR (clustered regularly interspaced short palindromic repeats), which is found naturally in the immune system of bacteria. A ribonucleoprotein endonuclease (Cas9) that can catalyse the cleavage of double stranded DNA binds to the target DNA site as determined by a guide RNA element and creates a double-stranded opening. Subsequently the cell can fill the excised section with the correct sequence through homologous directed repair(20, 42). Initial proof of concept studies for gene editing in CF were tested in intestinal organoids obtained from Phe50del paediatric patients (44). It has since been shown that gene correction can occur in generated induced pluripotent stems cells (iPSC) and these cells can then differentiated into mature airway epithelial cells with normal CFTR function(45). There is the potential for iPSCs to be used as cell grafts but to date there are ongoing concerns with regard to tumour risk and the potential to transmit genetic abnormalities. Ongoing work and research is required prior to its use in clinical practice.

CFTR modulator agents

The major advancement in CF therapy has been the introduction of small molecules that modulate the function of the abnormal CFTR protein. CFTR is a multi-domain protein belonging to the subfamily C ATP-binding cassette (ABC) transporters. It is the only one within the group to function as an ion channel (46). Class I mutations (Traditional classification system) result in no functional CFTR protein but the other classes lead to abnormally translated protein, which can be both misfolded and mis-assembled (Figure 1) (1, 13, 47). Agents to target the dysfunctional protein were developed via high-throughput drug discovery programs. The molecules identified were optimised and subsequently evaluated in terms of pharmacokinetics and toxicology (48-50).

The first CFTR modulator agent for individuals with CF following these drug development strategies was Ivacaftor (Kalydeco®) (51). It was originally developed for the Gly551Asp-CFTR mutation (G551D, a class III mutation), which causes defective CFTR channel gating. Gating refers to the opening and closing functions of the channel, and when defective leads to a low probability of CFTR channel opening and thus reduced CFTR function. Ivacaftor increases chloride transportation by prolonging the time period that activated CFTR channels at the apical cell membranes remain open(51). Initial phase 3 studies STRIVE and ENVISION, evaluated ivacaftor in individuals aged 12 years and older and in those aged 6 to 12 years of age respectively. STRIVE found a substantial improvement in absolute percentage predicted (pp) FEV_1 of 10% at 24 weeks (the primary endpoint), which was maintained until 48 weeks. This was alongside a 3kg weight gain, an 8-point increase in CFQ-R (where an increased score out of 100 reflects a reduced impact on patient quality of life and a 4 point change is a clinically relevant difference) with a reduction in sweat chloride (SwCl) to below diagnostic threshold to a mean of 47.8 mmol/l (51). Similar findings were demonstrated in the children recruited to ENVISION. Participants from both studies were enrolled in the open-labelled extension study (PERSIST), with those receiving placebo switched to ivacaftor therapy. The changes were maintained for up to 144 weeks(52).

Ivacaftor has subsequently been identified as being effective in almost 40 variants, including other specific gating mutations, missense or canonical splice mutations(53-55). Some of these mutations were identified as being eligible from in vitro data, which

is an alternate pathway to enabling patients with rare, difficult to study mutations from being able to access newer therapies(56). The development of ivacaftor was a considerable treatment advance for individuals with CF but it is only effective for approximately 8% of CF patients. Furthermore, ivacaftor and its M1 metabolite is associated with some drug-drug interactions, resulting in the inhibition of both cytochrome P450 3A (CYP3A) and, or the P-glycoprotein (P-gp) substrates. Caution and monitoring must be maintained when using such agents alongside ivacaftor(57).

Agents targeting the commonest CFTR mutation, Phe508del (a class II mutation), would provide modulator therapy for a greater proportion of CF individuals. However, the Phe508del-CFTR mutation results in a more complicated protein defect with destabilisation of protein folding leading to protein degradation together with altered CFTR gating and reduced cell membrane surface stability(1, 13, 50). As multiple stages in CFTR protein development are affected, different approaches were required to treat such individuals. Lumacaftor is an oral corrector agent, which in vitro corrects the misprocessed protein leading to increased cell membrane localised protein (58). Oral monotherapy of either ivacaftor or lumacaftor did not yield positive outcomes in Phe508del homozygotes(59, 60). However, lumacaftor in combination with ivacaftor (Orkambi®) did result in a modest gain in absolute ppFEV$_1$ of 3% at 24 weeks (the primary outcome) in phase 3 studies (TRAFFIC and TRANSPORT) along with significant increases in body mass index (BMI)(61). In comparison with ivacaftor, this was a disappointingly small increase in ppFEV$_1$. However, the 96-week extension study (PROGRESS) did elicit a 42% reduction in the annual rate of lung function decline when compared with a US registry control group(62). This study recruited the TRAFFIC and TRANSPORT participants and treated those who had initially received placebo with lumacaftor/ivacaftor. Although not an exceptional outcome, it is still important because rate of lung function decline is known to correlate with morbidity and mortality in CF(63, 64).

Unfortunately, lumacaftor/ivacaftor is associated with respiratory related side effects and 7% of patients discontinued treatment in PROGRESS. Real-world experiences with lumacaftor/ivacaftor have found even higher discontinuations rates of up to 30% (65, 66). Lumacaftor is also a potent inducer of CYP3A4 enzymes, which can have a significant impact upon concurrent medication use (67). Other modulator treatments

with better side-effect profiles and greater FEV_1 improvements for homozygous Phe508del-CFTR patients were required. Therefore, another corrector, tezacaftor, was developed. Tezacaftor in combination with ivacaftor (Symdeko®/Symkevi®) elicited a 4% absolute change in $ppFEV_1$ with significantly lower pulmonary exacerbations rates, compared with placebo, for Phe508del homozygote adults (68, 69). Again, this is only a modest improvement in FEV_1 but tezacaftor/ivacaftor does have a better side-effect profile with only a 2.9% discontinuation rate in the active treatment arm and none secondary to respiratory events. The open-labelled extension study is currently awaited. It is also approved for individuals with 26 specific residual function and splice mutations(70). In children aged 6-11 years for both Phe508del homozygotes and Phe508del/RF mutations, SwCl levels decreased and mean $ppFEV_1$ remained stable within the normal range along with normal growth parameters(71).

Although tezacaftor/ivacaftor is more promising for Phe508del-CFTR homozygotes, it also does not dramatically improve CFTR function. These dual combination therapies are thus not suitable for the 30% of individuals with CF who are Phe508del heterozygotes and have a minimal function (MF) mutation(13). MF mutations comprise class I and II mutations, which result in no function CFTR protein production or produce defective protein that are unresponsive to ivacaftor, lumacaftor/ivacaftor or tezacaftor/ivacaftor. These include insertion, deletion, nonsense and canonical splice and certain severe protein misfolding mutations(72). If increased functional protein could be achieved in those with at least one Phe508del mutation, this may provide higher rates of chloride transport in a greater percentage of individuals with CF.

Next generation CFTR correctors used in combination with tezacaftor/ivacaftor have given rise to remarkable clinical outcomes. Phase 2 trials of VX-659 and VX-445, each combined with tezacaftor/ivacaftor elicited a 9.7% and 11% absolute change in $ppFEV_1$ respectively for Phe508del homozygotes versus tezacaftor/ivacaftor and a 13.3% and 13.8% respectively absolute change in $ppFEV_1$ in Phe508del-MF versus placebo (72, 73). The subsequent phase 3 studies with the use of elexacaftor (VX-445) in combination with tezacaftor/ivacaftor (Trikafta™) maintained such responses. For Phe508del homozygotes there were increases in ppFEV1 of 10% and in CFQ-R RD of 17.4 points together with a 45.1 mmol/l decrease in SwCl after 4-weeks(74). In the Phe508del-MF cohort ppFEV1 increased by 14.3% together with a 20.2 point increase in CFQ-R RD and

a SwCl decrease of 41.8 mmol/l at 24-weeks(75). It is exciting to see such results for severe mutation classes.

Specific therapies for premature termination codon class I mutations

Nonsense mutations cause truncated non-functional or partially functional protein. The nonsense mutations in DNA insert premature translation stop codons (PTCs), which interrupts ribosomal translation of the protein. In CF approximately 5-10% of the alleles carry a nonsense mutation that leads to these PTCs. Ataluren was developed to enable ribosomes to read through PTCs during mRNA translation to produce functional protein. Phase 2 studies demonstrated an improvement in the electrophysiological abnormalities seen in the lungs in adults with CF and an increase in functional CFTR protein production in children (76, 77). However, phase 3 studies did not find an increase in FEV_1 or quality of life scores (78). Ataluren is currently not a treatment option for CF individuals although it is used for nonsense mutations in Duchenne muscular dystrophy. Aminoglycosides, which are widely used both intravenously and nebulized to treat *P. aeruginosa*, also have been found to suppress the PTCs (79, 80). However, their side effects prohibit long-term usage, particularly with the potential for oto- and nephro-toxicity. Synthetic aminoglycosides are a potential avenue for further investigation due to their better side effect profiles (81).

Targeting non-CFTR channels

Some mutations result in no functional protein and no mRNA and thus are considered to be 'unrescuable' mutations. For these types of mutations, an alternative approach is to target the non-CFTR anion channels such as ENaC. Denufosol tetrasodium acts on P2Y receptors expressed on the surface airway epithelium and in vitro has been found to stimulate chloride secretion via calcium activated chloride channels, inhibit sodium absorption via ENaC and stimulate ciliary beat frequency. A phase 3 trial did not demonstrate any improvement in FEV_1 over 48 weeks(82). The topical application of the sodium channel blocker amiloride has also been investigated in several studies, with no evidence that it improves lung function or mucus clearance(83).

Implications of novel treatment therapies

The novel therapies discussed, specifically modulator therapy, have resulted in important advances in the treatment options available for individuals with CF. However, they are associated with a substantial cost burden and on a global scale, these medications are far from being available for all even within developed countries (13).

CFTR modulator therapy targets the dysfunctional CFTR protein and has resulted in some exceptional outcomes. However, treatment responses are not comparable across all CF individuals, in part related to the specific CFTR mutations but also due to other less well understood gene-environment interaction heterogeneities(23). The CF phenotype is known to be affected by modifier genes and specifically in the case of ivacaftor, there is evidence of interindividual variability in respiratory treatment responses in the context of the Solute Carrier Family 26 member 9 (*SLC26A9)* gene variants (84-86).

In routine clinical practice, it is not possible to assess true CFTR protein function throughout all cells. Surrogate markers of protein function are currently monitored; through the testing of sweat chloride, basic lung function testing and change in nutritional status and energy expenditure (87, 88). However, these markers do not necessarily provide a complete picture of the impact of these therapies, nor do they robustly predict the treatment effect each individual may or may not expect to experience(89, 90).

IV. General Cystic Fibrosis Care in the setting of Gene Specific Therapies: ***Integrated Excellence***

With an increasing proportion of patients receiving modulator therapy, life expectancy should continue to rise. However, disparities in survival between patient groups, based on mutation class and access to modulator therapy, may start to emerge. Disease trajectories will potentially be very different amongst individuals depending on the age

at which highly effective gene modulator therapy is initiated. Increasing longevity will also herald a new set of problems that will need to be managed.

As CF is a multi-system disease, modulators have an impact upon multiple organs with a variety of changes ensuing. Considerations of these various changes are discussed below.

1. Respiratory Disease

Modulator therapy favourably alters stepwise respiratory disease progression and improves lung function baseline status and rates of decline. However, complete reversal of the parenchymal lung destruction and bronchiectasis is not possible. Two Irish studies identified improvements in the extent of the peri-bronchial thickening and mucus plugging on CT imaging, together with a reduction in the pro-inflammatory cytokines (IL)-1β, IL-6 and IL-8 and the relative abundance of *Pseudomonas* species after ivacaftor treatment (91, 92). However, other studies have not found ivacaftor to have an impact upon the lung microbiome or sputum inflammatory markers(93-95). The effect of other modulators upon the different aspects of structural lung disease is as yet unknown. However, it is hypothesised that early treatment during the neonatal period with agents such as ivacaftor or elexacaftor/tezacaftor/ivacaftor in appropriate patients could prevent the initial development of CF-related lung disease.

Lung function is the traditional parameter for evaluating respiratory disease. However, cardiopulmonary exercise testing (CPET) provides a more complete picture of the integrated cardio-respiratory, muscular and metabolic response to exercise. An increased aerobic exercise capacity is known to correlate with a reduced mortality rate(96, 97). Ivacaftor was found to improve exercise times in patients who have at least one copy of the Gly551Asp-CFTR mutation but an increase in VO_{2max} (maximal oxygen consumption) when compared with placebo was not demonstrated after 28 days of treatment(98). A short case series found an improvement in exercise tolerance in 3 Phe508del homozygotes treated for 2 years with lumacaftor/ivacaftor(99). Further assessment of the effects of modular therapies upon exercise is required.

Patients with extensive lung disease on modulator therapy will still require standard CF care in terms of antibiotic therapy and airway clearance, with lung transplantation the treatment of last resort for those with end-stage lung disease (26, 100, 101). Modulator therapy, however, does reduce both pulmonary exacerbation and hospitalisation rates. In patients treated with ivacaftor, hospitalisation rates have decreased in the region of

40-55%, with CF related admissions reduced by 75-81% (102) and lower annualised hospitalisation rate have been seen in patients with advanced lung disease treated with lumacaftor-ivacaftor(103). This reduction in inflammation and infections prevents the lung function decline that is linked with morbidity and mortality. As a consequence, patients might only develop end stage lung disease later in life, leading to a more elderly CF population being referred for lung transplantation. A change in referral demographics in patients with additional confounding factors, secondary to a different ageing-related profile, may have an adverse impact on transplantation outcomes.

2. Gastrointestinal Disease

2.1 Weight/nutrition

Intestinal malabsorption and pancreatic insufficiency are classic features of CF disease resulting in malnutrition and improvements in nutritional status are associated with decreased mortality (35). Ivacaftor has been shown to significantly improve weight, BMI and quality of life (98, 104). Significant BMI improvements have been found with lumacaftor-ivacaftor use in those aged 12 years and older but not in children aged 6-11 years or with tezacaftor-ivacaftor (61, 68, 105).

The current strategies to combat malnutrition in CF have been a high-energy, high-fat diet together with pancreatic enzyme replacement therapy(106). With the correction of CFTR dysfunction, patients are at risk of becoming obese, in line with the increasing prevalence of obesity in the general population. In 2015 a single-centre in the US found that in 2-18 year old patients with CF, 8% were obese(107). This was significantly different to the 1% obesity rate found in the UK in children ten years previously(108). The population groups between the two studies are not entirely comparable but it does reflect increasing obesity rates in the CF population. In part, this could be related to patients prescribed a high-energy, high-fat diet primarily achieving this through overeating energy-dense, nutrient-poor foods rather than nutrient dense foods(109). It does not bode well for individuals started on modulator therapies that have been accustomed to obtaining calories from such food sources. Having a normal BMI is important for ensuring maintenance of lung function but being overweight or obese does not confer additional benefits (110). Education relating to appropriate food sources and the number of calories that should be ingested for each individual patient is important.

2.2 Luminal Gastrointestinal Disease

CF individuals have abnormal gastrointestinal physiology with delayed gastrointestinal transit, luminal hyperacidity and abnormal small and large bowel colonisation secondary to frequent antibiotic use(111, 112). A decrease in the vicious cycle of abnormal luminal mucus, bowel dysmotility and dysbiosis should result in symptomatic improvements. Ivacaftor has been found to decrease intestinal inflammation and favourably alter the gut microbiome in individuals with at least one gating CFTR mutation(113).

CF is also associated with increased gastrointestinal reflux with a prevalence of 25% in children and 85% in adults. This is significant because micro-aspiration can lead to a deterioration in lung disease(111). A small observational study evaluating reflux symptoms post ivacaftor demonstrated a beneficial response but the study did not include any objective reflux measurements(114). Other studies have found a significant improvement in the early ability to neutralise gastrointestinal pH following ivacaftor(115).

CF is associated with an increased risk of gastro-intestinal malignancies compared to the general population. In non-transplant CF patients there is almost a 20 times increased risk of small bowel cancer and a 10 times increased risk of colon cancer, with transplanted patients with CF experiencing a 2-5 times increased risk compared to non-transplant CF patients(116). Although the exact mechanism underlying the susceptibility to gastrointestinal malignancy in these individuals is unclear, particularly with respect to specific CFTR related effects on cell growth, differentiation and apoptosis, oncogenesis appears to be promoted by the chronic gastrointestinal inflammation and gut microbial dysbiosis (117, 118). Modulator therapy could hypothetically have the potential to reduce this malignancy risk, which is important as CF life expectancy continues to increase.

Intestinal epithelia tissue can be propagated in vitro to create patient-derived stem cell cultures, which that can then be grown into functional epithelia organoids. These organoids can then be used as a functional CFTR assay to assist with CFTR-drug discovery and to assess response to treatments(119). This in vitro testing can be used as a prospective means of selecting efficacious treatments for individuals and can provide patients with a personalised treatment regime(120). Such approaches will become increasingly necessary to provide personalised patient care and particularly for those

individuals who do not respond to standard modulator therapy or who experience side-effects. Furthermore, such strategies will be helpful in drug discovery for individuals with rare mutations.

2.4 Pancreatic Exocrine Function

The majority of individuals (85%) with severe CFTR mutations have pancreatic insufficiency. The loss of pancreatic function occurs early in life due to mucus obstruction within the pancreatic and biliary tree leading to chronic obstructive pancreatitis. In the ivacaftor safety study for children aged 2-5years with CF and a gating mutation, there was a non-significant improvement in pancreatic insufficiency as assessed by faecal elastase measurements at 24 weeks (p=0.0504)(121). It is unknown whether longer treatment periods in these children or initiation as a neonate could prevent the development of pancreatic insufficiency.

For those individuals who are pancreatic sufficient approximately 10-20% will developed pancreatitis(122). A notable reduction in pancreatitis frequency has been seen in a small case series of patients taking ivacaftor (123).

2.5 Hepatobiliary

CFTR dysfunction leads to impaired bile acid hydration, resulting in thick inspissated secretions causing biliary obstruction. Bile salt accumulation can cause heptatocyte damage and inflammation and injury to the portal tracts. The commonest liver manifestations in CF are hepatic steatosis and focal biliary cirrhosis(122). Overall hepatobiliary complications have been found to be reduced with ivacaftor use in a US and UK registry study(124). There has also been a case-report of improvement in hepatic steatosis in an adolescent patient treated with ivacaftor(125). It is unclear whether such changes would occur in adults or with other modulator agents.

3. Metabolic Disease

3.1 Metabolic rate/energy expenditure

Energy balance is determined by basal metabolic rate, physical activity and diet-induced thermogenesis. To maintain neutrality, food intake must match daily energy expenditure, and this is complicated in CF by pancreatic insufficiency. Studies assessing energy expenditure in CF monitor resting energy expenditure (REE), which is generally increased compared to the general population(126). Small studies have identified a reduction in REE by 5% following ivacaftor treatment (127, 128). The impact of other modulator therapies is yet to be formally evaluated.

3.2 Obesity

Obesity is a heterogenous group of conditions with multiple causes and has a significant impact upon physiological function. It causes an increase in risk for type 2 diabetes mellitus, hypertension, coronary heart disease, certain malignancies, obstructive sleep apnoea and osteoarthritis of large and small joints(129). Individuals with CF who are obese will be at risk of all the metabolic complications that ensue from excess body fat accumulation, especially on a background of a high-fat diet. These issues could be further exacerbated with modulator therapy.

3.3 Diabetes mellitus

CF related diabetes (CFRD) is secondary to loss of pancreatic beta cell numbers and the direct effect of the CFTR mutation upon insulin secretion. It is a common extrapulmonary complication, with rates as high as 50% in adults with CF(130). Small studies have found that ivacaftor improves oral glucose tolerance tests by 66-178% after one month of treatment(131, 132). Addtionally, there are case reports of diabetes resolution following ivacaftor initiation(133). It remains to be seen whether other modulator agents will be able to achieve such results for more severe genotypes(134).

3.4 Bone Disease

CFTR is expressed within human osteoblasts, osteocytes and osteoclasts and its dysfunction impacts bone cell activity(9). Low bone mineral density (BMD) is common within the CF population. The pooled prevalence of osteoporosis in adults with CF is

23.5%. As a consequence, fracture rates are higher than the general population; the pooled prevalence of radiologically confirmed vertebral fractures are 14% and nonvertebral fractures are 19.7%(135). The cause of the low BMD is multi-factorial; vitamin D and vitamin K insufficiencies, calcium malabsorption, malnutrition, glucocorticoid usage, delayed puberty and hypogonadism, infection and CFTR dysfunction are all known to be causative factors. Individuals with a normal nutritional status and preserved lung function generally have normal bone mineral density(136) and those individuals with the Phe508del-CFTR mutation have an independent risk factor for low BMD(137). A retrospective analysis of patients treated with ivacaftor demonstrated improvement in CFTR-related bone disease and bone remodelling. Improved nutrition and a reduction in infection rates are likely to be contributory factors together with the direct activity of CFTR within bone cells(138). Furthermore, G551D CF patients have been found to have higher levels of blood monocyte osteoclast precursors compared to healthy individuals and which decrease post ivacaftor treatment(139).

3.5 General Endocrine Disease/Salt-Fluid Balance/ Renal Function

Vitamin D is integral to bone health and vitamin D deficiency is present in more than 90% of individuals with CF. Decreased vitamin D absorption secondary to exocrine pancreatic insufficiency, impaired metabolism and reduced sunlight exposure, are all at play(140). Alteration of these processes through modulator therapy has the potential for hypercalcaemia developing if vitamin D supplementation is not monitored, which in turn could lead to proteinuria or acute renal disease.

CFTR is also highly expressed within the kidney and there is evidence of its involvement with chloride secretion in the distal tubule(141). A range of renal phenotypes have been described, with nodular glomerulosclerosis reported in patients with normal glucose metabolism(142). Despite this, the prevalence of renal disease in individuals with CF is only approximately 5%(143). Modulator therapy could lead to an increase in this, through the unmasking of renal disease as a result of salt retention. Salt intake will need to be closely monitored in these patients. In addition, it may have a significant impact on hypertension and cardiovascular disease in individual patients, as described below.

4. Cardiovascular Disease

Blood pressure elevation is a potential side-effect of treatment with lumacaftor/ivacaftor. Treatment over 96 weeks saw increases in mean systolic blood

pressure (BP) by 5mmHg and diastolic BP by 4mmHg (62). This could be related to salt retention and the unmasking of renal disease. However, if the rises in BP continue unchecked following prolonged modulator usage, CF patients will have an increased risk of complications secondary to hypertensive disease.

CF is associated with chronic inflammation and systemic oxidative stress, both integral in cardiovascular disease processes(144). A recent study has identified CF patients with more severe genotypes to have a greater impairment in cardiovascular function(145). The cardiovascular impact of long-term modulators either started early and/or in older CF patients has yet to be evaluated.

5. Neuropsychosocial factors

CF is not classically considered to affect the nervous system. However, there is increasing evidence of neuropsychiatric abnormalities in individuals with CF that is not necessarily fully explained as an indirect consequence of chronic disease manifestations(146). The CFTR protein is present in the human nervous system, both in the brain and spinal cord neuronal cells, with widespread expression throughout brain tissue(6-8). CFTR transports chloride, an ion involved in the regulation of neuronal excitability. Investigation is still required to identify CFTR's exact role in this context.

Modulators could directly act on the CFTR proteins within the nervous system, altering the pathways affected by dysfunctional CFTR proteins. It is unclear though if they cross the blood-brain barrier. Ivacaftor and its metabolites have been shown to have significant affinity for the 5-hydroxytryptamine (5-HT) and dopamine transporter receptors, with mouse models of depression displaying improved outcome measures similar to that exhibited by fluoxetine(147). Initial modulator trials did not report any deterioration in depression or anxiety rates during treatment (51, 68, 148) and in fact anxiety rates improved post ivacaftor therapy (147). However, there have been reports of deterioration in mental and pulmonary health in some individuals with CF on lumacaftor-ivacaftor therapy who have depression or anxiety being treated with psychotropic medications. These changes are likely to be multi-factorial in nature, with a combination of drug-drug interactions, the direct actions of CFTR modulators on the central nervous system together with the psychological impact of starting new medications all being contributory factors(149). This needs to be taken into account particularly as individuals with chronic diseases, such as CF, are known to be at an increased risk of depression and anxiety. A large multi-centre study across Europe and

the US identified depression rates in the range of 5-19% for adolescents and 13-29% in adults and anxiety rates of 22% in adolescents and 32% in adults (150). These high rates highlights the importance of screening for these conditions within the CF population, especially as depression is associated with decreased lung function and increased CF-related hospitalisation rates(151). Careful planning and monitoring of mental health should be undertaken in high risk individuals starting on modulator therapy.

6. Pregnancy and CF

As survival in CF has increased, so have the rates of pregnancy among women with CF. In the US between 2005-2014, the pregnancy rate was 25.5 per 1000 women with CF(152). Pregnancy is well tolerated by many women but there can be an unpredictable impact on lung function and difficulty in maintaining an adequate nutritional status (153, 154). There is evidence of a downward trend in pregnancy rates in women with CF post ivacaftor availability(152), which is likely secondary to prescribing guidelines advising that modulator therapy is contraindicated during pregnancy. However, there have been a few case reports of the use of both ivacaftor and lumacaftor-ivacaftor use during pregnancy without delirious outcomes(155, 156). As an increasing number of individuals receive modulator therapy, so the implication of these treatments during pregnancy and upon contraceptive usage needs to be evaluated. However, to date these important areas have not been rigorously investigated. The discontinuation of modulator therapy is likely to be undesirable for individuals who have received a treatment from childhood, especially if therapy has maintained disease stability and prevented deterioration in health and well-being. Lumcaftor-ivacaftor interacts with all hormonal contraceptives and current recommendations are for the use of barrier methods or the copper intrauterine device (IUD), which limits appropriate contraceptive options for females of a reproductive age(152, 157). These interactions upon contraceptive efficacy have not been identified for ivacaftor, tezacaftor/ivacaftor or elexacaftor/tezacaftor/ivacaftor. However, there is evidence of an increased frequency of rash in individuals treated with elexacaftor/tezacaftor/ ivacaftor whilst on hormonal contraception(158).

Future Directions

The widespread introduction of modulator therapy is likely to result in both individual

and overall changes in the CF phenotype. Although we might predict that the number of phenotypic manifestations should decrease as fewer patients have dysfunctional CFTR, there is always the possibility that other gene-environment heterogeneities may be unmasked. In this setting, both overall and differential treatment effects represent opportunities for improved understanding of CF pathophysiology and disease manifestations. This can hopefully be translated into improvements in management approach and health-related patient outcomes. Although the latter are to be expected, we also anticipate many new challenges both clinically and within CF research.

From a clinical perspective, the predicted increase in less severe phenotypes will shift the focus of CF care to an increasingly outpatient system. This should lead to a reduction in health care spending for these individuals, particularly when adjusted for number of years lived and quality of life improvements during those years, which need to be recognised when evaluating the cost effectiveness of modulator therapy. Flexibility with service delivery systems and a corresponding change in the current standard model for CF care delivery will be required. Alternatively, this may be re-stated as next generation physicians for next generation thinking and practice in CF (25, 159).

Current CF models of care are predominantly hospital-based with stable patients being reviewed every 2-3 months. These services are largely provided at tertiary centres or when geographical distance is a problem, at general hospitals with links to the tertiary centres. The recommendation is for these centres to provide timely emergency assessment and access for inpatient treatment(26). As life expectancy continues to increase, there will be a larger demand on adult services and an even greater requirement for highly skilled health professionals to manage these patients. In parallel with this will be the need to ensure that high-quality training in the multi-faceted condition of CF is maintained(25). In the context of highly effective gene modulator therapy received from birth, patients may no longer require such regular clinical review. Many services already utilise telemedicine to enable ease of review for patients lively in geographically remote areas. However, despite its utility alongside direct clinical review, some places are slow to initiate its use (160-162). It may become even more important to engage with such strategies when more individuals with CF are stable and have near normal lung function. If patients are receiving modulator therapy during the neonatal period, they may not develop the multi-system complications of CF and thus will have a significantly reduced treatment burden compared CF individuals living today. In line with this, constant review and monitoring of medications must occur as some therapies may be able to be withdrawn as stability with modulator therapy is maintained over

many years.

From a research standpoint, there are still many unanswered questions regarding CFTR dysfunction. Individuals with CF are well-known to have a range of phenotypes. In part, this is related to the amount of functional CFTR protein but as discussed, other factors are at play that are likely to be very important, particularly in specific individuals (Figure 2a & 2b) (163). The focus of modulator therapy has been on increasing the amount of functional cell surface protein. It is not clear what overall and/or differential effect this may have on other CFTR-dependent cellular processes. Although this "revealing" may only occur after prolonged usage and systematic cohort analyses (Figure 2), it may further explain genotype-phenotype heterogeneity and ongoing symptoms whilst on these agents, thereby having the potential to drive further novel, targeted therapies in CF.

CF carriers have been classically thought of as being disease free and indeed there is evidence that the carrier status confers a survival advantage(164-166). However, heterozygotes have also been found to have an increased prevalence of asthma and respiratory infections when compared to the general population. This may arguably result from a degree of CFTR dysfunction in the context of other specific genetic or environmental predisposing factors (167, 168). Additionally, some CFTR dysfunction could also be acquired as a result of specific acute insults or general ageing and life-long accumulated "hits". Smoking confers a 68% reduction in CFTR activity in human bronchial epithelial cells(22). These observations raise the question as to whether patients with borderline sweat chloride values would benefit from CFTR modulation, either in the short or long-term. It also raises the ethical dilemma as to how to use modulator therapies in the setting where an individual continues to smoke or inhale other noxious agents despite counselling. Detailed systematic clinical audit and ongoing hypothesis driven research will need to go hand in hand in order to best address these questions.

Conclusion

Cystic Fibrosis care has advanced dramatically over the last thirty years and indeed since it was first noted as an entity in the 1930s(169). If this revolution is to continue to its extreme and therefore achieve maximum real impact upon CF morbidity and mortality, treatment options need to be available from birth. Animal models have identified that inflammation is absent in the lungs of newly born pigs with CF. Once exposed to bacteria, their ability to eradication these organisms is inferior to controls (170). The impaired pulmonary host defence is comparable to that which is seen in infants with CF. Controversy exists as to the precise processes that lead to inflammation, but what is clear is that the ensuing cycle of inflammation and infection results in lung parenchymal destruction, bronchiectasis and respiratory failure, albeit at varying rates in different individuals (1, 12). Ensuring that patients are diagnosed as early as possible is integral in preventing this vicious cycle from developing. The introduction of newborn screening programmes for CF in some countries is a positive step, but those with less common mutations may still have delays in their diagnosis (171). The treatments that are then offered must be targeted at favourably altering the dysfunctional CFTR protein. With over three hundred disease causing mutations now identified, patients would ideally have individualised treatment plans. Modulator therapy is currently in the strongest position for achieving this goal for most CF individuals – but not all. Additionally, it is unlikely that a single CFTR modulator therapy will be able to treat all aspects of CF pathology. Arguably the ideal CF treatment would be a one-off gene therapy agent that could fully restore CFTR protein function and regulation and thus treat all aspects of abnormal CFTR dysfunction. However, this is by no means an easily achievable goal. Either way, an ongoing commitment to research and development should provide ever-improving answers for patients with CF and their families as the CF community continuously strives for longevity and improvements in quality of life.

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Reimagining Cystic Fibrosis Care Figure Legends

Figure 1 CFTR Classification Table

The classification systems divide mutations into discrete groups determined by the predominant CFTR defect. However, these systems may not be mutually exclusive for all mutations. For example, the p.Phe508del-CFTR is predominately class II but does also have some class III & class VI properties

Figure 2 Cell biology of CFTR - abnormal CFTR protein results in the uncoupling of CFTR dependent processes at all levels from intracellular dynamics to cell membrane function

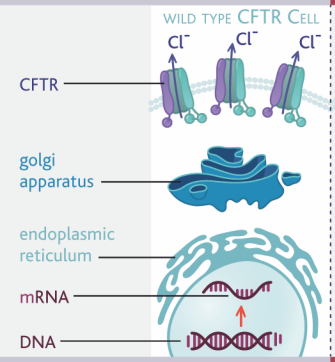
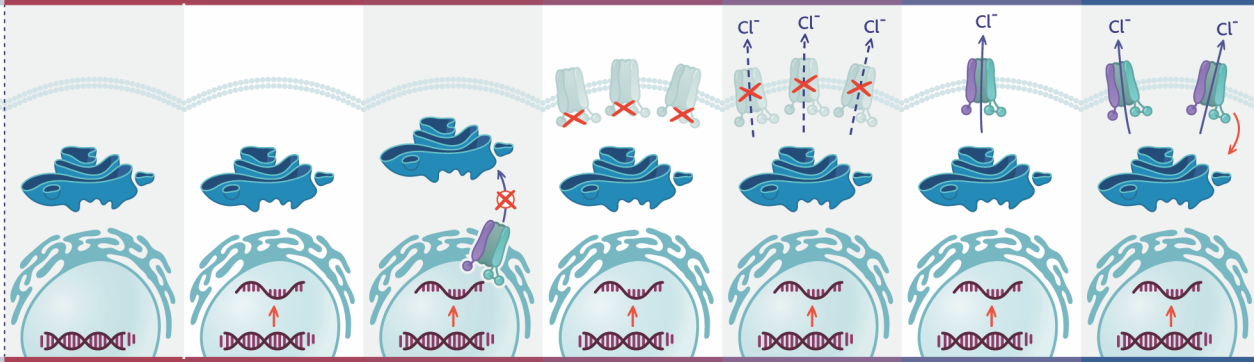
Various cell processes are dependent upon CFTR as schematically represented in the above diagrams (A & B). Intracellular dynamics of CFTR processing include protein synthesis, protein folding and trafficking leading to CFTR proteins reaching the cell membrane. Significantly misfolded or dysfunctional CFTR is redirected to the proteasome for degradation and recycling.

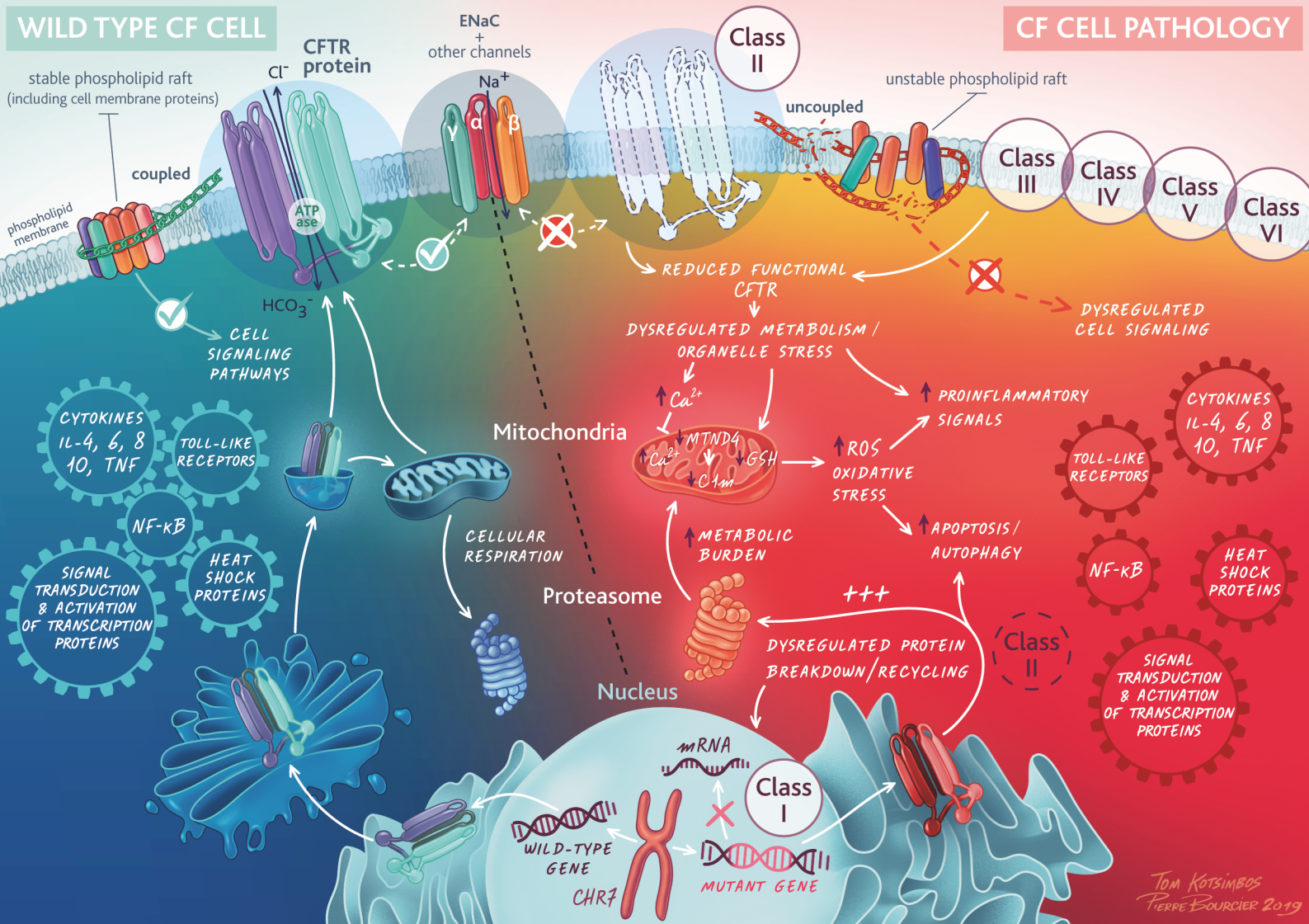
Ubiquitination of abnormal proteins targets them for the proteasome where degradation occurs. Abnormal CFTR proteins (large complex proteins) requiring degradation via the proteasome necessitate an increase in the metabolic requirements of the cell. It is hypothesised that CFTR both ‘burdens’ and ‘blocks’ the proteasome. This impacts upon multiple processes, particularly antigen processing, cell cycle and division, apoptosis and the modulation of cell surface receptors/channels and secretory pathways, at the same time as increasing the metabolic requirements of the cell.

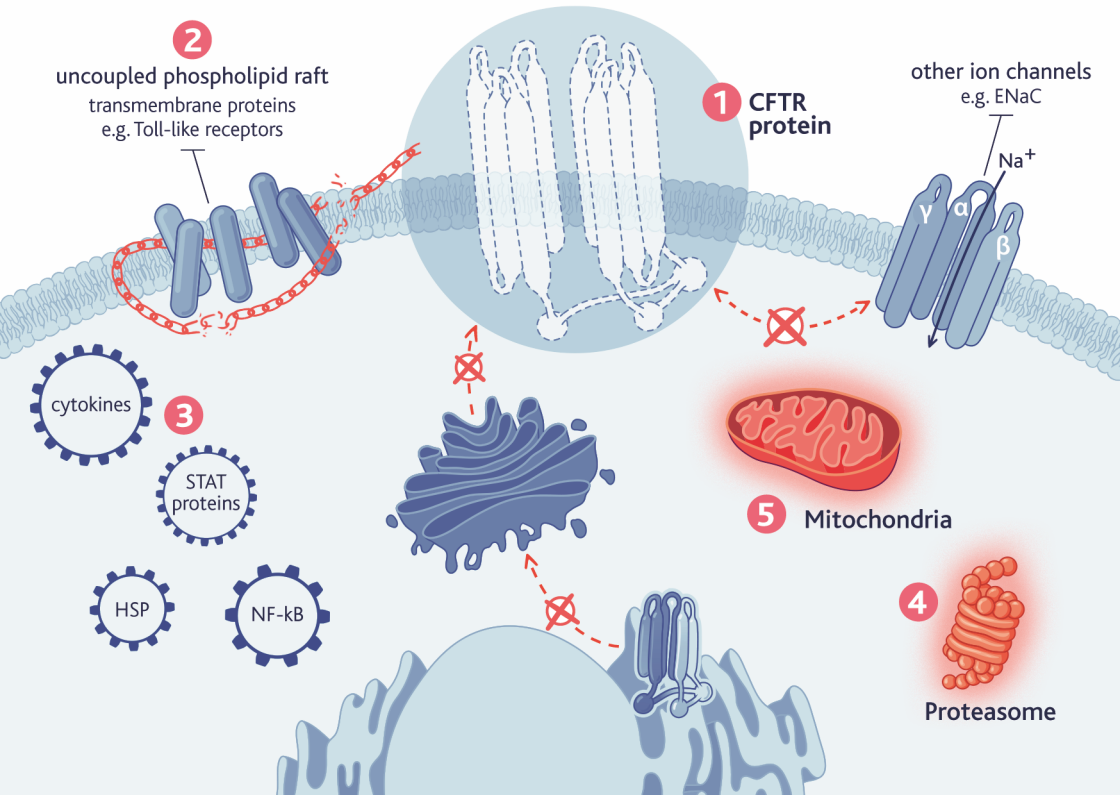
Figure B

Schematic of the abnormal cell processes occurring within the cell:

1) Channelopathy – resulting in reduced chloride and bicarbonate transport across the cell membrane with subsequent uncoupling of other ion channels, for example, unopposed sodium transport via the ENaC channel
2) Abnormal lipid raft stabilisation and cell surface signal transduction dysfunction - system 'uncoupled'
3) Intracellular signal transduction processes 'uncoupled' in either positive or negative directions
4) Increased CFTR turnover due to an increase in misfolded CFTR or increase CFTR turnover dynamics. This hypothetically causes proteasome dysfunction due to the increased CFTR being degraded and thus the inability to process and degrade other proteins within the cell.
5) A high energy state within the cell due to the uncoupled processes. This is exacerbated by mitochondrial/metabolic dysfunction, either indirectly through the inability to meet the increased metabolic demands, or directly through CFTR dependent gene associated 'uncoupling' of oxidative phosphorylation.

Traditional classification	CLASS I		CLASS II	CLASS III	CLASS IV	CLASS V	CLASS VI
Marson, Bertuzzo and Ribeiro's classification	CLASS IA	CLASS IB	CLASS II	CLASS III	CLASS IV	CLASS V	CLASS VI
De Boeck and Amaral's classification	CLASS VII	CLASS I	CLASS II	CLASS III	CLASS IV	CLASS V	CLASS VI
							
CFTR defect	No mRNA	No functional protein	No protein trafficking	Impaired channel gating	Decreased channel conductance	Reduced protein synthesis	Decreased protein stability
Specific mutation examples	Dele2,3(21kb), 1717-1G → A	Gly542X, Trp1282X	Phe508del, Asn1303Lys, Ala561GLu	Gly551Asp, Ser549Arg, Gly1349Asp	Arg117His, Arg334Trp, Ala455Glu	Ala455Glu, 3272-26A → G, 3849+10 kg C → T	c.120del23, rPhe508del
Treatment strategies	Unrescuable	Rescue synthesis	Rescue protein trafficking	Restore channel activity	Restore channel activity	Correct splicing	Promote protein stability
Medications	None	None	Lumacaftor-Ivacaftor, Tezacaftor-Ivacaftor	Ivacaftor	Ivacaftor (some mutations)	Tezacaftor-Ivacaftor (some mutations)	Tezacaftor-Ivacaftor (some mutations)
Clinical features	**More-severe disease**					**Less-severe disease**	


WILD TYPE CF CELL
CF CELL PATHOLOGY
CFTR protein
ENaC + other channels
Class II
stable phospholipid raft (including cell membrane proteins)
coupled
phospholipid membrane
ATP ase
Cl⁻
Na⁺
γ
α
β
uncoupled
unstable phospholipid raft
Class III
Class IV
Class V
Class VI
HCO3⁻
CELL SIGNALING PATHWAYS
REDUCED FUNCTIONAL CFTR
DYSREGULATED CELL SIGNALING
DYSREGULATED METABOLISM / ORGANELLE STRESS
↑Ca2+
Mitochondria
↓MTND4
↑Ca2+
↓GSH
↓C1m
↑ROS
OXIDATIVE STRESS
↑PROINFLAMMATORY SIGNALS
CYTOKINES IL-4, 6, 8 10, TNF
TOLL-LIKE RECEPTORS
NF-κB
HEAT SHOCK PROTEINS
SIGNAL TRANSDUCTION & ACTIVATION OF TRANSCRIPTION PROTEINS
CELLULAR RESPIRATION
↑METABOLIC BURDEN
↑APOPTOSIS / AUTOPHAGY
Proteasome
+++
DYSREGULATED PROTEIN BREAKDOWN/RECYCLING
Class II
Nucleus
mRNA
Class I
WILD-TYPE GENE
CHR7
MUTANT GENE
Tom Kotsimbos
Pierre Bourcier 2019


2
uncoupled phospholipid raft
transmembrane proteins
e.g. Toll-like receptors
1
CFTR protein
other ion channels
e.g. ENaC
Na+
γ
α
β
cytokines
3
STAT proteins
HSP
NF-kB
5
Mitochondria
4
Proteasome