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Perspectives on the translation of *in-vitro* studies to precision medicine in Cystic Fibrosis

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ABSTRACT

Recent strides towards precision medicine in Cystic Fibrosis (CF) have been made possible by patient-derived *in-vitro* assays with the potential to predict clinical response to small molecule-based therapies. Here, we discuss the status of primary and stem-cell derived tissues used to evaluate the preclinical efficacy of CFTR modulators highlighting both their potential and limitations. Validation of these assays requires correlation of *in-vitro* responses to *in-vivo* measures of clinical biomarkers of disease outcomes. While initial efforts have shown some success, this translation requires methodologies that are sensitive enough to capture treatment responses in a CF population that now predominantly has mild lung disease. Future development of *in-vitro* and *in-vivo* biomarkers will facilitate the generation of new therapeutics particularly for those patients with rare mutations where clinical trials are not feasible so that in the future every CF patient will have access to effective targeted therapies.

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Introduction

CF is an autosomal recessive disease caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene [1]. CFTR functions as a chloride and bicarbonate anion channel at the apical membrane of epithelial tissues [2]. To date, 360 disease causing gene variants of the *CFTR* gene have been validated of a total of over 2000 identified (cftr2.org). Disease causing variants have been sorted into seven classes with respect to their molecular consequences [3]. The major CF causing variant: F508del, leads to misprocessing with aberrant trafficking and altered channel function [2]. CF affects multiple organs include the lungs, pancreas, liver, intestines, and the reproductive tract, but lung disease defines the long-term prognosis in most subjects [4]. The pathogenesis of CF lung disease is thought to be initiated by altered properties of the airway surface fluid, compromised innate immunity and mucus obstruction of the airways, eventually leading to recurrent infections, inflammation, fibrosis and loss of lung function [5]. Lung disease starts as early as infancy and progressively worsens throughout the individual's life [6,7]. Standard therapies aimed at treating symptoms and effects of CFTR dysfunction include chest physiotherapy, inhaled mucus clearance targeting therapies such as hypertonic saline and dornase alfa, as well as the treatment of infections with antibiotics [8]. Small molecule-based modulator compounds that target and ameliorate the basic defective protein have become available more recently changed the care of people with CF (pwCF) and its progression over time [4].

The first licensed CFTR modulator was ivacaftor, a potentiator that improves CFTR function by increasing the probability of the channel opening at the cell surface [9]. When administered alone, clinical efficacy of ivacaftor was limited to mutations with apical channel expression, class III gating mutation, such as G551D [10]. Correctors, such as lumacaftor or tezacaftor, increase protein processing, and trafficking to the membrane surface [11]. Subsequent drug development programs used combination therapies targeting the most common CFTR mutation (F508del) for which up to 90% of pwCF carry at least one copy in North America [11]. Compared to normal Wild Type (WT)-CFTR, F508del-CFTR has reduced membrane expression, decreased function and higher rate of turnover at the cell surface due to intramolecular instability caused by protein misfolding and misassembly during synthesis [12]. The combination of a corrector and a potentiator, such as lumacaftor and ivacaftor (LUM/IVA) () or tezacaftor and ivacaftor (TEZ/IVA), led to modest clinical benefits for patients

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with two copies of the F508del mutation [13,14]. Subsequently, the triple modulator combination therapy, TRIKAFTA™, containing two correctors: elexacaftor and tezacaftor, and the potentiator ivacaftor (ETI) led to substantial improvements in lung function, reduction in respiratory symptoms and pulmonary exacerbations in pwCF with at least a single copy of F508del [15]. However, variability in response exists and not all F508del CF patients derive the same benefit from ETI treatment [15].

There are multiple, relatively rare, missense mutations that induce similar (but not identical) defects in CFTR as the F508del mutation [16]. The US Food and Drug Administration (FDA) has accepted the concept that positive drug responses in the cell line: the Fisher Rat Thyroid (FRT) cell line, may be used as a surrogate for clinical efficacy. The use of these *in-vitro* data enabled the label extension of ivacaftor, ETI and TEZ?IVAto multiple rare mutations [16], but a heterologous expression system is not representative of individual drug responses; therefore, drug testing in relevant, patient derived tissues may be necessary for precision medicine in CF.

While *in-vitro* systems can provide proof of concept evidence for choosing a potentially promising compound, confirmation of efficacy in clinical studies is needed to determine the safety and efficacy of modulators in individual pwCF. This is especially challenging early in the disease process where many outcome measures are not sensitive enough to capture treatment effects. Here, we summarize the current status of *in-vitro* systems used evaluate CFTR modulator response and describe novel clinical outcome measures that will help to evaluate drug efficacy in individual subjects.

In-vitro models

Primary Bronchial Cells

Human Bronchial Epithelial (HBE) cells are primary airway epithelial cells isolated from the bronchi of lung explants and when differentiated at the air-liquid interface (ALI), exhibit many of the functional properties expected for the epithelium of the proximal airways. As a result, cultures derived from CF patients are well suited to investigations of the primary defects and secondary phenotypes associated with CF-causing mutations. Bioelectric measurements have captured CF-induced defects in anion conductance and associated alterations in apical sodium conduction through ENaC [17]. Optical techniques have been effective in measuring the reduced airway surface fluid accumulation, ciliary beat frequency, and mucostasis that occur as a consequence of the primary defects [18-20].

Preclinical studies of CFTR modulators or other interventions using primary bronchial cultures from pwCF, have shown rescue effects on both the primary and secondary defects. The molecular and physiological basis for patient-specific in-vitro and clinical responses, even amongst those with identical CFTR genotypes, remains unknown. This is highlighted by the potential role of genetic modifier [21-24] but other many other variables, including the inflammatory status of each individual's airway tissues are likely to influence individual drug responses [25]. Future studies that integrate whole genomic, transcriptomic, and proteomic data will contribute to our understanding of the basis of differential in-vitro drug responses.

However, the role of CF bronchial epithelial cultures in precision medicine for CF is limited by relatively poor accessibility to bronchial explants, particularly for testing therapies targeting rare CF-causing mutations. Further, post-transplant lung tissue from CF and non-CF individuals may carry underlying co-morbidities that could confound the interpretation of *in-vitro* testing. Finally, the field acknowledges the need for accessible and scalable tissue models with which to understand disease pathogenesis and to test interventions for personalized therapies (Table 1)

Primary Nasal Cells

The *in-vitro* expansion and culture of airway cells was first developed through the reprogramming primary epithelial cells using both Rho kinase inhibitors and feeder cells [26]. More recent developments have bypassed the need for feeder cells through ampliation of basal cells and the directed differentiation of airway tissue expressing functional CFTR [27]. Human Nasal Epithelial (HNE) brushings and nasal cultures differentiated at ALI have also been employed in CF therapy testing [28]. These samples are more accessible than bronchial tissue explants and recapitulate many of the bioelectric properties described for differentiated bronchial epithelial cultures as measured in the Ussing chamber.

Importantly, Pranke and colleagues showed that there is a positive correlation between patient specific modulator responses measured in primary nasal epithelial cultures and bronchial cultures [28,29]. Further, because the nasal cultures model a complex tissue with multiple cell types, including ciliated and secretory cells, they can be used to evaluate the effects of CFTR modulators on reversing secondary CF phenotypes such as decreased air surface liquid height, pH, viscosity, and mucociliary movement [24,30,31]. Hence, HNE cells are recognized as a relevant surrogate for HBE cells in preclinical studies of CFTR modulators [28,29,32].

Interestingly, studies have shown that *in-vitro* responses to modulators in patient derived HNE are variable amongst donors harbouring identical CFTR genotypes, variability that may be dependent on other status of modifier genes [33]. Debley and colleagues showed that the magnitude of *in-vitro* responses to modulator treatment in nasal epithelial cultures correlated with patient specific clinical outcomes such as FEV1 [34]. Other studies supported the application of drug testing in HNE cultures as proof-of-concept for follow-up, N=1 clinical trial for individuals with rare CF mutations [34,35]. Hence, HNEs may provide a useful model for drug testing and therapy development for those people harbouring understudied CF causing mutations [34-36].

Beyond the 2D monolayer model, HNE cultures have been utilized for making HNE organoids. Lui et al, demonstrated HNE organoids as an effective *in-vitro* model to in studying CFTR fluid transport function. The authors found significantly less luminal area and forskolin induced swelling in CF HNE organoids compared to non-CF controls over 1-hour, greater differences were seen over 8 hours [37]. Organoids swelling in F508del CF HNE organoids can be rescued with CF modulators. Both baseline and modulator rescued organoid swelling has been shown to correlate with clinical outcome, FEV1 and sweat chloride concentrations (SCC) [38].

CF Centres around the world have established nasal culture biobanks for the purpose of validating the use of this model to predict therapeutic outcomes and to advance the development of therapies for rare mutations [28,34,35,39,40]. In real time, the potential of nasal epithelial cultures to provide a predictive biomarker of the clinical efficacy of CFTR modulator drugs is being tested, as access to the relatively effective modulator, TRIKATA™broadens, globally.

Rectal organoid models

Stem cell derived organoids have revolutionized basic biomedical and translational research in many fields [41-43]. The seminal paper by Dekkers et al [44], prompted international study of rectal organoids and recognition of the potential for this model to facilitate drug development and precision medicine for CF. Stem cells in the crypt epithelium of patient derived intestinal tissue provide a renewable source of tissue containing the expected cell types [41]. Organoids, oriented such that the apical membrane faces inward, and the basolateral membrane is supported by extracellular matrix like Matrigel, generate a fluid filled lumen [41]. The size of this lumen is an indirect measure of CFTR channel activity, and it increases with agonists of CFTR like the forskolin [44]. The directional movement of chloride

Table 1
Description of current *in-vitro* models and assays measuring CFTR channel function and drugs response

Tissue Models	Assays (in-vitro)	Opportunities	Challenges
Human Bronchial Epithelial 2D Cultures (HBE) at Air/Liquid Interface (ALI)	• Bioelectric • (Ussing Chamber, TEVV) • Mucociliary movement	• Bioelectric assays of CFTR modulators in CF-HBE correlate with clinical efficacy for certain CF-causing mutations • Potential to study complex airway phenotypes (other ion channels, mucociliary movement etc.)	• Tissue from transplant donors with end stage lung tissue • Limited availability of primary cultures from people with rare CF-causing mutations • Limited scalability for reproducing in-vitro responses to investigational compounds
Human Nasal Epithelial 2D Cultures (HNE-ALI)	• Bioelectric • (Ussing Chamber, TEVV) • Mucociliary movement	• Bioelectric assays of CFTR modulators in CF-HNE correlate with clinical efficacy for certain CF-causing mutations • Potential to study complex airway phenotypes (altered ion transport, mucociliary movement etc) • Nasal tissue is less affected than bronchial tissue by disease • Reasonable availability of nasal cultures from people with rare CF-causing mutations	• Limited scalability for reproducing in-vitro responses to investigational compounds
Rectal Organoids (3D)	• Imaging of forskolin-induced swelling (FIS)	• FIS assays of CFTR modulators in CF-rectal organoids correlate with clinical efficacy for certain CF-causing mutations. • Opportunity to test multiple interventions simultaneously • Reasonable availability of organoid cultures from people with rare CF-causing mutations.	• Limited potential to study other ion channels
Induced Pluripotent Stem Cells differentiated to lung, intestine, ducts (2D & 3D)	• 2D: Bioelectric and Fluorescence based assays of chloride conductance • 3D: FIS	• Scalable tissue models and fluorescence-based assays provide opportunity for medium to high-throughput comparisons of investigational compounds. • Isogenic controls (with CF mutation corrected by genome editing) provide patient specific controls • Multiple CF-relevant tissues can be generated and studied from the same iPSC lines	• Current differentiation protocols lead to incomplete maturation of tissue. • Generation of patient specific iPSCs and isogenic controls costly, highlighting the advantage of open-access biobanks.

through CFTR followed by cation uptake may contribute the osmotic driving force for luminal swelling. Organoid swelling is lacking in CF organoids but can be rescued by treatment using modulators [44]. However, forskolin-induced swelling (FIS) may underestimate the function of Wt-CFTR function in organoids due to saturation effects, and therefore over-estimate the proportional rescue of mutation CFTR by CF modulators. Dekkers et al, demonstrated organoids with greater the Steady-state Lumen Area (SLA) experienced less overall swelling with forskolin stimulation in non-CF organoids and in CF organoids expressing highly functional CFTR mutants [45]. Therefore, the FIS and SLA assays together provide a robust *in-vitro* method for measuring the intrinsic defect caused by CFTR mutations and the response to CFTR therapies in patient-derived tissue.

To date, several studies have shown that patient specific, organoid swelling responses correlate with clinical outcomes after treatment with CFTR modulators. Correlation with clinical biomarkers, FEV_1 and SCC suggest that organoid swelling can potentially, be used to predict individual therapeutic outcomes [45-47]. Such a predictive model is required for those people lacking approved therapies because they harbour rare, understudied CF causing mutations [45,48]. CF research centres around the world have established rectal organoids biobanks for the purpose of validating the use of this model for therapeutic efficacy of approved drugs for rare CF-causing mutations and discovery new therapies [45,46,49]. However, the correlation by forskolin-induced welling of rectal organoids and patient-specific clinical improvement measured through *in-vivo* biomarkers such as, SCC, FEV1, nasal potential difference and intestinal current measurements, are not universally observed [50]. These discrepancies may reflect the modest effect size for CFTR modulators like LUM/IVA and TEZ/IVA. Hence, the ongoing assessment of correlations between in-vitro and clinical outcomes in response to the highly effective modulator, ETI, will be particularly informative. Recent studies found a significant positive correlation in modulator rescued CFTR response between rectal organoids and HNE cells. Thus, supporting the use of either *in vitro* models as potential personalized preclinical translational models in testing the efficacy of CF modulators [38,51].

Airway organoids

Primary airway organoids (AO) can be derived from tissue resections or broncho-alveolar lavage fluid from CF patients [52] and they can be used to study CF lung disease and response to CFTR modulators. Interestingly, in addition to exhibiting defective CFTR mediated swelling as first described in rectal organoids, AOs from F508del CF individuals also exhibit secondary phenotypes such as mucus accumulation [52], making them a more faithful model of CF lung disease. Importantly, CFTR modulators do partially rescue defective forskolin stimulated swelling of AOs, as in the case of rectal organoids [52]. However, due to safety concerns related to obtaining bronchial cells, the widespread use of this model for preclinical trials of CF therapies has been limited.

iPSC derived organoid models

Induced Pluripotent Stem Cell (iPSC) derived models are also recognized as useful tools for understanding CF pathogenesis in different tissues. iPSCs are created by reprogramming somatic cells to regain

their pluripotency [53]. In this state, patient-specific iPSCs are infinitely expandable and hypothetically, they can be differentiated to any of the tissues that are impacted in CF. Hence, they provide a renewable source of tissue with which to model patient-specific disease and therapies [54].

iPSC derived lung models

are still evolving toward to the goal of recapitulating the complex properties of the proximal and distal airway [55-58]. Importantly, the functional expression of CFTR channel activity served as an early benchmark for success of directed differentiation toward mature lung tissue. Further, the loss of this function in CF iPSCs differentiated toward lung and the positive response to CFTR modulators, heralded the usefulness of these tissues for therapy development. Given the renewal capacity of iPSCs, this feature can be exploited for high-throughput screening of novel therapies [59]. Both two dimensional models of airway tissue differentiated under fluid submerged conditions or at ALI and 3D models can report the primary defect in CFTR function caused by CF associated mutation. For 2D cultures, defects in CFTR channel activity associated with mutations are measured in the Ussing chamber or using fluorescence-based assays of chloride conductance or membrane potential dye, FLIPR [21,59]. In 3D cultures, defective CFTR channel activity has been reported as poor forskolin-induced swelling [56].

There has been significant progress in understanding the developmental cues that drive iPSC differentiation toward tissues that model the proximal or distal airways, (as reported by transcriptomic and tissue imaging studies) [56,58,60]. However, the fidelity with which recent differentiation protocols generate mature lung or can serve to model CF pathogenesis beyond the loss of CFTR channel activity, remains unclear [61]. iPSC differentiation protocols which result in more mature tissue need to be developed. However existing iPSC derived immature lung models are still able to predict patient specific response to drug modulators [62]. Other differentiation protocols focusing on the differentiation of iPSCs to basal cells have been developed enabling the *in-vitro* modeling of airway diseases [63].

Transcriptomic studies of native tissues continue to instruct the field regarding normal lung biology and cell type composition [61]. The first, single-cell RNA sequencing studies led to the identification ionocytes and created an updated list of major cell types found the airway epithelium [64,65]. Only recently, are single cell transcriptomic studies of native CF airways starting to emerge to reveal possible triggers of CF pathogenesis in addition to defective channel activity [66]. Single cell proteomics studies will also be required to fully contextualize CF disease progression in the lung [67]. Altogether, these insights will define new molecular benchmarks with which to evaluate the fidelity of iPSC derived lung tissue models to recapitulate normal lung tissue and the effects of CFTR mutations.

iPSC derived intestinal models

have become another well-established model for precision and personalized medicine [68-70]. There has been substantive progress toward developing protocols for differentiating iPSCs to intestinal or colonic tissue [70-72]. Similarly, CFTR channel activity, measured directly in 2D format or indirectly as forskolin-induced organoid swelling, has been used as a benchmark for epithelial tissue generation of patient derived intestinal and colonic organoids [70]. CF and gene edited isogenic control iPSC derived intestinal organoids have been used for to test existing CFTR modulators and for high throughput screening of new potential modulators [59]. However, similar to the situation with airway cultures differentiated from iPSC, the transcriptomic profile for iPSC intestinal organoids is more representative of the immature intestinal state [73]. Further differentiation from the immature state requires transplantation into an animal model and engraftment [73].

iPSC derived ductular models

Stem cell-based models provide the opportunity to study CFTR function and drug response in organs that are not readily accessible for sampling, such as the pancreas and the liver. The pancreas is composed of exocrine cells (acinar cells), endocrine cells, including beta cells, and duct cells [74,75]. CFTR is expressed in the luminal membrane of ductular cells and loss of its channel activity is thought to be responsible for CF disease progression with duct obstruction, pancreatic enzyme insufficiency and eventually, degradation of the ductular and exocrine and later in life- the endocrine pancreas [76]. iPSC can be singly differentiated to each of these pancreatic tissue types in-vitro as well as complex pancreatic organoids consisting of mixture of acinar and ductal cells [77-81]. Reminiscent of the progress in modeling other CF-affected tissues, CFTR channel function and the loss incurred by disease-causing mutations, was used to provide evidence for differentiation to ductular epithelium. However, the promise of these models to inform disease pathogenesis remains somewhat limited. Transcriptomic studies suggest that the differentiated tissues resemble fetal pancreatic state [78]. Further, there is a need to reconstitute the complex tissue comprised of multiple cell types with fidelity in order to understand CF pancreatic disease progression and the effect of therapies on organ regeneration. iPSCs have also been differentiated to cholangiocytes, those cells that express CFTR and act to modify bile composition in the biliary duct [82]. The connection between loss of CFTR on the luminal membrane of bile ducts and the associated liver disease (including cholestasis and cirrhosis) is incompletely understood. iPSC-derived models of cholangiocytes promise to provide insight into CF related liver disease. Notably, a recent publication describes a protocol that is highly efficient in generating mature, ciliated cholangiocytes [83]. This accomplishment enabled studies of CFTR function in a relevant environment, namely, in the presence of cilia-bending sheer force. Interestingly, sheer force alone was sufficient to stimulate F508del-CFTR channel function after correction of its trafficking defect without the need of exogenous potentiators [83]. These findings highlight the importance of generating high fidelity models, capable of responding to natural mechanical stimuli as these models will better predict disease progression and therapeutic needs.

Future application of stem cell-based models for treating CF by gene editing or cell replacement

Genome Editing

Although there are clinically approved CFTR modulators available for most patients, such modulators are not approved for certain rare mutations, including premature stop codon mutations. Repair of CF-causing mutations by recent gene editing strategies, including Base and Prime editing, promises to repair almost 99% of mutant CFTR alleles and allow the normal version of the CFTR is permanently expressed according to endogenous regulatory mechanisms. While the successful repair of a CF-causing variant by gene editing was achieved in a cell line almost 10 years, there is a need to determine the best delivery vehicle for optimal efficacy and safety as well as the consequences of editing on the complex tissues. Organoids that model the complex consequences of CF disease are ideally suited for testing genome editing strategies. Geurts and colleagues showed in proof-of-concept studies, that a rare, CF-causing nonsense mutation (W1282X) could be corrected by Base editing in rectal organoids, restoring the functional expression of CFTR [84]. Yet, the impact of repairing CFTR on tissue homeostasis remains understudied and present an exciting challenge for future research.

Cellular Replacement Therapy

Going forward, stem-cell derived organoids offer opportunities for cell replacement therapy. A recently published study showed the

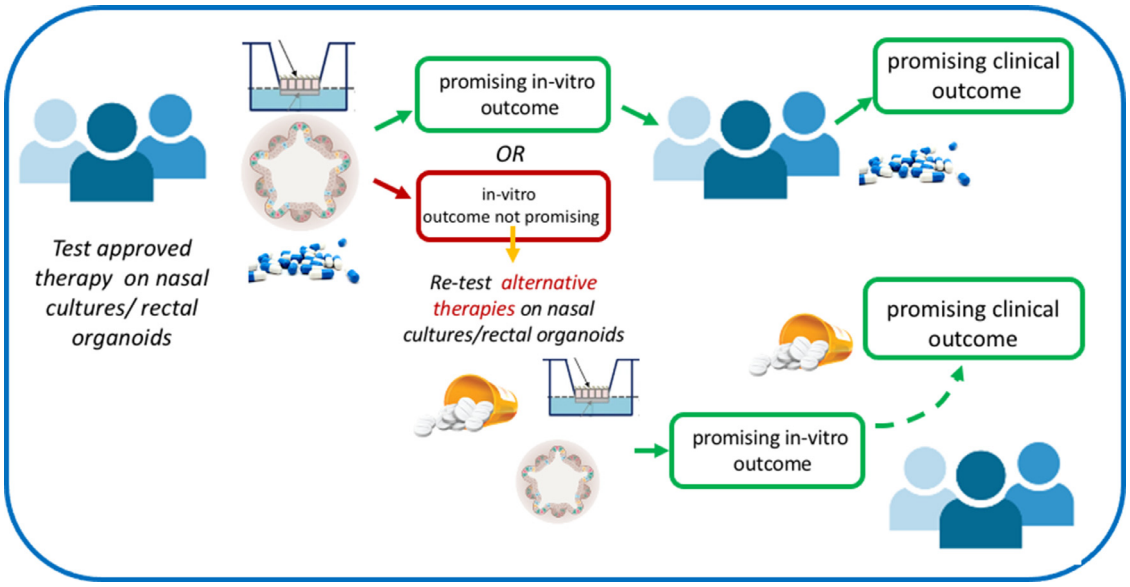

Figure 1. Patient derived primary and iPSC derived epithelial culture have the potential to inform precision medicine through increasing confidence in existing therapies and discovery of customized alternative therapies (BioRender generated the image of the organoid) .

effective treatment of short bowel syndrome in a mouse model of this disease with transplantation of human ileal organoids. Therefore, allogenic transplantation of mutation corrected iPSC derived organoids of CF patients may be the first step to tissue replacement and organ regeneration [85]. However, a limitation being the requirement of decellularized tissue for the engraftment of organoids in host tissue. This needs to be addressed before broad scale application of cell replacement therapy.

Translation from preclinical studies to clinical care

In the previous sections, we discussed the challenges associated with generating, patient-specific, high-fidelity *in-vitro* models of disease and therapies. To realize their promise to inform precision medicine, their development must occur in a coordinated manner with clinical research aimed at detecting multi-system clinical outcomes with sensitivity and specificity for individuals with CF (Figure 1).

Clinical outcome measures of CF disease and functional outcome measures of therapeutic effects of CFTR modulator therapies in the lung

Sweat chloride analysis is used in clinical trials of CFTR modulators as a measure of CFTR function [10,15,86,87] as it is widely available and non-invasive. Studies have demonstrated that sweat chloride captures the efficacy of CFTR modulators as it decreases shortly after initiation of therapy. Trials with the new CFTR modulator ETI demonstrated large changes in sweat chloride (up to -60.9 mmol/L in children 6 to 11 years of age) [15,87] whereas the changes were more modest in clinical trials on lumacaftor/ivacaftor [86,88]. However, although the changes are significant in between drug and control groups the sweat chloride changes do not correlate with measures of lung function on an individual basis [89].

Phase 3 randomised controlled trials of CFTR modulator therapies in pwCF included participants 12 years and older with confirmation of safety in subsequent trials involving younger individuals. These studies have used spirometry, more specifically absolute change in percent predicted FEV_1 as a primary outcome measure [15,90]. FEV_1 is widely available and correlates with long term outcomes such as mortality in pwCF. Its most significant limitation is its lack of sensitivity in early disease [91]. Most pwCF have normal FEV_1 results in early childhood, but evidence of progression of structural lung disease, demonstrating the difficulty to detect early changes in lung disease with this outcome measure [92].

Therefore, although correlation between in-vitro models, sweat chloride analysis and FEV_1 have been demonstrated more sensitive measures of lung function are necessary to identify changes on an individual basis. The different clinical outcome measures for assessing CFTR modulators are shown in Table 2.

Outcome measures beyond FEV_1

Multiple breath washout

Multiple Breath Washout (MBW) is more sensitive in detecting changes in lung function compared to spirometry. Its main outcome, Lung Clearance Index 2.5 (LCI), measures ventilation inhomogeneity by calculating the number of functional residual capacity turnovers needed to reach the point at which 1/40 (2.5%) of the initial gas concentration remains in the exhaled breath [93].

Davies et al. performed a placebo-controlled, double-blind crossover study of ivacaftor in pwCF with at least one G551D CFTR mutation and mild lung disease (NCT01262352). LCI and FEV_1 were both significantly improved from baseline at all time points of the study but post-hoc analysis demonstrated that the sample size needed was three to four times smaller for LCI compared to FEV_1 [94]. For an efficacious drug such as ivacaftor in pwCF with G551D CFTR mutation, the treatment effect and variability of LCI in this study suggest that a sample size as small as five is require to achieve 80% power for between-group comparison [94].

LCI was also sensitive to detect the effect of ivacaftor in an observational study in pwCF $\geq$12 years of age and at least a class III CFTR gating mutation starting ivacaftor with baseline FEV_1 % predicted from 38% to 122% (mean 70%) [95]. Although the mean FEV_1 and LCI both improved significantly at 1 month compared to baseline, in this group of patients with a wide range of lung function, only LCI showed consistent improvement in all participants [95].

LCI was first used as a regulatory approved primary outcome measure in a phase III randomized placebo-controlled clinical trial on LUM/IVA in children (age 6-11 years) homozygous for F508del (NCT02514473). Lumacaftor/ivacaftor significantly improved LCI and FEV_1% predicted compared to placebo [86]. In the clinical setting, MBW testing was performed in addition to spirometry in the open

Table 2
Clinical outcome measures of therapeutic effects of CFTR modulators

	Opportunity	Limitation
Sweat chloride	Widely available Reflects a CFTR specific response Rapidly changes after initiation of therapy	Not correlated with measures of lung function on an individual basis
Spirometry (FEV_1)	Widely available Well established	Lacks sensitivity in early disease
Multiple breath washout (LCI)	Sensitive Captures response in therapeutic trials of short duration	• Requires specialized equipment and training • More time consuming than spirometry • Unable to identify regional changes
Chest Computed tomography (CT)	Widely available	• Requires specialized training of observers for scoring • Exposure to ionizing radiation
Functional MRI	Sensitive measurement to monitor disease progression Can capture regional changes in ventilation	Requires specialized equipment Currently at an early stage of validation

label observational study in patients homozygous for F508del starting LUM/IVA (PROSPECT study) [96]. LCI was the only outcome measure that captured a treatment response, and the change was observed as early as the one-month visit suggesting LCI is a suitable outcome measure for short term therapeutic trials [96].

MBW is also feasible in young children as it requires only tidal breathing [97]. In an observational study in CF children 3 to 5 years of age with at least one gating mutation who were initiated on ivacaftor, LCI was able to detect a significant improvement demonstrating that MBW is also feasible, and sensitive in preschool children [98].

While LCI appears to be a more sensitive outcome measure with milder disease, its utility in capturing individual drug responses still needs to be explored further. Whether LCI is the most suitable outcome measure to assess in-vitro: in-vivo correlations is part of the CFIT program [99].

MBW provides an overall assessment of ventilation homogeneity but is unable to identify the location of structural changes, nor the regional changes related to mucous plugging. This complementary information can be obtained by imaging studies.

Chest computed tomography

Chest computed tomography (CT) scan demonstrates structural changes in the lungs quantified with scoring methods such as CF-CT and the more sensitive Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF)[100-102] The cumulative effect of ionizing radiation dose limits its repeatability especially in young children but dose optimisation strategies are available to reduce this risk [103]. Forskolin induced organoid swelling has been shown to correlate with disease severity quantified by the PRAGMA-CF score [103], but the utility of CT to capture individual treatment responses is still unclear.

Functional MRI

Functional magnetic resonance imaging (MRI) of the lungs with hyperpolarised ^{129}Xenon gas (^{129}Xe-MRI) is used to assess ventilation inhomogeneity as it shows signal voids in the areas that the hyperpolarised gas does not reach due to localized lung disease [104]. Ventilation defect per cent (VDP) is calculated from regional ventilation obtained with ^{129}Xe-MRI. Marshall et al. demonstrated that ^{129}Xe-MRI was the most sensitive measurement of abnormalities in children with CF (6 to 16 years of age) when compared with CT, LCI and conventional MRI [105]. In subsequent follow-up, ^{129}Xe-MRI showed the most change over time compared to LCI, and spirometry when repeated 1 to 2 years later [106]. Therefore, functional imaging may be especially suitable for monitoring disease progression.

The Hyperpolarized Imaging for New Treatments (HyPOINT) study is currently under way to clarify the utility of functional imaging to monitor treatment response to CFTR modulators (ETI) (NCT 04259970). Larger studies are needed to define the best indices of functional MRI in patients with CF as well as the expected variability of these imaging techniques over time.

Therapeutic effects of CFTR modulator therapies on other organs. The review of the impact of CFTR modulators on other organs and the clinical outcome measure to assess them is beyond the scope of this review. Nutritional status (weight and BMI) improves significantly with treatment with ETI suggesting a positive effect on pancreatic, and gastrointestinal function [15,87]. Its benefits on exocrine pancreatic function need to be further established [10,107]. Studies on ivacaftor in young patients aged 1 to 5 years old with CF have demonstrated some recovery of pancreatic function, and improvement of stool fecal elastase concentrations within normal thresholds (>200 ug/g) in 23% of participants [108]. This suggests recovery of pancreatic function is possible with early treatment with CFTR modulators. Longitudinal observational studies such as PROMISE (NCT04038047), and RECOVER (NCT04602468) will allow us to learn more about the long-term impacts of triple combination CFTR modulators on lung function and other organs.

Conclusion

Multiple in-vitro models have become available and have proven to be a valuable tool in facilitating the process of bringing modulators targeting primary defects associated with CF causing mutations into clinical care. CFTR channel activity assays in patient derived tissue models have the potential to reveal variations in drug response size amongst individuals and support the development of personalized interventions. While no single assay will likely be perfect in predicting clinical response, comprehensive testing of CF modulator effect can be assessed using combination of in-vitro assays monitoring mutant protein function and processing, such as mutant CFTR protein processing, channel function and mucociliary movement, will ultimately lead to more robust predictions [24]. This concept has yet to be rigorously tested and widely applied. However, the true potential of stem cell derived tissue models for precision medicine has yet to be realized and requires innovation in the areas of tissue modeling, phenotypic assays and functional genomics. Technological advances in these disciplines will facilitate discovery of the molecular basis for variation amongst individuals and enable the development of alternative or complementary therapies. Further, the translation of these discoveries to clinical care requires further innovation in clinical research. We anticipate that the integration of customized, patient-specific therapies will require the development of sensitive imaging technologies that detect subtle changes in lung function throughout the life of each person. Developing precise *in-vitro* and *in-vivo* tools is a research priority to assure that in the future individualized targeted therapies will become available for every single person with CF.

Outstanding questions

1 Identifying the the best in-vitro tool (or combination of tests) predicting clinical response to CFTR modulators

2 Validation of in vitro test platforms to be used in future drug development
3 Assessing the role of sensitive functional tests and imaging tolls that can be used to evaluate individual response to disease modifying therapies.

Search strategy and selection criteria

Data for this review were identified by searches of MEDLINE, PubMed, and references from relevant articles using the search terms "Cystic Fibrosis" and selected based on their relevance to the topic. Only articles published in English between 1989 and 2021 were included.

Contributors

M.P.D, S.X., C.E.B, and F.R. contributed to the conceptualization, manuscript writing, revisions, and literature review. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

M.P.D, S.X., C.E.B, have nothing to disclose. F.R. acts as a consultant to Vertex Pharmaceuticals and Proteostasis; companies that produce CFTR modulators.

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