HHS Public Access

Author manuscript

J Cyst Fibros. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

J Cyst Fibros. 2019 January ; 18(1): 22–34. doi:10.1016/j.jcf.2018.05.004.

"CFTR Modulator Theratyping: Current Status, Gaps and Future Directions"

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Abstract

Background—New drugs that improve the function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein with discreet disease-causing variants have been successfully developed for cystic fibrosis (CF) patients. Preclinical model systems have played a critical role in this process, and have the potential to inform researchers and CF healthcare providers regarding the nature of defects in rare CFTR variants, and to potentially support use of modulator therapies in new populations.

Methods—The Cystic Fibrosis Foundation (CFF) assembled a workshop of international experts to discuss the use of preclinical model systems to examine the nature of CF-causing variants in

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Conflict of interest statement:
No authors reported conflicts of interest

CFTR and the role of in vitro CFTR modulator testing to inform in vivo modulator use. The theme of the workshop was centered on CFTR theratyping, a term that encompasses the use of CFTR modulators to define defects in CFTR in vitro, with application to both common and rare CFTR variants.

Results—Several preclinical model systems were identified in various stages of maturity, ranging from the expression of *CFTR* variant cDNA in stable cell lines to examination of cells derived from CF patients, including the gastrointestinal tract, the respiratory tree, and the blood. Common themes included the ongoing need for standardization, validation, and defining the predictive capacity of data derived from model systems to estimate clinical outcomes from modulator-treated CF patients.

Conclusions—CFTR modulator theratyping is a novel and rapidly evolving field that has the potential to identify rare CFTR variants that are responsive to approved drugs or drugs in development.

Introduction, Background and Definitions

Cystic Fibrosis and the Cystic Fibrosis Transmembrane conductance Regulator.

Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an anion channel that regulates the activity of other ion transporters and governs the hydration and viscoelastic properties of mucus in several epithelial tissues [1]. CFTR variants that produce disease result in aberrant ion transport and mucus obstruction of the airways, leading to chronic infection, inflammation and ultimately damage progressing to respiratory failure. Over 2,000 variants in the *CFTR* gene have been identified and greater than 300 are known to cause disease [2]. A small number of well-studied variants are responsible for disease in the vast majority of people with CF; however, one third of CFTR variants have been found in five or fewer CF patients worldwide, and many remain largely uncharacterized and/or untreated.

Symptom-based therapy and CFTR-based therapy with CFTR modulators.

Management of CF disease has traditionally relied on symptom-based treatments. These are therapies that address signs and symptoms that result from the loss of CFTR function, such as mucus obstruction, inflammation and infection; they have led to steady improvements in patient longevity and quality of life. There are numerous available treatments for CF symptoms across the various affected organ systems, and these continue to be areas of active therapeutic development. Despite these advances, the median predicted survival of newborns with CF is well below that of unaffected newborns in developed countries [3]. This single observation supports the rationale for novel therapeutic approaches in CF. Recently, a class of drugs termed CFTR modulators have become available for subgroups of people with CF [4]. There are several modes of action of CFTR modulators, but they differ fundamentally from other CF therapies in that they aim to improve or even restore the function of defective CFTR protein and are effective for people with only certain CFTR variants (or mutations). Highly effective CFTR modulators can offer transformational benefit to people with CF, producing improvements across numerous relevant endpoints in clinical trials and care [lung function, risk of pulmonary exacerbation, respiratory symptoms, weight, linear growth, rate

of lung function (e.g. FEV_1) decline over time, mucociliary clearance, intestinal pH, sweat chloride (SC), inflammatory burden, detection of CF pathogens, etc.] [5–9]. Therefore, an important goal of the CF research and care community is to provide CFTR-based therapies to every individual with CF. Conceptually, this includes highly effective CFTR modulators (the focus of this review), but can also be extended to nucleotide and cell-based strategies (e.g. mRNA correction or replacement, gene transfer, chromosomal mutation correction by gene editing, stem cell replacement). These approaches may or may not address the systemic nature of CF; moreover they will still require significant scientific advancement to be used safely and effectively.

CFTR Modulators.

CFTR modulators are small molecules that aim to improve the function of mutant CFTR proteins by different approaches [4]. Examples include: i) potentiators that improve the channel gating of CFTR variants (increase open channel probability), ii) correctors that augment trafficking of CFTR processing variants to the plasma membrane, iii) stabilizers that increase the residence time of variant CFTR at the plasma membrane, iv) amplifiers that increase the amount of variant CFTR available for subsequent modulation by protein-active small molecules, and v) readthrough agents of in frame premature termination codons (PTCs) that suppress PTCs, produce translational readthrough by the ribosome and subsequent full length protein. This final approach is particularly challenging to address, as PTCs lead to both truncated protein and mRNA instability. Two classes of modulators have gained regulatory approval to treat CF caused by discrete CFTR variants, including: 1) potentiators (approved for individuals with CF who have CFTR defects attributable to abnormal gating and/or conductance, and also variants with residual function that demonstrate improved activity following potentiation); and 2) correctors (rescue maturational processing) combined with potentiators (that together improve the function and clinical outcomes of individuals homozygous for the F508del CFTR variant or have one F508del CFTR and a responsive variant). Current clinical trials are examining CFTR modulators from several classes, including potentiators, amplifiers, correctors, and ‘next generation’ correctors that combine with first generation correctors (e.g. tezacaftor) and potentiators (ivacaftor) to further increase the activity of F508del CFTR in individuals with CF who carry at least one copy of F508del.

Theratype definition.

The term ‘theratype’ has been described as a means to group *CFTR* variants according to their effect on the CFTR protein and their response to corrector and potentiator compounds [10]. In this framework, unclassified *CFTR* variants would initially be assigned to theratype groups based upon their effect on CFTR quantity and function (via traditional cell- based characterization). The modulator/compound effects on the unclassified CFTR variant would serve to validate the appropriate assignment of the variant. More recently the term has been used to describe an approach to characterize mutations by their response to CFTR modulators across various model systems, which can include functional and biochemical characterization. While similar functional responses to common CFTR modulators may be achieved by disparate *CFTR* variants, the molecular mechanism of action responsible for the common functional readout may vary. As an example, missense mutations (e.g. gating

mutations) may achieve similar chloride transport in response to ivacaftor compared with a noncanonical ('leaky') CFTR splice variant, but the mechanisms underlying the response differ. In the former, defective gating is improved, while in the latter low levels of normal CFTR are hyperactivated. Theratyping can be achieved by testing modulators on a variety of laboratory or patient-derived cells, which includes testing in different model systems that are under development (described in detail below). Historically, studies of CFTR modulators have been performed in heterologous expression systems (e.g. stable cell lines expressing mutant CFTR cDNA) and explanted human bronchial epithelial (HBE) primary cell cultures from CF patients undergoing lung transplantation. Each of these has their strengths and limitations (summarized below). Tissues collected during transplantation or autopsy cannot be relied upon to provide HBE cells from patients with rare variants; additional models and tools must therefore be developed. Currently, there are several patient-derived model systems in different stages of development that will be considered in this review.

The goals of theratyping are to provide a means to: 1) more completely characterize complex CFTR variants; 2) assess modulator responsiveness of rare or even unique CFTR variants *ex vivo* or mutation combinations not available from lung explants; and 3) compare several modulator responses of various variants using either a mutation- or patient-specific approach. This data could provide a path for testing of CFTR modulators in individuals who are unlikely to be included in traditional clinical trials, and help guide selection of modulators for patients for whom multiple options exist. At the current time, validation of these theratyping concepts is not complete. It is important to note, however, that the US Food and Drug Administration recently approved the use of modulators in persons with CF possessing several rare CFTR variants. This approval was based (in part) on results from studies of mutant CFTR cDNAs expressed in a standardized heterologous expression system, indicating that theratyping concepts can be considered during regulatory review of CFTR modulators (see Section 5 of this document). Therefore, this approach may be appropriate in some cases to expand patient access to CFTR modulators, provided that there is sufficient clinical experience, the drug mechanism of action is well understood, and the accompanying safety data is supportive from studies in other populations [11]. It is not yet clear, however, whether any *in vitro* test will be predictive of clinical benefit of novel therapies for CFTR variants that may require expression in native epithelia to fully represent a molecular phenotype (e.g. canonical splice variants, PTC variants, intronic single nucleotide polymorphisms or SNPs). Advancement and validation of theratyping technology will be necessary to determine whether *in vitro* surrogate assays can predict *in vivo* clinical response in settings such as these.

Potential Applications of Theratyping to Research and Care

There are several care and research circumstances where theratyping concepts may be applied to ultimately help understand the basic defect caused by CFTR variants and potentially enable access to appropriate CFTR modulators.

Use of patient-derived model systems for drug discovery/early development.

Some types of CFTR variants are highly dependent on the study of chromosomal *CFTR* in the context of patient-derived cells (e.g. splice variants, PTC variants, poorly characterized variants with potentially multiple defects). Therefore, drug development for variants in these classes may benefit from incorporation of patient-derived model systems and the use of theratyping principles.

Evaluation of modulator efficacy on rare variants not captured in clinical trials or traditional drug development.

As rare variants are not feasibly studied via traditional clinical trial designs, data from theratyping studies may provide support for clinical evaluation in individuals not represented in clinical trials. This could also include individuals with two CFTR variants (in *trans*) that each have variable responsiveness to CFTR modulators. This could include studies in patient-derived cells or co-transduction in established cell lines. Heterologous expression systems can provide clear data regarding isolated CFTR variants, but have not routinely been adapted to study modulator effects on greater than one variant simultaneously. This has been used for ivacaftor and ivacaftor/tezacaftor label expansion into new missense CFTR variant populations using standardized studies in Fisher Rat Thyroid cells (see Sections 3.2 and 5, and the respective product inserts).

Selecting subjects/enrichment for clinical trials.

Cellular responses to modulators may be used to enrich clinical trials for likely responders to drugs in development as well as to include subjects with different variants into ‘basket trials’. This concept is already applied to drug development for other diseases (e.g. cancer therapies) and CF [6,12].

Optimizing modulator selection (‘personalized medicine’) when choices exist.

The treatment response to a particular modulator can differ remarkably among individuals with the same genotype. Furthermore, it is projected that greater than one CFTR modulator option will be available for some people with CF (e.g. lumacaftor/ivacaftor vs tezacaftor/ivacaftor for those with two copies of F508del). Theratyping may help provide a rationale for one option compared with another, particularly when considering long term benefits over the lifetime of a person with CF. This general approach is underway with the HIT CF program, using patient derived rectal organoids to assess cellular responses to various CFTR modulators (see Section 3.4, and reference 41). It could also be implemented if optimization of drug exposure is required to exact maximal efficacy and safety. Depending on assay performance, theratyping of patient derived cells could also be used to identify patients who are not responsive to given modulator regimen (despite appropriate CFTR variants). Clearly, applying theratype principles to include or exclude CF patients from modulator treatment would require extensive validation of model systems and their predictive capacity for long term clinical outcomes.

Use of theratyping for regulatory purposes.

In principle, data collated from theratyping could be used to support applications of drug expansion to new populations, or provide a path using a standardized laboratory panel that could be implemented to achieve access on an individual level without blanket approval for a given variant. This could require studies in native epithelia to accurately reflect their variants' behavior.

Examining the benefit of CFTR modulators in individuals with CFTR-related disorders.

It is currently unknown whether CFTR modulators have a positive impact on the clinical course of subjects with CFTR-related disorders outside of CF. Data generated from patient-derived materials that demonstrate *in vitro* modulation of CFTR may be the first step toward formal clinical trial development in these understudied populations.

Preclinical Model Systems to Test CFTR Therapies

Table 1 below provides a summary of the preclinical model systems discussed at the Theratyping Workshop, including source and level of technical advancement to support theratype studies. Animal models have not played a significant role in CFTR modulator development (beyond toxicology studies). There are a number of likely contributors to this fact, including the lack of humanized *CFTR* variant animal models, the cost and scarcity of expertise to perform such studies in recently developed animal models, and the ready availability of informative model systems (described below). In addition, advanced molecular techniques have not routinely been applied to the assessment of different model systems or responses to modulation. This is likely due to the defined functional response criteria of current CFTR theratyping model systems (ion and fluid transport) relative to other diseases. However, genomic studies may become more valuable in future theratyping efforts, particularly for those model systems derived from patients and used to select chronic therapy (e.g. personalization of modulator therapy). The subsequent sections discuss each model system in terms of their relative advantages, disadvantages, future directions and roles in theratype testing.

Laboratory derived/heterologous expression systems have been the backbone of high-throughput screens (HTS) to identify lead compounds for subsequent modulator development. This is particularly true for cell lines stably expressing missense CFTR variants, which can be standardized to enhance assay performance.

Transient CFTR expression in cell lines (e.g. lipid/DNA, electroporation, viral transduction)

There are several advantages to transient expression of mutant CFTR cDNA in cell lines to characterize the impact of variants on CFTR behavior, including high flexibility, speed, and comparably simple assays. Disadvantages include the potential for non-physiologic and/or variable expression. Furthermore, cDNA-based expression may not capture fundamental aspects of certain CFTR variants (e.g. splice, PTCs, intronic SNPs). Depending on the cell type chosen, they may or may not have epithelial behavior relevant to CF. While this may not be of high importance to initially characterize CFTR behavior, it is critical to examining downstream consequences of CFTR dysfunction (e.g. impact on fluid secretion, airway

surface liquid regulation, mucociliary clearance, etc.). Finally, mutant CFTR rescue can vary across different cell lines, complicating analysis and translation to more advanced model systems [13,14]. In summary, the results from transient expression systems can be informative regarding defects of CFTR variants, and modulator effects for subsequent validation in more physiologic model systems. It is unclear if data from these systems could be the sole source to support modulator development/extension.

Stable CFTR transduction in cell lines (e.g. FRT, HEK, 3T3, CFBE41o-, MDCK cells).

These systems have several advantages, including flexibility and the capacity to control/standardize transgene expression. They have demonstrated the capacity to have precise assay performance in cells that are easy to culture, which has been the basis of HTS to identify CFTR modulators. CFTR variants can be readily introduced into epithelial cell lines, but underlying chromosomal *CFTR* activity may require consideration depending on the cell type used. Evidence suggests that the transcriptome of CFTR-expressing cell lines is similar to that of primary cells, and isogenic cell lines may also permit comparison between distinct CFTR variants, or direct comparison to wild type CFTR [15]. As noted previously, the FDA has recently accepted results from studies of variant CFTRs stably expressed in cell lines (FRT cells), supporting label expansion to CF subjects with these uncommon variants. Potential disadvantages include cDNA-based expression that may not capture fundamental aspects of certain CFTR variants, or variants that require native cells to fully capture complex defects (e.g. canonical vs noncanonical splice variants, translational readthrough and nonsense mediated decay for PTCs, intronic SNPs). Some of these challenges may be overcome with advances in gene expression studies. Heterologous expression of splice site variants in mini-genes has recently been shown to replicate splicing patterns seen in primary tissues [16]. Thus, studies in non-native tissues may be informative for the complex variants. In addition, the cell type may or may not have CF epithelial behavior when using non-human, non-respiratory cells. This may theoretically impact the predictive value of these model systems, as studies of correctors have demonstrated that cell background may differentially impact effects on F508del CFTR [14]. Finally, establishing these systems takes time for standardization, validation, and linking preclinical studies to patient outcomes. FRT cells have performed well to predict clinical modulator responsiveness for many missense variants, but modulator response of G970R CFTR in vitro failed to correlate with clinical response in CF subjects with this variant studied with ivacaftor [6,17]. Whether confirmatory studies in patient-derived epithelial cells are needed for some CF-causing variants is still to be determined. In summary, heterologous expression systems are an excellent vehicle for HTS efforts depending on the nature of the CF-causing variant (e.g. missense, gating such as G551D CFTR, trafficking such as F508del CFTR).To date, many (but not all) modulator results in stable cell lines have relied on validation in explant primary HBEs; however this may not be necessary in certain circumstances (e.g. rare CFTR gating variants, and recent ivacaftor label expansion for several variants). These models have proved to be an excellent (but not perfect) system for understanding the mechanism of action of modulators, and CFTR responsiveness of variants where primary HBE cells are not available due to their rarity.

Primary HBE planar cultures grown at air-liquid interface (from explant lung tissue obtained at the time of lung transplantation).

Primary HBE planar cultures grown at an air-liquid interface (ALI) have been a critical bridge between CFTR heterologous expression systems and testing in human subjects. Specifically, measurements of G551D CFTR chloride conductance in response to ivacaftor in HBEs faithfully predicted bioactivity of ivacaftor in vivo, including CFTR biomarkers (sweat chloride, nasal potential difference) and important clinical outcome measures (lung function, growth, disease stability) [5,18–21]. Furthermore, G551D CFTR HBEs treated with ivacaftor demonstrated downstream effects of improved CFTR function that are believed to be critical steps in CF disease pathogenesis, including sodium transport inhibition, ASL volume regulation, and mucociliary clearance [21]. For the development of lumacaftor/ivacaftor, F508del/F508del CFTR HBEs treated with lumacaftor/ivacaftor demonstrated improvements in F508del protein maturation and chloride conductance [22]. These effects on F508del CFTR function were less than that observed with ivacaftor in G551D CFTR HBEs. The lumacaftor/ivacaftor effects were also reduced in HBEs with only one F508del CFTR allele, and these findings were largely recapitulated in lumacaftor/ivacaftor clinical trials [23]. Finally, ivacaftor had small effects on F508del CFTR activity in HBEs from F508del homozygous donors, which was similar to the small effects of ivacaftor observed in CF patients homozygous for F508del CFTR [24].

There are several advantages of HBE planar cultures, as they currently are the ‘gold standard’ for preclinical testing and a checkpoint for CFTR modulators entering the clinic based on the successful path of FDA-approved CFTR modulators. Importantly, there has been a lack of published HBE data for modulators that have failed in clinical trials (e.g. ataluren to treat PTC-mediated CF, cavasonstat to stabilize and enhance mutant CFTR activity, riociguat to enhance trafficking of F508del CFTR) [25–30]. The rationale for its use is clear since the bronchial epithelium is an established site of CF lung disease. Mean results of modulator induced improvement in CFTR activity in HBEs correlate well with mean group *in vivo* improvements in FEV_1 (see Table 3). There are some ongoing disadvantages, including a lack of available lung explants from individuals with rare CFTR variants, cumbersome growth conditions and variable epithelial behavior that is dependent on culture conditions (that are partially but not fully standardized and can be modified to accentuate different aspects of the CF phenotype). Intersubject variability among individuals with a common variant has been described, but it is not clear if this reflects biological differences between subjects or technical differences in the manner or circumstances that the cells were obtained. Furthermore, cells are typically derived from organs with end stage disease that may or may not reflect cell behavior in early disease. Airway epithelial cells grown in planar culture are a mix of epithelial cell types and lack glandular structures, and thus are an imperfect reflection of the CF airway [31,32]. In summary, HBE planar cultures will continue to be a standard for comparison to other technologies for years to come due to their successful translational role for currently approved drugs. It is currently unclear if this model applies to nonsense directed therapies, mRNA transfer, gene transfer and/or other nucleotide-based therapies in development. The potential risk and complexities of systematically obtaining brushed and expanded bronchial epithelial cells from subjects with rare CFTR variants is likely insurmountable (see Section 3.5 below – HNEs).

Gastrointestinal organoids and enteroids.

The rapid emergence of organoid technology has made this a particularly attractive patient derived model system to assess modulator effects [33–39]. Advantages of these gastrointestinal-based systems are that cells are obtained from stable individuals through an endoscopy or suction biopsy procedure. These cells are not impacted by disease state, and are a very exciting alternative to HBEs for testing of CFTR modulators. They offer an essentially 'limitless' supply from the donor based on the isolation of progenitor cells from rectal biopsies, thus allowing biobanking and exchange of materials across different labs [33,34]. Organoids are also an attractive model system for modulator testing in genotypes that requires native epithelial tissue [36,38]. They are sensitive to modulator effects and have a large dynamic functional readout. There is growing experience with their predictive performance *in vivo* (examples that predict efficacy as well as lack of efficacy), including the capacity for quality control, precision and centralized analysis. The primary readout (forskolin-induced swelling or FIS) is CFTR dependent, and there is accumulating evidence that the modulator-induced FIS response correlates with change in FEV_1 and SC in vivo [39]. Furthermore, plasma samples from modulator-treated CF patients have been used to personalize pharmacokinetics and pharmacodynamics by organoid testing [37]. In addition, studies of CFTR modulation in rectal organoids have more rapid assay throughput as compared to short circuit current measurements in HBE planar cultures. Enteroids derived from duodenal biopsies can provide a rapid readout within a few days of acquisition, but currently the lack of progenitor cell isolation has limited their use [40]. T his may be able to be overcome with further experience. There remain some disadvantages for the widespread use of GI-based model systems**.** The current assays are not easily transferable, with unique culture needs and significant investment in equipment and training of personnel required. Culture reagents are commercially available, but they are expensive and have not been fully validated. The stimuli used may need to be adjusted based on the variant type (e.g. dose/response stimulation of nonfunctional vs partial function CFTR variants) to clearly demonstrate modulator activity [36,39]. The primary readout (FIS) is an indirect measure of CFTR activity. Colonic tissue may not be appropriate to test therapies aimed at activating Ca^{2+}-activated Cl^- channels (CaCCs) or inhibiting ENaC, as these are not expressed consistently in the colon [41]. If channel cross talk is important to a particular agent's activity, this could be limiting [42]. Rectal epithelia is not clearly relevant to disease pathology, which may or may not impact its clinical predictive capacity. Rectal organoids typically require approximately one month until samples have been expanded sufficiently for biobanking and functional testing. There is also some risk of culture failure. In addition, there is reticence by some patients to have rectal biopsies performed, and duodenal biopsies require upper endoscopy. In summary, rectal organoids have great promise for theraptyping for numerous reasons. There are currently a number of active next steps in process, including i) expansion to clinical research sites for validation and coordination of central analysis; ii) testing the role of organoids in assigning modulators to patients (e.g. Human Individualized Therapy: HIT - CF program in Europe); iii) standardization of culture conditions (media compositions and CFTR stimulation); and iv) determining if the time to readout can be shortened for rectal tissue-derived organoids [43,44]. The RARE study (NCT03161808) seeks to bank a number of patient derived cell types centrally (iPSCs, nasal

cells, rectal organoids) from CF donors with two PTC CFTR variants to aid in future drug discovery and development efforts.

HNE planar cultures grown at air-liquid interface (primary nasal cells obtained from CF individuals by nasal brushings).

One of the main advantages of HNE cells is that they are easily accessible by nasal brush/curettage, and can therefore be used for testing from essentially any CF donor (regardless of age). Significant expansion in cell numbers can be accomplished using conditional reprogramming conditions (CRC) and other complimentary methods [45–47]. HNE cells have similar ion transport features compared with HBE cells, and studies of HNE cells in planar culture at air-liquid interface can use standardized equipment and culture conditions [48–50]. There is accumulating evidence of relationships between HNE cell CFTR short circuit currents, CF subject SC values and FEV_1 during modulator treatment, but this data is not as advanced as seen with traditional HBE cell planar culture from lung explants and rectal organoids [48–52]. Disadvantages include the need for significant investment in equipment, reagents and training. The expansion and maturation into nasal epithelial planar cultures is relatively time-intense (appx two months), with some loss of ENaC and eventually CFTR expression that occurs with increasing passage (with or without CRC). Reproducibility across different labs has not been carefully assessed, and shipping of fresh and frozen cells is being investigated in several labs. There are risks of contamination and epithelial squamous transformation, and both can lead to culture loss. The optimal growth and propagation conditions are not fully defined or standardized at this time. Compared with organoids, the expansion of cultures is rather limited and there is a smaller dynamic range due to lower CFTR expression levels in nasal cells. In summary, HNE cells grown in planar cultures are an exciting potential model system for application to theratyping. Standardization is early in development, with early steps achieved through the activities of an HNE Workgroup. These early steps have allowed application to clinical studies seeking to bank brushed HNE cells for future study (the RARE, PROSPECT and GOALe2 studies including NCT03161808, NCT02477319 and NCT0152133, respectively), but further standardization is required regarding cell acquisition and growth conditions. It is also important to determine the impact of cryopreservation and storage of specimens on the performance of cells in culture and assays as compared with fresh, non-frozen specimens. There is a need for comparison of HNE monolayers with gold standard explant HBE cells from lung explants grown in planar culture. Opportunities exist to advance HNE cells as a predictive testing tool by determining whether CFTR currents in modulator-treated HNEs derived from modulator-treated subjects correlates with *in vivo* CFTR biomarkers. This work is ongoing in several laboratories.

HNE cell spheres

Studies of HNE cells grown as spheres rather than planar cultures are a very recent development. The HNE cells are obtained and expanded in the same way as described above, but seeded into three dimensional matrix culture for subsequent study (similar to GI enteroids and organoids) [53,54]. The major advantages include a faster readout than studying ion transport in planar cultures (three to four weeks), fewer cells are needed to form spheroids vs. planar culture, and there is potentially less risk of squamous transformation (as

the structures are much smaller and achieved more rapidly than a mature sheet of epithelial cells). HNE cell spheres can be grown with the apical (luminal) surface facing inward or outward. HNE spheres have a measurable forskolin-induced response in the presence versus absence of CFTR. Spheres with an inward facing luminal surface swell following CFTR activation in the presence of functional CFTR (similar to organoids). Spheres grown with the luminal surface facing outward shrink following CFTR activation. Disadvantages for HNE cell spheres are similar to all sphere-based studies, as they provide an indirect measure of CFTR function (swelling or shrinking due to fluid transport). The assay is early in development in terms of optimized growth conditions, assay and data interpretation. Data generated thus far suggests that there is a smaller dynamic range than monolayers and enteroids/organoids. Fewer research laboratories have experience with this approach compared with more traditional planar cultures, and standardized protocols have not been developed. Furthermore, there is currently little data regarding assay reproducibility. In summary, HNE cell spheres offer some advantages relative to other patient derived model systems, but it is unclear if HNE cell spheres are a better option than HNE cells studied under planar culture conditions. It is possible that they could serve a complimentary role for simultaneous studies performed under planar culture conditions, providing two independent assays to evaluate modulator effects (one focused on ion transport, the other focused on fluid transport).

Studies of CF-affected epithelia derived from iPSCs

It is clear that there are numerous potential advantages for using iPSCs to address theratyping questions [55–59]. Samples can be obtained via blood draw (or other cell sources) and therefore are theoretically available from all CF donors. Since cells require specialized culture conditions to achieve a pluripotent state, they are well suited for process centralization and banking. iPSCs can in principle be differentiated into any CF-relevant epithelia, and they have the potential for limitless supply. iPSCs also have the ‘biomass’ appropriate for adaptation to a HTS platform (that is a potential limitation of all other primary human model systems discussed here). It is anticipated that iPSCs should retain donor- specific features, but this is yet to be carefully assessed in CF. Once differentiated into respiratory or other epithelial cells, they lack the secondary disease effects observed in primary tissues from CF patients. This can be either an advantage or disadvantage depending on what questions are being addressed. The current disadvantages center around the methodology of producing mature target epithelia. These protocols are largely still in development, and current protocols to fully differentiate iPSCs to various CF-relevant epithelia are lengthy and difficult. Furthermore, there have not been direct comparisons with primary human epithelia (such as HBE planar cultures from lung explants). Only a few labs worldwide regularly differentiate iPSCs into fully differentiated respiratory cells, and therefore this model system has not demonstrated that it is easily transferable. Future directions include focus on refining and optimizing differentiation protocols to appropriate epithelia. Subsequent steps would include comparison of performance with established model systems. A powerful validation step would include comparison of iPSCs derived from donors who have undergone lung transplant and have functional and biochemical data from their explant HBE cells.

Comparing model systems

There has been little effort to date to compare the performance of different model systems and their capacity to predict clinical outcome measures. These would best be accomplished by testing the different model systems (particularly the patient-derived model systems) simultaneously from a given patient. One limitation is that most laboratories do not have expertise across all assays simultaneously. This should be considered an important goal for future theratyping research, and is highlighted in the Summary and Future Directions Section. Criteria for comparison between systems and with the HBE planar culture monolayer system is largely the same as those included in Table 2 [assay dynamic range, sensitivity, specificity, positive and negative predictive value (PPV and NPV, respectively), precision, accuracy, and portability].

Linking Pre-Clinical Testing to Clinical Outcomes

One of the key goals of using theratype principles to personalize therapies is to link results from preclinical model system testing to clinical outcome measures. This may be done retrospectively or prospectively using data collected during clinical care, in patient registries, or during clinical trial participation [60].

Preclinical model systems hold the promise to provide support for expansion of CFTR modulators to new CF populations. Key principles that should be considered in evaluating their performance include the dynamic range of the assay, sensitivity and specificity to detect CFTR function, assay precision and accuracy, PPV and NPV of the assays, and their portability. Most of these have not been comprehensively assessed across the preclinical model systems. Table 2 provides a qualitative comparison of preclinical model system performance and prediction of clinical benefit. These are largely based on expert opinion at this point. Table 3 furnishes a list of potential clinical outcome measures for correlation with preclinical model results. It is possible that further standardization of these model systems and validation of their performance and predictive nature will help when considering modulator use in understudied CF populations. It will be important to determine the PPV and NPV of these model systems, particularly when using theratype data to justify therapeutic trials of CFTR modulators with third party payers or regulators. Just as important as matching the right modulator to the right patient(s) is ensuring that patients who might benefit from modulator therapy are not restricted due to erroneous theratyping results.

Regulatory Considerations for Theratyping

Data from theratyping, i.e., FRT cell lines expressing mutant CFTR cDNA, have already been successfully used to support drug discovery, development, and labeling based on CFTR mutation type [74]. More refined theratype systems have the potential to target drug therapies to the individual patient level.

The level of supportive data required and regulatory scrutiny encountered with a specific approach would depend on the intended use of the testing system. For example, use of in vitro systems in early drug discovery to screen candidate drugs for CFTR modulating

activity would generally fall below the regulatory “radar screen”. On the other hand, development and ultimate marketing of a theratype system as an *in vitro* companion diagnostic device intended to provide information for the safe and effective use of a corresponding therapeutic CFTR modulator would entail detailed review by both drug and device regulatory authorities [75]. It is likely the regulatory considerations that would need to be addressed for the types of theratyping applications discussed in this paper would fall somewhere in between the two uses described above [76].

Two such applications come to mind, use of theratyping as a patient enrichment tool for determining a patient population for the purpose of conducting a clinical study in a patient subpopulation, and extension of a drug indication to a patient subpopulation based on theratype data in the absence of additional clinical data. As an enrichment tool, one would need evidence supporting the hypothesis that patients with particular CFTR variants would be more likely to respond to the therapy based on a drug’s mechanism of action, previous clinical experience, or other strong scientific rationale [77]. Use of theratyping as a means to extend a drug indication to patient subpopulations without additional clinical data would entail meeting additional regulatory considerations [69], including:

- A solid understanding of the drug’s mechanism of action and consequences of specific CFTR defects intended to be targeted
- An established drug risk/benefit profile based on an existing efficacy and safety data
- Adequate characterization and standardization of the specific theratype system
- Reasonable evidence based on existing clinical data that achieving a predetermined theratype response threshold would be likely to predict clinical benefit

The recent FDA label expansion of ivacaftor to include CF subjects with several additional rare variants was based on use of a standardized in vitro assay (variant CFTR cDNA expression of a common construct in FRT cells), and functional results exceeding a threshold of 10% of wtCFTR activity (see fourth bullet, above). This relatively low threshold helps CF patients with rare variants (but no clinical efficacy data) gain access to potentially beneficial drugs, but prescribing providers will need to more intentionally consider the relative benefits of these expensive and life-long drugs in these understudied groups.

Summary and Future Directions

Numerous preclinical model systems have been established or are in development for testing of CFTR modulators. Several have been adapted for the purpose of theratyping (characterizing and classifying variants by their response to CFTR modulators), and for some variants this is best accomplished with donor-derived materials. Recommendations for studies in the short-, medium- and long-term are listed below.

Short term

- Standardize conditions for growth and testing in preclinical model systems used to evaluate CFTR modulators. This is most advanced for stable cell lines expressing missense mutations, HBE cells grown in planar cultures, and rectal organoids. This is least advanced for duodenal enteroids, brushed HNE cells, and iPSCs.
- Develop Standard Operating Procedures for preclinical testing and therapeutic trials of modulators in individuals with CF (including those with common and rare CFTR variants). These data should be centralized in data repositories for potential support of regulatory expansion of CFTR modulators.
- Perform standardized testing of available CFTR modulators, examining CFTR missense mutations in transiently transfected model systems as well as the better established stably expressing model systems (e.g. FRT, HEK, MDCK, CHO, CFBE41o- cells). Clarifying what background cell lines are most informative to characterize rare mutations that translate to human subjects could accelerate future theratyping efforts.
- Define clinically relevant thresholds for CFTR responses in all pre-clinical systems, including CF donor-derived and stably transduced cellular models.

Medium term:

- Test the predictive nature of preclinical model systems simultaneously (both independently and compared with one another) in individuals who have been prescribed CFTR modulator therapies, examining assay sensitivity, specificity, PPV and NPV. Clear input from clinical researchers and regulators will be needed to define what constitutes a meaningful response in the laboratory, what constitutes a clinical response, and to determine experimental thresholds for clinical response or nonresponse.
- Capture data centrally and in common format(s) from individual labs/ investigators testing CFTR modulators in CF donor-derived model systems. This includes both *in vitro* and *in vivo* studies.
- Perform inter-laboratory and repeated measure validation for model systems.

Long term:

- Conduct prospective studies of theratyping strategy to predict, select or optimize CFTR modulator therapy regimen.
- Accumulate real world data from patient registries to determine the relationship between CFTR modulator responses from preclinical testing with long term outcome measures, including pulmonary exacerbation risk, lung function trajectory, microbiology, CF co-morbid conditions (e.g. diabetes, bone disease, mental health), and mortality/progression to lung transplant. This goal comes with inherent challenges because of differences in adherence to prescribed

therapies, age and disease state at start of treatment, and stage of comorbid conditions.

Acknowledgements:

This workshop was funded by the Cystic Fibrosis Foundation

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Highlights:

- ‘Theratyping’ helps classify CFTR variants by response to modulation
- Use of theratyping may help understand and treat CF caused by rare variants
- This expert workshop summarized the state of the art and knowledge gaps
- Numerous model systems are in varying stages of development for theratyping
- Recommendations for priorities and future research directions were identified

Table 1:
Preclinical model systems for theratyping

Model system	Source	Level of advancement	Most common uses
Transient CFTR expression in heterologous cell lines	Established cell lines	high	Characterize CFTR variants and CFTR biology
Stable CFTR transduction in cell lines	Established cell lines	high	HTS screening, evaluate common and rare CFTR variants in standardized system
Human bronchial airway epithelial (HBE) planar cultures	Lung explant	high	Validation of CFTR modulation in primary human cells, assess downstream effects (e.g. mucocilary clearance, airway surface liquid height)
Rectal organoids	Rectal biopsy	moderate	Validation of CFTR modulation in primary human cells, and patient-specific responses to modulators (fluid secretion)
Duodenal enteroids	Duodenal biopsy	low	Similar to rectal organoids Above
HBE planar cultures from brush	Bronchial brush	low	Similar to HBEs from lung explant, and patient-specific responses to modulators (ion transport)
Human nasal epithelial (HNE) planar cultures from brush	Nasal brush	low	Similar to HBEs from lung explant, and patient-specific responses to modulators (ion transport)
HNE spheres	Nasal brush	low	Similar to HBEs from lung explant, and patient-specific responses to modulators (fluid transport)
Induced pluripotent stem cells (iPSCs)	Blood	low	Differentiation into CFTR- expressing epithelial cells and tissues

Table 2:
Preclinical model system performance and relationship to clinical benefit

Model system	Dynamic range	Sensitivity/ specificity	PPV/NPV**	Precision/ accuracy	Portability*
Transient CFTR expression in heterologous cell lines	Large	Mod to high	Moderate	High	High
Stable CFTR transduction in cell lines	Large	Mod to high	Mod to High	High	High
HBE planar cultures (explants)	Moderate	High	High	High	Moderate
Rectal organoids	Large	Sens. high Spec. unk	Mod to High	Moderate	Low
Duodenal enteroids	Moderate	Unknown	Unknown	Unknown	Low
HNE planar cultures (brush)	Small	Moderate	Unknown	Unknown	Moderate
HNE spheres	Small	Moderate	Unknown	Unknown	Moderate
iPSCs	Unknown	Unknown	Unknown	Unknown	Low

*Portability considers the ability of a technique and/or assay to be successfully transferred from one investigator to another.

**PPV and NPV have not been systematically assessed, as unresponsive variants in preclinical models have generally not been studied in clinical trials. However, some populations with CFTR variants unresponsive or less responsive to specific CFTR modulators in preclinical model systems have been examined in clinical trials (e.g. ivacaftor monotherapy in F508del homozygous CF adults, ivacaftor/lumacaftor in F508del/minimal function CF adults [23,24]). These studies have generally confirmed that variants unresponsive/poorly responsive to modulation *in vitro* are poorly responsive *in vivo*. However, some variants studied in transient or stable expression systems have not aligned with clinical findings (e.g. G970R CFTR variant response to ivacaftor in stably transduced FRT cells compared with results from clinical trials [6,17]. Of note, VX770 studies of this variant in rectal organoids did correlate with absent/low clinical response [39]).

Table 3:

Relevant clinical outcome measures for comparison with preclinical model results*

Clinical outcome measure	Comments
CFTR biomarker	Genotype group changes in sweat chloride (and NPD, but more limited number of studies) during modulator clinical trials have correlated with CFTR responses from many preclinical model systems [5,18,19,60,61,62]. ICM measurements of G551D CFTR activity have correlated with benefit for patients treated with ivacaftor [63], or F508del CFTR activity in homozygous patients treated with lumacaftor/ivacaftor [64]. This biomarker is most valuable for demonstrating modulator bioactivity.
FEV_1	Genotype group changes in FEV_1 during modulator clinical trials have correlated with CFTR responses from many preclinical model systems, including heterologous expression systems, HBE cells, rectal organoids, and HNE cells [5–9,24,36,39,49,50]. Change in FEV_1 over time (i.e.: FEV_1 trajectory) compared with untreated patient registry controls has been assessed in open label studies of ivacaftor in G551D CF patients and lumacaftor/ivacaftor in F508del CF patients. Both analyses have demonstrated reductions in FEV_1 decline trajectory [65,66]. This outcome measure is the gold standard for pulmonary drug development, but has limitations in young patients, and those with early and advanced lung disease.
Multiple breath washout/ lung clearance index (MBW/LCI)	The use of MBW/LCI has been limited to studies in young people with CF and those with preserved lung function, including ivacaftor in CF subjects with gating and the R117H mutations, and lumacaftor/ivacaftor in F508del homozygotes. Genotype group changes in LCI during modulator treatment have aligned with CFTR responses in many preclinical model systems [67–69]. This outcome measure is most valuable in early lung disease and potentially younger CF subjects.
Nutrition/Growth	Genotype group changes in BMI during modulator clinical trials have aligned with CFTR responses from many preclinical model systems for ivacaftor and lumacaftor/ivacaftor [5,7–9,67,68]. In contrast, tezacaftor/ivacaftor produced similar F508del CFTR correction and potentiation *in vitro*, but did not demonstrate weight/BMI benefits in F508del homozygous adults relative to placebo [70]. Ivacaftor has been demonstrated to be associated with increased linear growth [71]. Growth is a valuable outcome measure (typically a secondary efficacy endpoint in modulator clinical trials), particularly in pediatric studies.
Risk of acute pulmonary exacerbation (APEx)	The data for this endpoint is less developed than that for other outcome measures. When included in clinical trials, genotype group changes in risk of APEx during modulator clinical trials have generally aligned with CFTR responses from many preclinical model systems [5,9,70]. The frequency of APEx is typically not included in crossover trials, which have been necessary for studies in rare CFTR variant groups (e.g. subjects with non-G551D gating mutations). Furthermore, many CFTR modulator trials in young CF patients do not have a placebo group, limiting assessment of APEx risk. Monitoring APEx can be complicated by variable definitions, and is most valuable outside of the young pediatric age group (since they are poorly defined in this population).
Microbiology	Detection of CF pathogens has only been carefully assessed in open label studies of ivacaftor in subjects with highly responsive CFTR vairants (e.g. gating mutations) [8,72]. The results of these studies have been mixed, and thus the relationship regarding the modulation of CFTR variants in preclinical model systems to CF microbiology is unknown. Changes in the detection of known CF pathogens is an important secondary outcome measure in clinical trials of CFTR modulators, but is limited in younger patients or patients with mild disease who fail to expectorate.
Patient Reported Outcomes (PROs)	PROs are typically included in CFTR modulator trials, and are an important secondary efficacy endpoint. The Cystic Fibrosis Quality of Life Questionnaire Revised (CFQ-R) has most commonly been included in modulator clinical trials, frequently demonstrating significant improvements in the respiratory domain that exceed the minimal clinically important difference (MCID). (5,9,68)

* The experiential hierarchy for preclinical model system comparisons with clinical outcome measures includes primary HBE cell planar cultures > rectal organoids ~ stable CFTR expression in cell lines > transient CFTR expression in cell lines > primary HNE cell monolayers > primary HNE cell spheres.