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CFTR BIOMARKERS: TIME FOR PROMOTION TO SURROGATE ENDPOINT?

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ABSTRACT

In patients with cystic fibrosis, CFTR biomarkers such as sweat chloride concentration and/or nasal potential difference are used as endpoints of efficacy in phase III clinical trials with the disease modifying drugs ivacaftor (VX-770), VX809 and ataluren. The aim of this project was to review the literature on reliability, validity and responsiveness of nasal potential difference, sweat chloride, and intestinal current measurement in patients with cystic fibrosis.

Data on clinimetric properties were collected for each biomarker and reviewed by an international team of experts. Data on reliability, validity and responsiveness were tabulated. In addition, narrative answers to 4 key questions were discussed and agreed by the team of experts.

Data collected demonstrated the reliability, validity and responsiveness of nasal potential difference. Fewer data were found on reliability of sweat chloride concentration, however validity and responsiveness were demonstrated. Validity was demonstrated for intestinal current measurement, however further information is required on reliability and responsiveness. For all three endpoints, normal values were collected and further research requirements were proposed.

This body of work adds useful information to support the promotion of CFTR biomarkers to surrogate endpoints and to guide further research in the area.

INTRODUCTION

Outcome measures fall into three classes: clinical endpoints, surrogate endpoints and biomarkers.

Clinical endpoints reflect how a patient feels, functions or survives (1, 2) and detect a tangible benefit for the patient. The improved life expectancy in cystic fibrosis CF (CF) has rendered survival, the gold standard clinical efficacy measure, an impossible endpoint to use in clinical trials. Therefore, intermediate clinical efficacy measures, such as the frequency of respiratory exacerbation were introduced. The latter has been used in registration trials for rhDNase (3), tobramycin solution for inhalation (4) and aztreonam lysinate (5). Clinical endpoints particularly useful for young children include anthropometric measures. Quality of life as measured by the Cystic Fibrosis Questionnaire-Revised (CFQ-R) is also accepted as a measure of treatment benefit in CF (6, 7) however it is considered only an optional endpoint by the European Medicines Agency (6, 8).

A surrogate endpoint is a laboratory measurement used as a substitute for clinical endpoints and predicts the efficacy or toxicity of therapy (1, 2). It is an indirect measurement of effect and is used when direct measurement of clinical effect is not feasible or practical. Surrogate endpoints can be used complementary to measures of treatment benefit and may shorten the period of follow-up required. The link between the surrogate endpoint and survival, long-term prognosis or accepted measures of treatment effect (both improvement and deterioration) must be proven. FEV_1 has been widely used as a surrogate endpoint due to the established link with survival (9). However, in many patients with CF, the rate of decline in FEV_1 has slowed (10), limiting the current sensitivity of the measure, particularly in children or in patients with mild lung disease (10, 11).

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to a therapeutic intervention” (e.g. nasal potential difference NPD, mucociliary clearance, inflammatory markers, sputum bacterial density) (1, 2). These measures are mainly used in phase I or II clinical trials when proof-of-concept for a specific compound is explored. Biomarkers are useful for gaining information about the mechanism of action of potential drugs, for identifying treatment responders and for dose selection. Some biomarkers are currently being considered for “promotion” to the status of surrogate endpoint. They are often used as secondary outcome measures in phase III trials which provides data on responsiveness, confirms mechanism of action, and compiles information for promotion of biomarkers to surrogate outcome measure. During phase III trials with ivacaftor, CFTR

correctors (Vertex Pharmaceuticals Ltd, USA) and ataluren (PTC Therapeutics Inc, USA) in patients with CF, CFTR biomarkers are being used as endpoints. These new therapies address the basic defect in CF and may be particularly well suited for people with early or mild lung disease.

To gain acceptance by researchers and licensing bodies, an outcome measure must be assessed for clinimetric properties such as reliability, validity and responsiveness to treatment (Table 1). Reliability (e.g. an assessment of the consistency of a given measurement), is important, both in terms of inherent biological variation (repeatability) and also in relation to differences across different assessors and centres (reproducibility). To optimise reliability in multiple centre trials, standardised operating procedures (SOPs), and training are needed (12). Validity refers to clinical and biological relevance; in other words, there must be a direct link with the disease process and the mechanism of action of the intervention (13). The outcome measure should correlate with established measures of treatment benefit or a gold standard (i.e. concurrent validity and predictive validity) and reflect clinical severity (i.e. discriminate validity) (14). When a gold standard is not available, evaluation of convergent validity can be performed (i.e. an outcome measure can be compared with another which measures the same attribute). Prediction of prognosis is also important, for example, the ability to predict survival (predictive validity). Responsiveness refers to the ability of the measurement to detect change due to an intervention known to alter the attribute of interest.

Also important in the development of surrogate endpoints is feasibility, referring to financial, practical and ethical considerations as well as patient and assessor acceptability (15). A feasible endpoint should be cost-effective, pose minimal risk/discomfort to the patient and should be applicable throughout the entire range of ages and disease severities. Feasibility will determine whether outcome measures gain acceptance into research practice. Clinimetric properties and feasibility are population and situation dependent.

Scope and purpose of the guideline

This guideline documents the European Cystic Fibrosis Society Clinical Trial Network ’s (ECFS-CTN) current agreement on aspects of CFTR biomarkers for use in clinical trials in the area of CF. After preparatory work during a period of six months, participants met twice to discuss their results and conclusions (17/18th November 2010, 9th June 2011). This resulted in a draft document that was circulated among all participants and further amended.

After a description of the CFTR biomarkers, we explore the clinimetrics and the feasibility of the chosen outcome measures, we report on their use in clinical trials and we conclude by

answering the following questions: 1) Do CFTR bio-assays have the potential to become surrogate outcomes? 2) For what kind of therapeutic trial is this outcome appropriate (therapeutic aim; phase of trial, target population, trial duration, number of patients involved, number of sites involved)? 3) Within what time frame can change be expected and what treatment effect can be considered clinically significant? 4) What are the most needed studies to further define these outcome measures in patients with CF?

The guideline also provides an inventory of the literature on selected CFTR bio-assays. We chose to include papers published since 1980 only. It is hoped that this document will offer some guidance for pharmaceutical companies, investigators and regulatory authorities.

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CFTR bio-assays

CFTR biomarkers measure the presence and/or function of the CFTR protein in different organs. We chose to discuss the sweat chloride test, the nasal potential difference measurements (NPD), and intestinal current measurements (ICM) because they are functional assays and not only document the presence of CFTR, but also its ion transport activity. As such, they are most appropriate for use in clinical trials of compounds aiming to correct the basic defect in patients with CF, e.g. gene therapy and small molecules such as CFTR potentiators, correctors, and premature termination codon suppressors (16-21). These biomarkers of CFTR function are currently used to confirm the diagnosis of CF (22-25). Since values for these biomarkers differ in CF versus non-CF subjects, it seems logical to hypothesise that, when treatments correct the basic CFTR defect at the protein level, the values for these biomarkers will change as well.

After stimulation of sweat production by pilocarpine iontophoresis and collection of sweat in a gauze or collector Macroduct® (Wescor), the sweat chloride concentration is determined by original titration with colorimetric end-point (QPIT), by titration with coulometric end-point (chloridometer), by *in situ* selective electrode (Exsudose®, TemSega) or by indirect potentiometry (ISE) (26). The increase in sweat chloride concentration in CF is the consequence of decreased chloride re-absorption via CFTR in the water impermeable sweat ducts (27). NPD and ICM measure the voltage potential or electrical current, respectively, resulting from epithelial ion fluxes at the mucosal surface *in vivo* and *ex vivo,* respectively. The NPD measurement is thought to provide information on both sodium absorption and chloride secretion (28, 29). In normal airway epithelia, sodium absorption is the primary ion transport activity so that the resulting airway surface PD is negative with reference to the

interstitium. Perfusion of the ENaC channel blocker amiloride will lead to a less negative PD. Creating a chemical gradient for chloride by superfusion of chloride free solution followed by activation of the CFTR channel with isoproterenol, will lead to chloride secretion and thus again a more negative PD. In contrast, in CF subjects there is heightened ENaC mediated sodium absorption due to absent or dysfunctional CFTR (30-32). The resultant baseline PD is thus more negative. The change with application of amiloride is larger, where-as minimal or no change in PD is seen upon stimulation of chloride secretion through CFTR dependent pathways. Recently the notion of increased sodium absorption in CF epithelia has been challenged by data in the newborn CF pig and in cultured tracheal epithelia (33, 34). In these models, the defective CFTR chloride current seemed sufficient to explain all phases of the NPD measurement. For ICM, an intestinal (usually rectal suction) biopsy and special micro-Ussing chamber are needed for measurement of *ex vivo* transepithelial short-circuit current (Isc) as a measure of net ion fluxes across the tissue. In CF, the intestinal CFTR-mediated chloride secretion is impaired, while absorptive processes remain unchanged or may be enhanced. In CF, the normal Isc response to forskolin, an activator of CFTR, is absent or reduced. The Isc responses to carbachol and histamine consist of two components: a lumen-positive current that is most likely caused by the apical potassium efflux, and a lumen-negative current, caused by apical chloride secretion. In ICM of healthy individuals, the apical potassium efflux in reaction to carbachol and histamine is masked by the much larger chloride efflux. In CF, the response is reversed due to the apical potassium efflux in the absence of a chloride efflux, or biphasic due to residual CFTR-mediated chloride efflux in milder forms of CF (35-37).

Clinimetrics of CFTR bioassays

For NPD, data were collected on reliability (Table 2), validity (Table 1 online supplement) and responsiveness (Table 3). Eight studies document reliability of NPD and demonstrate that with repeated measurements, the mean results per group and the diagnostic conclusions do not differ; however, the within-subject variability is considerable. There is strong evidence that NPD has excellent discriminate validity Twenty five studies consistently show a statistically significant difference in chloride and sodium conductance between patients with CF and healthy controls. In patients with “questionable” CF, NPD composite scores provided a highly sensitive tool to diagnose patients as “CF-likely” and “CF-unlikely”, with both cohorts having significantly different disease presentation (38-41). Data from studies with ataluren, ivacaftor, the CFTR corrector VX-809 and gene therapy confirm that NPD is a responsive

endpoint. In the earliest gene therapy trials, the overall results were not uniformly conclusive. The low subject numbers of subjects along with the relatively low bioactivity of the agents tested may explain these non-significant results for NPD. Tables 2 and 3 in the online supplement report reference values for the NPD measures in patients with CF and in healthy controls. The majority of the available data concerns adults.

Reliability data for sweat chloride are inconclusive as these are mainly from retrospective studies with few, or combined CF and non-CF individuals (Table 4 online supplement). Data clearly establish validity of sweat chloride, which discriminates between patients with CF and non-CF individuals, between patients with CF and carriers (Table 5 online supplement) and between patients with different disease severit (e.g. patients with pancreatic sufficiency and insufficiency). Individuals grouped according to their sweat chloride result had significantly different disease presentation. The sweat chloride concentration has been used as an endpoint in studies of ivacaftor and VX-809 which clearly demonstrated responsiveness of this parameter (Table 3). However, one study investigating ataluren demonstrated a significant difference in NPD but failed to show a difference in sweat chloride (18). Subsequently, the sweat chloride test was not included as an endpoint in additional phase II trials of ataluren, but is currently being evaluated in the phase III trial.

Few studies were found about clinimetric properties of ICM (Table 6 online supplement). No studies were found on reliability. ICM has been shown to discriminate between patients with CF and healthy individuals (37, 42-48) and –at a group level- can discriminate between pancreatic sufficient and insufficient patients (35). Similar to the sweat test and the NPD, patients with CF who were grouped according to their ICM result have been shown to differ in disease presentation: the more chloride secretion measured in the rectal mucosa, the milder the disease presentation (35, 37, 44). These data provide evidence of sound discriminate validity. ICM has been shown to correlate well with results from CFTR mutation analysis and moderately with sweat chloride (37). No studies used ICM as an endpoint. For reference values we refer to Derichs et al 2010 (37).

Feasibility of CFTR bioassays

The sweat test has a long tradition, is widely available, relatively non-invasive and easy to perform but for reliable performance rigorous adherence to standard techniques is needed (26, 49, 50). The more recent measurements of NPD and ICM are limited to selected centres with expertise. Given the complexity of these tests, strict adherence to SOP’s is important.

All three tests can be performed from infancy through to adulthood. However, NPD can be problematic in young children. NPD in infants can be done for diagnostic purposes in single centres with extensive experience in this age group (39, 51). As such, use of NPD as an outcome measure in clinical trials in infants and preschool children has a limited role. Conversely, ICM may be better tolerated by younger children than in adults because it involves rectal sampling. Obtaining a sufficient amount of sweat can be an issue in some (mainly young) patients. Obtaining valid NPD measurements may be impossible or (temporarily) unreliable in subjects with acute upper respiratory tract infection, extensive nasal polyps or after prior sinus surgery.

The risk of infection is minimal in all three tests when care is taken to discard, disinfect or sterilise equipment as appropriate. Electrical equipment for sweat testing and NPD, should be checked annually for current control and leakage. The sweat test is viewed as a comfortable and very safe as it uses a low voltage electrical current produced by a battery. Some local erythema lasting a few hours is expected; skin burns can occur when sweat test equipment is not properly handled (26). A small scab or skin scar can occur when too deep a skin abrasion is performed during NPD measurement. Rarely a rectal bleed can occur after biopsy taking for ICM (52) which is contra-indicated in patients with abnormal hemostasis or portal hypertension.

The cost for equipment is lower for the sweat test than for the NPD or ICM. The sweat test requires staff time to cover sampling and assay. The NPD requires staff time to prepare solutions and catheters/bridges and to perform the procedure. ICM requires an experienced gastroenterologist/CF specialist to obtain the sample, a research nurse and a technician. The time required to perform each test is approximately the same (90 to 120 minutes). The sweat test and ICM require clinical space for sample collection and laboratory space for assay. NPD requires sufficient clinical space to accommodate the equipment along with the personnel due to the *in-vivo* nature of the test.

Training is required to perform each test. Dedicated laboratory personnel can easily learn sweat collection and analysis assay. NPD and ICM require more extensive training and experience in order to minimise variability of the results (for NPD: correct placement and fixation of catheter, real time interpretation of readings including stable baseline and end of response to solutions, and troubleshooting; for ICM: biopsy taking, mounting the tissue in the Ussing chamber, real time interpretation of readings and checking biopsy viability).

Comparison of the different CFTR biomarkers

The advantages of using sweat chloride as outcome measure are its feasibility, availability and the assessment of CFTR function in an organ unaffected by chronic infection and inflammation. Results evaluating ivacaftor and VX-809 also suggest it is more sensitive to small changes in CFTR activity. The advantage of NPD is that it reflects CFTR function in the respiratory tract (albeit the upper respiratory tract), the organ strongly related to CF survival. Measurements in the lower respiratory tract can be performed bronchoscopically (53) but are too complex and invasive for use in large scale trials. Advantages of ICM include easy application in young children and the ability to measure both chloride and bicarbonate transport. It is anticipated that ICM may have a fast response to CFTR correctors because of the exceptionally high cell turnover in the intestinal epithelium that renews itself within 3 to 5 days).

Limitations of sweat testing and ICM include that they do not measure CFTR activity in the respiratory epithelium. Limitations of NPD include the large intra-subject variability and the difficulty of performing it in young children. Although it is a painless procedure, some adults are reluctant to undergo a rectal suction biopsy. Other limitations of the ICM are the very low number of centres with expertise and the short viability of the rectal biopsies, which precludes long-distance transport to a central laboratory for analysis.

Use of sweat test, NPD and ICM as outcome parameters in clinical trials to date

Sweat chloride is an appropriate biomarker in clinical trials for systemic therapies only. Marked changes in sweat chloride occurred after administration of the CFTR potentiator ivacaftor to CF subjects with the G551D mutation (20, 54). In subjects homozygous for the F508del mutation, small changes were seen after intervention with the CFTR corrector VX-809 (21) and moderate changes during combination treatment with ivacaftor and VX-809 (55). In patients with a nonsense mutation, ataluren improved NPD but not sweat chloride (18, 19). Therefore, the organ specificity or efficacy might differ between drugs.

In CFTR gene therapy trials, applications of viral and synthetic vectors to the nasal epithelium have resulted in significant changes in chloride secretion on NPD. Interventions with the nonsense mutation read-through drugs (aminoglycosides and ataluren) (17-19) have also been proven to change only the chloride response and not the basal potential nor amiloride response. An improvement in chloride but also sodium transport was observed with ivacaftor therapy, the latter only in the combined data set from the two parts of the trial (20). In patients exposed to ataluren for 84 days, both components of the total chloride response, the zero chloride response and the isoproterenol response, improved significantly, but the zero chloride

improvement was larger (56). In the ivacaftor trial where both have been measured, changes in sweat chloride concentration were more impressive than changes in NPD read out (20).

For ICM substantial experience has been made in preclinical human *ex vivo* corrector studies (57, 58). What follows can be taken into consideration when contemplating use of ICM as an outcome parameter. CFTR is the dominant- if not sole- apical chloride channel in the intestine and becomes rate-limiting for transepithelial chloride transport in rectal biopsies at CFTR protein levels below ~20% of wild-type controls. Therefore, a small gain in CFTR expression or function induced by CFTR corrector compounds (e.g. from 1% to 5% of wild-type values) will result in a large gain in chloride and bicarbonate secretory current (Isc) (e.g. from 5 to 25% of wild-type controls). In contrast to the sweat test and to NPD, ICM performed with bicarbonate rich perfusion fluid provides information on CFTR-dependent bicarbonate secretion, an important and CF-relevant determinant of mucus release, expansion and viscosity (59). ICM is the only biomarker that can directly assess the beneficial effects of pure CFTR correctors i.e. compounds that allow the mutant F508del-CFTR to reach the plasma membrane (60). Rescued F508del-CFTR, has major gating defects (61) that might be overcome by CFTR potentiators i.e. compounds that increase the opening of the CFTR channel. Since ICM evaluates CFTR activity *ex vivo* (e.g., by), potent potentiators (e.g., genistein, ivacaftor) can be applied directly on the tissue removed from the patient under corrector treatment to assess full CFTR activity and hence membrane rescue of the mutant protein. When using sweat test or NPD as outcome measure, pure correctors can only be tested properly *in vivo* by conducting combination trials of correctors with potentiators to overcome the gating defect of the rescued mutant protein (55).

Question 1: Do sweat chloride, NPD and ICM have the potential to become a surrogate outcomes?

Our view is that each of these measures has the potential to be a surrogate outcome since they are *in vivo* (sweat chloride and NPD) and *ex vivo* (ICM) markers of CFTR function. To achieve this, long-term studies with disease modifying drugs need to demonstrate that improvement in CFTR function correlates with improvement in clinically relevant outcomes (increased longevity, patient reported outcomes, decrease in pulmonary exacerbations) or surrogate outcomes (improvement in FEV_1). In patients 12 years and older, treatment with ivacaftor during 48 weeks lead to large improvements in sweat chloride and clinical as well as surrogate outcome measures: a decrease of sweat chloride concentration from a mean of 100 mmol/L to below 60 mmol/L, a mean weight gain of 3.1 kg, a 55% decrease in likelihood of

experiencing a pulmonary exacerbation and a mean improvement of 10.6 % predicted in FEV_1 (54). The intermediate results of the ivacaftor trial in 52 children 6-11 years of age demonstrate the same improvement in all outcomes: a drop in sweat chloride concentration from a mean of 104 to 60 mmol/L a large weight gain, a mean improvement in FEV_1 of 12.7% predicted or 17.4% change from baseline (62). Concurrent overall changes in the clinical outcome, surrogate outcome and the sweat test result are expected, rather than a close correlation between the improvement in sweat chloride concentration and the improvement in clinical or surrogate outcome. Indeed, the latter are dependent on many variables (including those unrelated to disease mechanism, such as environment, adherence, and exposure to respiratory infections). The ongoing phase III ataluren trial is expected to provide additional information, and may offer further data supporting the use of sweat test and NPD as surrogate outcome measures.

Question 2: For what kind of therapeutic trial are CFTR bioassays appropriate (therapeutic aim, phase of trial, target population, trial duration, number of patients involved, number of sites involved)?

Sweat chloride concentration and NPD are particularly well suited for phase II trials with disease modifying therapies aimed at correcting the basic CFTR defect via gene therapy or strategies to rescue or potentiate CFTR protein. Power calculations need to take into consideration the moderate (sweat chloride concentration) to large (NPD) intra-subject variability (for specific values consult the online table) and the uncertainty of the effect size that should be aimed for (see further). For phase III studies involving systemic drugs, sweat chloride concentration may be the most feasible choice. Given the complexity of the NPD technique and the large intra-subject variability even in sites with great expertise, a large, multicentre trial using NPD as outcome can be challenging and costly, but is presently in progress for the ataluren phase III trial.

For similar therapies, ICM may be useful in phase II clinical trials in adults, children and infants with CF. But more information on reliability is required before firm statements can be made. ICM has, at present, most application in *preclinical* drug testing of potentiators and correctors. As stated above, for ‘pure corrector compounds’, only ICM is appropriate.

CF is a rare disease with at present a slow lung disease progression, especially in young patients. This makes demonstration of real clinical benefit in phase III studies extremely difficult in children. Therefore, sweat chloride and NPD, being in the causal pathway of the disease, could be considered as efficacy outcome measures in such phase III trials with

disease modifying drugs, especially if a compound has proven efficacy and safety in adults. Efficacy and further safety testing can follow during phase IV pharmacovigilance. Using a biomarker or surrogate outcome as preliminary proof of efficacy is also suggested in the EMA guidance for trials in small populations (63, 64).

Question 3: Within what time frame can change be expected and what treatment effect can be considered clinically significant?

The timeline in which changes in the measurement will be detected will depend on the mechanism of action of the drug and on the rate of renewal of the epithelium studied. The kinetics of such changes in humans have not been widely evaluated. During treatment with the potentiator ivacaftor improvements in sweat chloride concentration and NPD have been demonstrated at the earliest time point measured (3 days and 14 days respectively) (20). During treatment with the CFTR corrector VX-809 alone or in combination with the potentiator ivacaftor, decreases in sweat chloride concentration were reported at day 14 to 21(65). Also during treatment with ataluren, changes in NPD readout were present at the first time point measured i.e. 14 days (18, 19).

The magnitude of change which is of clinical significance has not been established for any of the CFTR bio-assays. The mean changes in sweat chloride concentration reported with ivacaftor were large (in the order of 50 to 60 mmol/L) (20, 54, 62) . Since, in these trials all clinical and surrogate outcomes improved one can conclude that such changes in sweat chloride are clinically meaningful. Further analysis of these data may help to determine if a cut-off value of improvement in sweat chloride concentration can be correlated to a change in clinical benefit. Determining the minimally clinically important difference will be an important parameter to guide the development of further agents active towards modulating CFTR. A zero chloride plus isoproterenol response above the threshold of -5 to -7 mV is considered significant because it is the cut-off between CF and non CF subjects in cross sectional evaluation. Prospective phase III studies still have to provide evidence for this assumption. To assess response in an individual, the correct approach may be to monitor whether a repeated test, measured to monitor the response to an intervention, has changed beyond its natural variability (66). In the phase II ivacaftor trial the improvement in NPD chloride secretion was small, i.e. only -3.5 mV (20), still the clinical benefit of this drug is very marked. In another trial, small sweat chloride changes were detected with VX-809 therapy (21), whereas no changes in NPD or lung function was observed. Therefore, the relative sensitivity of changes in different outcomes is at present unclear. Is NPD less

sensitive than sweat test? Will a CFTR measurement in the respiratory tract give a better prediction of respiratory outcome than e.g. the sweat test? Will modifier drugs differ in their organ specific efficacies? For NPD we need to keep in mind that changes in basal PD and changes in amiloride response reflect sodium transport, where as changes in zero chloride and isoproterenol response reflect chloride transport. Which of these is most important for disease amelioration remains to be determined.

Only theoretical considerations can be made about ICM. Because of the fast renewal rate of intestinal epithelium (3-5 days), test compounds which act by improving CFTR function through effects on *de novo* protein synthesis are expected to show full beneficial effects in less than one week, abolishing the need for prolonged testing.

Question 4: What are the most needed studies to further define this outcome measure in patients with CF?

For sweat test better knowledge of reliability in genetically well defined controls and CF patients is needed. For NPD and ICM further unification of test performance and establishment of normal values for use in multicentre trials are needed. These aims are being addressed by the new ECFS NPD and ICM SOPs and the ongoing multicentre reference data validation study in the ECFS Diagnostic Network Working Group. In addition, the track record of these biomarkers in longitudinal phase III studies is needed. We must understand which change in CFTR-bioassay is associated with long term clinical benefit of drug therapy, and how well this associates in individual responses.

Conclusion

This document provides a systematic review of the clinimetric properties of CFTR biomarkers and provides supporting evidence for promoting these biomarkers to surrogate endpoints. Data collected demonstrate the reliability, validity and responsiveness of NPD. Fewer data were found on reliability of sweat chloride concentration, however validity and responsiveness are demonstrated. Validity is demonstrated for ICM, however further information is required on reliability and responsiveness. Normal values are collected for all three endpoints. Further research requirements are proposed for each endpoint. In particular, sweat test and ICM require further supporting data.

There is great interest in biomarkers and surrogate endpoints in CF. Already over a decade ago, participants in an NIH workshop challenged statisticians to develop robust metrics to study relationships between surrogate endpoints, clinical endpoints and interventions (1). That

NIH workshop also highlighted the need to assess data from both epidemiological studies and randomised clinical trials as a source of information on biomarkers when considering promotion to surrogacy (1). In a small population such as CF, it is all the more important that valuable information is shared and that centres work together to improve clinical research.

Table 1: Definitions and justification of importance for clinimetric/psychometric properties

Clinimetric/ psychometric property	Definition	Justification of importance
Reliability	Degree to which a measurement is consistent and free from error	Important to quantify error (systematic and random) so that true changes can be discerned from changes due to normal fluctuations
Validity	Concurrent validity: Degree to which a test correlates with a "gold standard" criterion test which has been established as a valid test of the attribute of interest Convergent validity: Degree to which a test correlates with another test which measures the same attribute Discriminate validity: Degree to which a test differentiates between groups of individuals known to differ in the attribute of interest Predictive validity: Degree to which an attribute can be predicted using the result of a predictor test/ or degree to which prognosis can be predicted	The gold standard outcome measures are often not feasible. Therefore it is important to know how an alternative outcome measure compares to the gold standard, and how different outcome measures compare. It is important to know the ability of outcome measures to discriminate between different groups
Responsiveness	Degree to which a test changes in response to an intervention known to alter the attribute of interest	Important attribute of tests used in clinical practice or research to assess treatment benefit (e.g. to identify improvements response to an intervention)

Table 2: NPD Reliability							
When NPD measurements are repeated in patients with CF, non-classic CF or questionable CF, the mean results per group do not differ.							
N and subject type		**N measurements**	**Basal potential *p*-value**	**Δ Amiloride *p*-value**	**Δ Low Chloride + Isoproterenol *p*-value**	**Statistic**	**Author**
14 16	CF (floor) CF (turbinate)	2 2	ND ND	NS NS	NS NS	Wilcoxon	(67)
17	Questionable CF	2	NS	NS	NS	paired t-test	(38)
25 43	CF Non-classic CF	2	NS 0.008	NS NS	0.07 NS	paired t-test	(68)
When NPD measurements are repeated in patients with CF, non-classic CF, questionable CF or non-CF, the individual 'diagnostic' conclusion remains the same but the intra-patient variability for individual NPD parameters is considerable.							
6 6 46 40	CF Non CF CF Non CF	≥4 ≥4 2 2	7.5 2.1 18.8, -9.8 mV 11.2,-10.4 mV	ND ND 10.3, -15.3 mV 7.6,-7.2 mV	1.5 (low chloride only) 3.3 (low chloride only) 3.1, -4.1mV 14.0,-23.6 mV	Within subject SD B-A limits of agreement R an L nostril difference (mean difference ± 2SD)	(69)
14 16 34 38	CF (floor) CF (turbinate) Non CF (floor) Non CF (turbinate)	2	ND ND	ND ND	10.2, -11.5 mV 11.5, -12.7 mV 21.9, -19.0 mV 17.4, -20.2 mV	B-A limits of agreement (mean difference ± 2SD)	(67)
25 43	CF Non classic CF	≥2	ND ND	ND ND	7, -10 mV 12, -12 mV	B-A limits of agreement (mean difference ± 2SD)	(68)
±10	CF	9	6.0, 8.5	7.5, 11.4	3.5, 9.3	within subject SD (Min and max for 4 sites estimated from Fig 1)	(70)
35 18 16	CF Questionable CF Non CF	2	6.4 % 10.5 % 9.5 %	ND ND ND	ND ND ND	CV	(71)

14	CF (subcutaneous)	2 (3 minutes)	r=0.97 p<0.05 variation=3.9%	ND	ND	not reported	(72)
		2 (>1 month)	r=0.78 p<0.05 variation=11.3%	ND	ND		
82	CF (epicutaneous)	2 (3 minutes)	r=0.92 p<0.05 variation=4.3%	ND	ND		
		2 (>1 month)	r=0.73 p<0.05 variation=14.3%	ND	ND		
7	non-CF	6 (7 weeks)	16%	ND	ND	CV	(73)

NS not significant, CV coefficient of variation, CI confidence intervals, ND no data, SD standard deviation, B-A Bland-Altman, CF cystic fibrosis
R and L: right and left

Table 3: Responsiveness of NPD and sweat chloride concentration							
The total chloride response (low chloride + isoproterenol) improves during treatment with ataluren TID in phase II open label trials in children and adults with CF carrying at least one nonsense mutation.							
N, subject type, and intervention		**Basal potential *p*-value**	**Δ Amiloride *p*-value**	**Δ Low chloride + Isoproterenol *p*-value**	**Sweat Chloride**	**Statistic**	**Author**
30	CF with nonsense mutation					paired t-test (mean change)	(19)
	Ataluren 4, 4, 8mg (14d)	NS	NS	0.04 (-4.6mV)	ND		
	Ataluren 10, 10, 20 mg (14d)	NS	NS	0.05 (-3.9mV)	ND		
53	CF with nonsense mutation					paired t-test (mean change)	(18)
	Ataluren 4, 4, 8mg (14d)	NS	NS	0.0001 (-7.1mV)	NS		
	Ataluren 10, 10, 20mg (14d)	NS	NS	0.03 (-3.7mV)	NS		
Inconsistent findings whether systemic administration of aminoglycoside changes NPD or sweat chloride values in patients with CF. Local administration of gentamycin nose drops improves NPD read-out in CF patients carrying at least one nonsense mutation.							
15d intravenous gentamycin treatment							
9	CF with Y122X mutation	NS	0.09 (20 to 15mV)	0.04 (-0.8 to -4.6mV)	0.03 (109 to 85 mmol/L)	Wilcoxon (mean before and after)	(74)
4	CF with other nonsense mutation	NS	NS	NS			
5	CF without nonsense mutation	NS	NS	NS			
15d nasal aminoglycoside treatment							
11	CF with nonsense mutation	NS	NS	NS	NA	paired t-test	(75)
18	CF without nonsense mutation	NS	NS	NS	NA		
7d intravenous gentamycin treatment							
5	CF with nonsense mutation	NS	NS	NS (4/5)	NS	GLM for repeat measures	(76)
5	CF without nonsense mutation	NS	NS	NS (0/5)	NS		

						(#patients with ≥1 reading ≥5mV)	
14d gentamycin nose drops TID							
11	CF homozygous nonsense mutation	0.008 (-48 to -34mV)	0.05 (33 to 24mV)	0.001 (0.4 to -5.5mV)	NA	t-test/MWU p value (mean before and after)	(17)
8	CF heterozygous nonsense mutation	NS	NS	0.04 (-.05 to -4.8mV)	NA		
5	CF homozygous F508del	NS	NS	NS	NA		
14d gentamycin nose drops TID							
9	CF with nonsense mutation	NS	NS	<0.001 (-0.6 to -10mV)	NA	MWU (mean before and after)	(77)
Systemic administration of VX-770 to CF adults and children carrying at least 1 G551D mutation is associated with large drop in sweat chloride and a moderate improvement of total chloride response measured by NPD.							
20	CF with G551D mutation					paired t-test (mean change from baseline)	(20)
	VX-770 75mg BID 14d	ND	ND	0.003 (-4.7mV)	<0.001 (-40mEq/L)		
	VX-770 150mg BID 14d	ND	ND	0 .01 (-5.3mV)	<0.001 (-42mEq/L)		
	VX-770 150mg BID 28d	ND	ND	0.02 (-3.5mV)	0.008 (-60mEq/L)		
	VX-770 250mg BID 28d	ND	ND	0.05 (-5.5mV)	0.02 (-38mEq/L)		
161	CF with G551D mutation						(54)
	83 VX770 150mg BID 48wks 78 placebo	ND ND	ND ND	ND ND	<0.0001 (-49mEq/L) NS (-0.8mEq/)L	MMRA (mean change from baseline through 24 wks)	
52	CF (6-11 yrs) with G551D mutation						
	26 VX770 150mg BID 24wks 26 placebo	ND ND	ND ND	ND ND	<0.0001 (-56mEq/L) NS (-1.2 mEq/L)	MMRA (mean change from baseline	(62)

						through 24 wks)	
Systemic administration of VX-809 to CF patients homozygous for F508del is associated with a small, dose dependent drop in sweat chloride							
89	CF homozygous F508del mutation					paired t-test (mean change from baseline) *Linear trend test p.0013	(78)
	VX-809 25 mg QD 28d	ND	ND	NS	NS		
	VX-809 50 mg QD 28d	ND	ND	NS	NS		
	VX-809 100mg QD 28d	ND	ND	NS	<0.05 (-6 mEq/L)		
	VX-809 200mg QD 28d	ND	ND	NS	<0.01 (-8mEq/L)		
After treating patients homozygous for F508del with VX-809 for 14 days, the addition of ivacaftor 250 mg BID during 7 days, is associated with a further small but statistically significant drop in sweat chloride							
61	CF homozygous F508del mutation						(55)
	VX-809 200mg QD 14d; +VX-770 150mg BID7d +VX-770 250mg BID7d	ND ND ND	ND ND ND	ND ND ND	<0.01 (-4.2 mEq/L)* NS (-2.2 mEq/L) P<.001(-9.1 mEq/L)	paired t test mean change from D14 or baseline*	
NPD parameters detect effect of treatment in Phase II trials of various modes of gene therapy							
12	CF						(79)
Compacted DNA nanoparticles in saline 0.8mg, 2.67mg, or 8.0mg, single dose		No change	NR	8 out of 12 subjects showed partial to complete response	NA	Descriptive	
11	CF						(80)
EDMPC cholesterol complexed with CFTR cDNA 0.4375mg, 1.3mg or 4mg total dose		NS	NS	NS	NA	Paired t-test	
16	CF					MWU & Wilcoxin rank sum	(81)
pCF-1-CFTR cDNA complexed with 229mg GL-67/DOPE/DMPE-PEG_{5000} single dose		NS	NS	NS	NA		

9	CF						(82)
pCF1-CFTR plasmid 1.25mg, single dose		NS	NS	p<0.05 (3mV to -3mV)	NA	not reported (mean before and after)	
pCF1-CFTR plasmid 1.25mg complexed with 2mg GL-67:DOPE, single dose		NS	NS	p<0.05 (3mV to 0.5mV)	NA		
16	CF						(83)
400µg pCMV-CFTR complexed with 2.4mg DOTAP cationic liposome, single dose		NS for group 2/8 treated patients demonstrated partial correction	NS for group 2/8 treated patients demonstrated partial correction	NS for group 2/8 treated patients demonstrated partial correction	NA	Not reported	
6	CF					sign rank statistic	(84)
CFTR cDNA via adenovirus vector, single dose							
$2x10^9$ I.U.		NS	NS	p=0.04 (2 to -2mV)*terb	NA		
$6x10^9$ I.U.		NS	NS	p=0.03 (2 to -0.5mV) *terb	NA		
12	CF					not reported	(85)
CFTR cDNA via DC-Chol/DOPE		NS	NS	NS	NA		
9	CF					not reported	(86)
CFTR cDNA		NS	p<0.05 (+4mV)	NS	NA		
9	CF					paired t-test	(87)
AdCFTR cDNA via adenovirus vector, single dose		p=0.01 (-53 to -35mV)	p=0.02 (37 to 20mV)	p=0.05 (-5 to -9mV)	NA		
3	CF					not reported	(29)
DC-Chol:DOPE		NS	NS	NS	NA		
No observed effect of single dose of CPX on either NPD or sweat chloride parameters							
37	CF					ANOVA	(88)
CPX, single dose		NS	NS	NS	NS		
NPD total chloride response detects effect of Moli1901 (activator of alternative chloride channels)							

4	CF					paired t-test vs. vehicle	(89)
Moli1901 (1, 3 and 10µmol/L)		NA	NA	<0.05 for all doses	NA		
NPD total chloride response detects effect of CFTR activation in patients homozygous for F508del mutation							
10	CF homozygous F508del mutation					paired t-test (mean before and after)	(90)
sodium 4-phenylbuturate 6, 6, 7g (7d)		NS	NS	p=0.0055 (5.2 to 0.6)	NS		
Basal NPD detects effect of aerosolised sodium channel blockers							
10	CF					Two way ANOVA	(91)
Amiloride nasal spray		p<0.0001 (+20mV)	NA	NA	NA		
Benzamil nasal spray		p<0.0001 (+21mV)					
12	CF					Independent t-test	(92)
Aerosolised amiloride		p<0.05	NA	NA	NA		
41	CF					no statistics	(93)
(n=16) Aerosolised amiloride (10^{-3}M)		+35mV	NA	NA	NA		
(n=5) Aerosolised benzamil ($7x10^{-3}$M)		+35mV					
NPD detects effect of flavonoids on CFTR function							
12	non-cf					ANOVA	(94)
Quercetin 20µg:mL single dose		NR	P<0.05 (-7mV)	P<0.05 (-15mV)	NA		
25	non-cf						
(n=15) quercetin, (n=3) genistein, (n=3) kaempferol, (n=4) apigenin		P<0.05 (-3mV)	ND	ND	ND	Paired t-test	(95)
NPD detects effect of hypertonic saline							
7	non-CF					paired t-test	(96)
150mM		p<0.05 (6.6mV)	ND	ND	ND		
250mM		p<0.05 (7.6mV)					
500mM		p<0.05					

		(10.0mV)					
	1,200mM	p<0.05 (13.1mV)					
	2,000mM	p<0.05 (14.8mV)					
NPD detects effect of fluticasone propionate on epithelial sodium absorption							
6	non-CF						
	Fluticasone propionate	ND	p=0.03 (1.8 to 3.3 mV)	NS	NA	paired t-test	(97)
NPD detects effect of milrinone on epithelial sodium absorption							
6	CF					MWU	(98)
	Milrinone (perfused during NPD)	p<0.05 (52 to 57mV)	NS	NS	NA		
Total chloride response increases in response to increased temperature							
32	non-CF	NS	NS	0.01 (-4.4mV)	NA	paired t-test	(99)
PD recorded at the end of Ringers (i.e. basal) and at the end of isoproteronol were more polarised when using agar catheter versus perfusion method							
26	non-CF	p<0.05 (-15.9 vs. -14.0mV)	NS	p<0.05 (-31.2 vs. -24.8mV)	NA	paired t-test	(100)
Basal NPD and amiloride response detects effect of moderate exercise							
7	CF					paired t-test	(101)
Cycle ergometer exercise at 80%HR_{peak}		p<0.05	ND	ND	ND		
9	CF					paired t-test	(102)
Cycle ergometer exercise at 85% of VT		p<0.01 (-34 to -7mV)	p<0.01 (+26 to +16mV)	NS	ND		

Abbreviations: HRpeak = peak heart rate, VT = ventilatory threshold, NA = not applicable
Subject type: CF subjects with a specific mutation can be homozygous or heterozygous for this mutation unless specifically stated.

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