

Review

Biomarkers for cystic fibrosis drug development



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Abstract

Purpose: To provide a review of the status of biomarkers in cystic fibrosis drug development, including regulatory definitions and considerations, a summary of biomarkers in current use with supportive data, current gaps, and future needs.

Methods: Biomarkers are considered across several areas of CF drug development, including cystic fibrosis transmembrane conductance regulator modulation, infection, and inflammation.

Results: Sweat chloride, nasal potential difference, and intestinal current measurements have been standardized and examined in the context of multicenter trials to quantify CFTR function. Detection and quantification of pathogenic bacteria in CF respiratory cultures (e.g.: *Pseudomonas aeruginosa*) are commonly used in early phase antimicrobial clinical trials, and to monitor safety of therapeutic interventions. Sputum (e.g.: neutrophil elastase, myeloperoxidase, calprotectin) and blood biomarkers (e.g.: C reactive protein, calprotectin, serum amyloid A) have had variable success in detecting response to inflammatory treatments.

Conclusions: Biomarkers are used throughout the drug development process in CF, and many have been used in early phase clinical trials to provide proof of concept, detect drug bioactivity, and inform dosing for later-phase studies. Advances in the precision of current biomarkers, and the identification of new biomarkers with 'omics-based technologies, are needed to accelerate CF drug development.

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1. Introduction

Cystic fibrosis (CF) is caused by mutations in a gene coding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an ion channel regulating chloride, bicarbonate, sodium and fluid fluxes at epithelial surfaces [1]. While there have been steady improvements in outcome, median predicted survival for a newborn with CF in the United States in 2014 was 41 years, and median age of death in 2014 was 28 years (Cystic Fibrosis Foundation 2014 Patient Registry Report) underscoring the need for better treatments. CF lung disease is characterized by defects in ion transport, mucociliary clearance, inflammation, bacterial infection, and airway remodeling that culminates in bronchiectasis and respiratory failure. CFTR may also cause intrinsic abnormalities in host defense cells including epithelia, neutrophils and macrophages.

Given its complex pathophysiology, the U.S. CF Foundation (CFF), academic investigators, industry partners and other sponsoring agencies have taken a multipronged approach targeting the different elements of CF pathophysiology. In particular, efforts aimed at treating *Pseudomonas aeruginosa* infection, thinning CF mucus and restoring airway surface liquid have shown clinical benefit but have not halted decline in lung function. Recently, a new class of agents termed CFTR modulators has had clinical impact (dramatic lung function improvement with ivacaftor for patients with CFTR gating mutations; modest with lumacaftor–ivacaftor for F508del homozygous patients) [2–5]. However, other measures more sensitive than lung function may accelerate drug testing and biomarkers are becoming recognized as a critical tool for CF drug development.

A biomarker, by US Food and Drug Administration (FDA) definition, is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or biological responses to a therapeutic intervention” and offers great promise to speed drug development [6]. The potential uses of biomarkers in drug development include enabling assessment of safety, efficacy, and patient selection for the purpose of enrichment. The FDA recognizes the importance of biomarkers and drafted a qualification process that addresses aspects of biomarker development and clinical utility [7]; here are great challenges to meeting this rigorous qualification process for a rare disease such as CF [8].

To advance biomarkers in CF drug development, the CFF has convened a CF Biomarker Consortium consisting of

investigators with particular expertise in biomarker research. This document focuses on biomarker validation and qualification for assessing CFTR function and detection, infection, and inflammation, examines relationships of existing biomarkers to established clinical outcomes in CF, highlights new technologies for biomarker development, and summarizes areas requiring further development.

2. Biomarker validation and qualification within the context of utilization

As biomarkers for CF drug development are advanced, their application or utilization will dictate the necessary validation and qualification process. All biomarkers to be used in the context of drug development should reliably and sensitively capture the biologic activity of a therapy. Hence, any novel assay, procedure, or test must be validated for its ability to accurately and reproducibly measure a biologic process. Beyond biomarker validation, additional qualification of a biomarker would establish a linkage with clinical outcomes (clinical validity); validation of such a linkage strengthens the utilization of the biomarker throughout later phases of clinical development and in rare cases can promote a biomarker to the level of a surrogate endpoint that substitutes for a clinical endpoint in pivotal registration trials [8,9].

It is important to recall that the role of biomarkers throughout drug development, and particularly in early phase studies, is to demonstrate biological efficacy of new therapies, confirm mechanism of action, and inform dose selection. Biomarkers used in early phases of drug development do not require qualification, or even evidence of strong correlation with measurable clinical outcomes. In early phases they can be used to explore and inform go/no go decisions regarding later-phases, but notably the level of risk to the overall development program is ultimately dependent on confidence that promising biomarker results will predict clinical efficacy.

Biomarkers typically play a supportive role to confirm mechanism of action in later-phase studies; however, in some settings it may be desirable for them to replace traditional clinical efficacy measures in order to streamline drug development by enabling more rapid assessment of efficacy with potentially fewer numbers of patients. In most cases this would require that the biomarker meets qualification standards as a surrogate endpoint for clinical efficacy [7]. Surrogate endpoints serve as substitutes for accepted measures of clinical efficacy and can accelerate drug

approval by responding over a shorter duration (e.g. compared to survival), or by requiring fewer study participants because of increased measurement precision. The process of qualifying a biomarker as a validated surrogate endpoint for phase 3 trials requires that the marker correlates with a meaningful clinical endpoint and captures the net effect of the intervention or drug on the efficacy endpoint [10]. The latter is more difficult to achieve because it demands that late-phase studies simultaneously capture the biomarker and the clinical endpoint to evaluate predictive net effect [11]. To date, no biomarker has formally gone through the qualification standards of a surrogate endpoint for clinical efficacy in CF. Importantly, however, there are instances in other rare diseases, such as Fabry disease, for which a non-validated surrogate endpoint of a physiologic biomarker of disease has been used as a pivotal endpoint in a registration trial [12].

While more traditional drug development focuses on prognostic biomarkers that are correlates of disease outcome and are intervention-independent, there is also a role for predictive biomarkers in drug development which offer information that is more specific to a particular intervention. Predictive biomarkers have been an area of focus for many years in oncology research [13]. In a drug development program, a predictive biomarker may be an analyte, gene, or protein that identifies candidate study participants who are more likely to benefit from treatment and enable enrichment trials that are meant to optimize trial outcomes. As with the more general classification of biomarkers, development and validation of predictive biomarkers generally demand rigorous, multi-staged assessment of their clinical utility through retrospective and prospective controlled trials [14].

As in any rare disease setting, opportunities for flexibility in this rigorous process balanced against level of risk will be necessary given the limited patient population. Strategic utilization of biospecimens and well characterized phenotypic data in conjunction with rigorous study design and close collaboration with regulatory agencies are essential for advancing biomarkers in CF.

3. CF relevant pulmonary biomarkers: current status and opportunities

Progressive lung disease is the primary cause of CF morbidity and mortality, and a critical target for therapeutic development. Several therapies have been developed to address CF lung pathologies, and their clinical use has been associated with improved outcomes. The most common primary outcome measures supporting regulatory approval of CF pulmonary therapies are discussed below. Physiologic outcome measures have recently been published [15].

3.1. Accepted clinical efficacy measures in CF

Change in FEV₁ has become one of the most established endpoints to demonstrate clinical efficacy in CF clinical trials [2,4,16–18]. Pulmonary exacerbations (risk, frequency, time to) have also served as primary clinical efficacy endpoints, but typically require larger and longer studies to demonstrate

treatment impact. Important secondary measures that have supported approval and clinical use of pulmonary therapies include patient reported outcomes (e.g. CF Quality of Life—Revised instrument), weight gain and bacterial density [4,18–22]. To date, no CF-specific anti-inflammatory drug has gone through regulatory approval and thus there has been little guidance as to what the accepted clinical efficacy measures will be for these agents, which have not produced the type of immediate and sustained FEV₁ improvement observed with mucolytics, antibiotics, or CFTR modulators [23,24].

3.2. Need for more sensitive clinical efficacy endpoints

Important gaps that have emerged include relevant outcome measures for patients with either mild or advanced lung disease, and young CF patients who typically have both mild disease and poorly standardized outcome measures. Multiple breath washout testing and the Lung Clearance Index as recently published [15] has advanced as a putative endpoint with greater sensitivity than FEV₁ to detect biologic effect in younger patients with mild lung disease, and are currently included in several CF clinical trials. Imaging may serve as a correlate of structural lung injury (e.g. bronchiectasis). The remainder of this review will examine the existing data supporting the validity and qualifications of several biomarkers of CFTR activity, infection and inflammation, and the investigations needed to optimize these biomarkers for use in CF drug development.

4. CFTR activity and ion transport in vivo

There are three *in vivo* biomarkers of ion transport which have primarily been used to monitor restoration of CFTR function. These include sweat-chloride concentration, nasal potential difference (NPD) measurement, and intestinal current measurement (ICM). Sweat-chloride is a simple, portable, and reliable test of CFTR function that clearly discriminates between patients with minimal, partial and full CFTR activity [25,26]. It has been carefully standardized for both clinical use and for incorporation into clinical trials, and remains the mainstay of CF diagnosis. Different levels of CFTR function quantified by the sweat-chloride test also correlate with important markers of disease severity, including age of diagnosis (pre-newborn screening), pancreatic sufficiency, isolated male infertility, microbiology, and lung disease severity [25–30]. Profound reductions in sweat-chloride have been observed in all studies of ivacaftor monotherapy in CF patients with gating mutations [3,5,31–34]. Intermediate effects were observed with ivacaftor in CF patients with the R117H mutation, and smaller effects in CF patients with two copies of the F508del CFTR mutation treated with ivacaftor or ivacaftor/lumacaftor [35–37]. The effects of CFTR modulators on sweat chloride generally parallel the clinical benefits observed for these three populations (FEV₁, risk of pulmonary exacerbations—see Table 1). While individual changes in sweat-chloride have not correlated directly with FEV₁ improvements [38,39], aggregate data have demonstrated excellent assay performance and detection of biologic activity. Future studies utilizing sweat-chloride will need to monitor long term relationships between sweat-chloride

Table 1
Impact of CFTR modulators on sweat-chloride in clinical trials.

*Ivacaftor monotherapy	Enrollment (number on modulator)	Change in sweat [chloride] (treatment effect)
Accurso [4] (G551D, >18 years)	N = 8	–42.3 mM (p < 0.01)
Ramsey [5] (G551D, >12 years)	N = 83	–48.1 mM (p < 0.001)
Davies [34] (G551D, 6–11 years)	N = 26	–54.3 mM (p < 0.001)
Davies [36] (gating, 2–5 years)	N = 19	–46.9 mM (p < 0.001)
De Boeck [6] (gating, age > 6 years)	N = 39	–49.2 mM (p < 0.001)
Moss [37] (R117H, >6 years)	N = 34	–21.9 mM (p < 0.001)
Rowe [35] (G551D, >6 years)	N = 151	–53.8 mM (p < 0.001)
Flume [39] (F508del/F508del, >12 years)	N = 112	–2.9 mM (p = 0.04)
Lumacaftor monotherapy	Enrollment (number on modulator)	Change in sweat [chloride] (treatment effect)
Lumacaftor/ivacaftor co-therapy		
Clancy [45] (lumacaftor, 200 mg every 24 h, F508del/F508del, >18 years)	N = 19	–8.21 mM (p < 0.01)
Boyle [38] Lumacaftor 400 mg every 12 h and ivacaftor 250 mg every 12 h, F508del/F508del, >18 years)	N = 11	–10.3 mM (p = 0.002)

*Studies listed by lead author, with genotype and age of enrolled subjects as noted. Patients >6 years of age were dosed with ivacaftor 150 mg every 12 h. Patients age 2–5 years were dosed with ivacaftor based on weight. †Dose of lumacaftor and ivacaftor as noted.

and key clinical outcomes in CF to support this biomarker as a key measure to accelerate drug development.

NPD is a direct measure of CFTR function in respiratory epithelium, and isolates CFTR activity across the nasal mucosa independent of sodium transport and the activity of other chloride transporters [40,41]. It is more difficult to perform than sweat-chloride, and requires specialized equipment and extensive training. Recent efforts have standardized NPD performance and analysis across the US and Europe, including SOPs and centralized coordination and interpretation of trial data [42–46]. It has been incorporated into small investigator-initiated trials of CFTR and other ion transport modulators [47–50] and also into early phase trials of CFTR modulators that proceeded to seek regulatory approval [3,46]. In multi-center trials, NPD measurements had sufficient sensitivity to detect dose-dependent bioactivity of ivacaftor in patients with the G551D CFTR mutation, but failed to detect bioactivity of systemic ataluren or lower-dose lumacaftor monotherapy in phase 2 and 3 studies performed in patients with PTC and F508del mutations, respectively [3,44]. Neither of these interventions had measurable clinical benefits, suggesting that the NPD assay may be specific for clinically relevant modulator bioactivity. Studies focused on improving NPD reliability and examining relationships between CFTR (or other ion transporter) restoration and clinical response are gaps in CFTR biomarker development and would clarify the future role of this assay in CF drug development.

ICM is another assay that can isolate CFTR function, and has the advantage of a large dynamic range between CF and non-CF [28,51–54]. Rectal biopsies are dissected and then

studied in Ussing chambers to monitor CFTR-dependent ion transport *ex vivo*. Like NPD, efforts have standardized ICM performance, and a universal SOP has been adopted by both US and European centers with centralized data interpretation for use in multicenter clinical trials [53,55]. Like sweat-chloride and NPD testing, ICM can clearly discriminate different levels of CFTR function based on CFTR genotype (nonfunctional, partial and full function) with clinical correlates dependent on level of CFTR activity [28,51]. Demonstrating reliability of ICM is difficult due to the need for repeated biopsies, and performance of the assay is limited to centers with expertise in specialized electrophysiologic measurements. For these reasons, ICM will likely remain an early phase CFTR biomarker performed in a limited number of standardized centers.

5. Biomarkers of infection

Viral and fungal infections are critical aspects of CF lung disease, but infection with defined pathogenic bacteria such as *P. aeruginosa*, *Burkholderia cepacia complex* and methicillin resistant *Staphylococcus aureus* are linked to CF morbidity and mortality [56–60]. Thus, developing anti-microbial interventions is a key goal of CF therapeutics. Infection can be considered as a progression from early transient infections to chronic, often biofilm-dominated conditions that are poorly reflected *in vitro* [61,62].

The most direct biomarkers of infection are from the lower respiratory tract (bronchoalveolar lavage (BAL) fluid, sputum) but other sources such as cough swabs, oropharyngeal swabs and

nasopharyngeal (NP) samples are used in non-expectorating patients, albeit with unclear sensitivity and specificity for lower airway tract infection that limits their applicability to drug development.

Commonly used biomarkers for antimicrobial studies include bacterial density (CFU/g for sputum or CFU/mL for BAL fluid) and/or detection of CF pathogens as primary or secondary endpoints. Only drugs treating chronic *Pseudomonas* infection have sought regulatory approval, and relied on reduction in microbial density as a key supportive endpoint [17,63–65]. However in many trials change in FEV₁ is the primary endpoint and this may not always overlap with reductions in *Pseudomonas* density [66].

Targeted PCR-based detection, which is routine for viruses, is being introduced to detect CF bacteria, yet these methods have not been used extensively [67,68]. Panels to detect frequently encountered bacteria are in development, and are often based on detection of bacterial enzymes or virulence factors that may enable early detection of resistance.

5.1. The CF lung microbiome

Evaluation of the CF lung microbiome (through unselected detection of 16S ribosomal bacterial RNA using deep sequencing methods) is an emerging technology. Microbiome studies to date have generally shown that decreased measures of microbial diversity are associated with more advanced disease and may change prior to exacerbations [69–71]. Methodological differences in extraction and sequencing, poor distinction of live vs. non-viable bacterial DNA, and the lack of quantitation are current limitations to their clinical and biomarker use. Detection of bacteria using molecular methods has enhanced sensitivity and potentially may reduce processing times, but monitoring of disease status and drug effect via molecular detection and microbiome analysis remains exploratory (Table 2).

Future directions include functional evaluation of the microbiome either through interactions between taxa and/or changes in metabolic activity correlating to clinical outcomes. To examine metabolic activity, DNA and RNA based metagenomics or metatranscriptomics (i.e. the study of the function/activity of the transcriptome—RNA-seq) have been used. Technical challenges include contamination with human genetic material and mapping

of sequence data [72]. Early reports indicate that DNA based (total) and RNA based (metabolically active) community structure overlaps for the predominant taxa, but less prevalent organisms may be metabolically very active [73]. Further, metabolic activities in CF samples show less patient-to-patient variation than the total microbiome and differ from results in other pulmonary conditions [74], and metabolic profiles of bacteria are dynamic with adaptation to the lung disease [75]. Although limited to small study numbers, early findings suggest changes in disease status based on exhaled volatile bacterial metabolites [76]. Measuring bacterial volatile organic compounds and inflammation may allow rapid monitoring of lung disease progression. Methods may include ‘electronic noses’ that distinguish patterns of volatile organic compounds rather than specific compounds, and have the benefit of portability. Technical challenges, standardization, contribution of non-respiratory vs. respiratory factors, and defining CF vs. non-CF patterns remain barriers to overcome prior to their use as biomarkers for antimicrobial therapies [77].

6. Biomarkers of inflammation

Inflammatory biomarkers could play a critical role in the development of anti-inflammatory drugs, and reflect downstream improvements in CF lung disease for disease-modifying treatments. They could be used in early phase studies to confirm the proposed mechanism of action of drug candidates. Alternatively, as correlates of clinical endpoints, they could help select agents for further Phase 3 investigations. Results from previous CF clinical trials indicate that anti-inflammatory therapies may not result in immediate improvements in pulmonary function, but could slow the rate of lung function decline [23,24]. This requires many patients being studied over a prolonged period to demonstrate efficacy, highlighting the urgency of identifying biomarkers that can more rapidly screen candidate drugs.

6.1. Lung-derived biomarkers of inflammation

The most direct method to assess CF lung inflammation is via bronchoscopy with BAL. BAL inflammatory markers have been used as clinical endpoints in pathophysiological studies (e.g. Australian Respiratory Early Surveillance Team for CF) [78,79], and in clinical trials of inhaled tobramycin [80] and

Table 2
Microbial biomarkers in acute and chronic infection.

Infection biomarker	Early infection	Chronic infection
Culture: Presence or CFU/mg (mL)	Common biomarker for antibiotic development May be of interest in CFTR specific therapies	Small changes in density with most therapies. May show changes with antimicrobials and CFTR modulators.
*Molecular detection and diversity measures	No differences prior to onset of a primary pathogen, followed by decreasing diversity with advance in disease.	Long term decrease with advanced disease. Short term changes less pronounced. Internal consistency within a given patient.
Bacterial metabolites	Few data available and low bacterial biomass compared to host derived signal, thus many signals represent host inflammation.	Changes in bacterial phenotypes and metabolites with disease progression. Some could serve as markers of disease severity.
Measures of bacterial co-occurrence	Few data are currently available, but indicate competition and communication.	Need to develop network analyses—not yet informative for pathogenesis or disease severity.

* Diversity can be measured using extensive culture conditions, but typically considered a measure in microbiome studies measuring relative abundance.

recombinant human DNase [81]. A recently published document from the European CF Society Clinical Trial Network concluded that the use of BAL in clinical research should be limited due to its invasive nature, but that it may be applicable to early-phase clinical trials conducted in specialized centers and in trials involving young children with early/mild lung disease [82].

Due to drawbacks of bronchoscopy, the two most commonly collected biospecimens to measure inflammation are sputum and blood. Spontaneous sputum expectoration is generally limited to those with more advanced lung disease (adolescents and adults). Sputum induction (inhalation of hypertonic saline) improves sample acquisition in individuals who do not routinely expectorate sputum, and biomarker measurements are reasonably comparable between induced and spontaneously expectorated sputum [83–85]. For studies in which the primary outcome is a sputum biomarker in children and/or those with preserved lung function, sputum induction likely should be performed throughout the trial. When sputum biomarkers are secondary outcomes, collecting spontaneously expectorated sputum (with induction as a back-up) is a reasonable approach.

The validity of sputum biomarkers as endpoints in CF clinical trials has been extensively reviewed [85]. Neutrophil elastase (NE) is currently the most informative sputum biomarker to monitor CF lung disease. Sputum NE activity correlates with bronchiectasis in CF [86], tracks with and is predictive of future lung function decline [87,88], relates to treatment response in pulmonary exacerbations, and predicts time to next exacerbation [89,90]. Increased BAL NE is also a predictive biomarker of impaired lung function and bronchiectasis in young children with CF [79]. Other sputum biomarkers that are associated with and predictive of key clinical events in CF include calprotectin [91], myeloperoxidase [92], high-mobility group box 1 (HMGB-1) [93,94], and YKL-40 [95].

A proof of concept study assessing the responsiveness of sputum biomarkers to intravenous antibiotic treatment during pulmonary exacerbations demonstrated that decreases in sputum inflammatory markers correlated with pulmonary treatment response [89]. These findings though were not replicated in a more recent study of exacerbation treatment [96]. Differences in sputum NE were observed over a six month study of azithromycin compared with placebo [19], but more recent interventional trials using sputum inflammatory biomarkers have generally failed to show significant changes in NE activity and other sputum biomarkers (except for modest reductions in sputum IL-6 [97–99]). Also, ivacaftor, which has been shown to improve many clinical outcomes, did not change sputum inflammatory biomarkers in a G551D CF cohort [33]. The lack of treatment effect in these studies may be due in part to the intrinsic variability, particularly between-subject variance, of sputum biomarkers [99]. This variance makes it challenging to rely upon sputum biomarkers during drug evaluation over short treatment periods, and sputum biomarkers may only be sufficiently sensitive to demonstrate anti-inflammatory effects in longer trials (\geq six months). To minimize the potential effects of sputum collection and processing on biomarker variability, recommendations include using SOPs (sputum induction, processing), centralized

laboratories for sputum processing and analysis (CFF National Resource Centers: <https://www.cff.org/Our-Research/Therapeutics-Development-Network/Working-with-the-TDN/National-Resource-Centers/>), training of research personnel, and ensuring quality control [85].

6.2. Blood-based biomarkers of inflammation

Systemic inflammatory markers would be ideal since blood measurements are easily standardized, repeatable, and can be obtained from subjects of any age and disease severity. Systemic inflammation may also link pulmonary and non-pulmonary comorbidities of CF. While there are less data correlating systemic inflammation with clinical outcomes in CF, systemic inflammatory biomarkers correlate with key clinical events including pulmonary exacerbations and lung function decline [100–104]. Circulating biomarkers have consistently changed more than sputum markers of inflammation with exacerbation treatment [91,96,105], suggesting that systemic inflammatory measures may be more sensitive in short term interventional studies focused on mitigating exacerbations. Based on results from a multicenter exacerbation study [106], serum C reactive protein (CRP), serum amyloid A (SAA) and calprotectin declined during azithromycin treatment in a CF interventional trial [107]. These reductions correlated with improvements in lung function and weight gain, providing indirect evidence that the changes were associated with clinically meaningful outcomes. Other candidate systemic biomarkers relating clinical status to CF outcomes include neutrophil elastase antiprotease complexes (NEAPC) [101], various cytokines including interleukin-6 (many studies), IgG [100], and circulating mononuclear cell RNA transcripts [108].

Additional studies are required to develop sputum and systemic inflammatory biomarkers for CF drug development. We must determine associations between inflammatory biomarkers and key clinical outcomes (FEV₁ decline, pulmonary exacerbations) in broader CF populations, which will be facilitated through biospecimen collection in longitudinal studies. We need to know whether short term changes in inflammatory biomarkers predict longer term clinical outcomes, and how airway and systemic inflammation may change with CFTR modulators. Additional data examining the effects of freezing and delayed sputum processing on analyte measurements are required, and would inform multicenter trials that collect sputum samples and perform centralized processing and analysis.

7. New technologies and tools

7.1. 'Omics-based biomarker development for new CF therapeutics

The use of 'omics based tools to identify and validate biomarkers relevant to CF pathologies has only recently begun to be applied, and elevation to regular biomarker use is not established. These tools allow for an unbiased analysis of complex cellular processes in a variety of substrates. Discovery of novel molecular biomarkers in CF has to date been limited, and the majority of discovered biomarkers represent acute response markers. Furthermore, no molecular biomarkers have

been shown to reliably predict acute or chronic CFTR restoration. The advent of novel approaches in high throughput technologies coupled with advancements in analysis of “big data” may allow investigators to ask important questions with high sensitivity and precision.

Gene-array studies have suggested the dysregulation of numerous pathways in CF. In an elegant study Wright and colleagues examined gene expression in the nasal epithelia of CF patients with either mild or severe lung disease [109] and identified abnormalities in gene expression regulating lipid metabolism, ubiquitination, and mitochondrial/whole-cell redox regulation that segregated cohorts by disease severity. More recently, Nick and colleagues identified a panel of RNA transcripts that were predictive of pulmonary exacerbations [108]. All of these observations require validation prior to clinical extension.

Mass spectrometry (MS)-based proteomics and/or metabolomics have been used for either non-biased or targeted/selected biomarker discovery, with lipidomics gaining much recent interest. MS can precisely and rapidly examine markers of disease or response to therapy broadly, allowing for global analyses of individual samples in a non-biased, data-driven approach. Metabolomic analyses of non-fasted CF patients revealed a decrease in β -oxidation of fatty acids, which is a marker of mitochondrial dysfunction [110], and CF plasma lipidomic studies have detected significant decreases in anti-inflammatory lipids [111]. In CF sputum, a metabolomic/lipidomic analysis by Yang and colleagues identified a number of proinflammatory lipids (oxylipins) previously not identified in the CF lung [112]. These studies also found a significant decrease in lipoxin A4 in CF lungs, which had previously been identified and validated by other approaches [113]. The fact that these broad analysis studies also accurately detected the known lipoxin A4 deficiency in CF increases confidence in the application of MS-based lipidomic analyses in CF.

In the case of proteomics, no broad analysis of markers of CF disease progression in serum has been reported. Studies have focused on CF versus non-CF comparisons, which require less extensive analyses to identify differences versus studies where all cohorts have CF with varying degrees of disease. Proteomic analysis of CF sputum discovered a relationship between myeloperoxidase, protein oxidation and pulmonary inflammation [114], which was validated in BAL [115]. When the proteome of CF-patient nasal epithelia was examined, a significant decrease in the expression of a number of anti-inflammatory proteins was detected [116]. Proteomic screens have discovered a previously unknown mechanism for antioxidant downregulation in CF, namely Nrf2 dysfunction [117,118]. Follow-up biochemical studies in CF primary tissues linked this dysfunction to increased inflammatory signaling, and demonstrated that activation of Nrf2 in CF mice produced anti-inflammatory benefits [119]. These results highlight the potential of non-biased proteomic analyses in discovering novel biomarkers and previously unknown mechanisms of disease.

Although ‘omics approaches hold much promise for biomarker discovery in CF, it is important to note that MS-based analyses are semi-quantitative, and therefore it is essential that observations made in proteomic, metabolomic, and lipidomic studies are

validated by other approaches. Furthermore, while instrumentation has improved, the techniques used to conduct proteomic analyses also vary and can influence results significantly. Standardization of methodology, data analysis and incorporation of these tools into therapeutic clinical trials offers the opportunity to fully realize the promise of these technologies to advance CF care and drug development.

8. Summary, needs and conclusions

The status of biomarkers to advance CF therapies varies considerably across the different pathogenic targets. Many CF biomarkers have demonstrated their ability to inform early drug development by assessing drug bioactivity and potentially enabling dose selection for trials. Sweat chloride appears to be a biomarker with great potential to guide early phase CFTR modulator development and enable regulatory decision making, and further studies examining relationships between improvements in sweat-chloride and long term outcomes may broaden the utilization of this biomarker. NPD and ICM can also provide supportive data of bioactivity, but technical challenges limit these biomarkers to early phase trials. Biomarkers of infection including sputum bacterial density and pathogen detection remain valuable tools for early phase trials. Molecular platforms to assess the microbiome (and host response) also hold promise to advance biomarkers of airway infection and drug activity, and standardization of techniques coupled with their incorporation into studies of new therapies are required to understand their role in drug development. Inflammatory biomarkers in sputum (NE) and blood (CRP, calprotectin, SAA) correlate with clinical status and interventions, but gaps in our understanding of CF inflammation are a limitation to the development of novel CF anti-inflammatories. The revolution in ‘omic technologies over the past decade is beginning to identify novel biomarkers of various disease manifestations, but advances in data analysis and the need for candidate validation are critical before these technologies become mainstream players in CF drug development. We hope that this review serves as a valuable summary of biomarkers relevant to CF therapeutics, and guides future research to advance this field and to accelerate the development of new treatments for CF patients.

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References

- [1] Spielberg DR, Clancy JP. Cystic fibrosis and its management through established and emerging therapies. *Annu Rev Genomics Hum Genet* 2016.

- [2] Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor–Ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N Engl J Med* 2015;373(3):220–31.
- [3] Accurso FJ, Rowe SM, Clancy JP, Boyle MP, Dunitz JM, Durie PR, et al. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N Engl J Med* 2010;363(21):1991–2003.
- [4] Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011;365(18):1663–72.
- [5] De Boeck K, Munck A, Walker S, Faro A, Hiatt P, Gilmartin G, et al. Efficacy and safety of ivacaftor in patients with cystic fibrosis and a non-G551D gating mutation. *J Cyst Fibros* 2014;13(6):674–80.
- [6] (US) IoM. In: Micheel CM BJ, editor. Evaluation of biomarkers and surrogate endpoints in chronic disease. Washington (DC): National Academies Press (US); 2010.
- [7] FDA. Biomarker qualification context of use 2014. [Available from:] <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopment-ToolsQualificationProgram/ucm284620.htm>.
- [8] (US) IoM. In: Field MJ BT, editor. Rare diseases and orphan products: accelerating research and development. National Academies Press (US); 2010.
- [9] Fleming TR. Surrogate endpoints and FDA’s accelerated approval process. *Health Aff* 2005;24(1):67–78.
- [10] Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med* 1989;8(4):431–40.
- [11] FDA. Guidance for industry—patient-reported outcome measures: use in medical product development to support labeling claims 2009. [Available from:] <http://www.fda.gov/downloads/Drugs/Guidance-ComplianceRegulatoryInformation/Guidances/UCM193282.pdf>.
- [12] Desnick RJ. Enzyme replacement therapy for Fabry disease: lessons from two alpha-galactosidase A orphan products and one FDA approval. *Expert Opin Biol Ther* 2004;4(7):1167–76.
- [13] La Thangue NB, Kerr DJ. Predictive biomarkers: a paradigm shift towards personalized cancer medicine. *Nat Rev Clin Oncol* 2011;8(10):587–96.
- [14] Freidlin B, McShane LM, Korn EL. Randomized clinical trials with biomarkers: design issues. *J Natl Cancer Inst* 2010;102(3):152–60.
- [15] Stanojevic S, Ratjen F. Physiologic endpoints for clinical studies for cystic fibrosis. *J Cyst Fibros* 2016;15(4):416–23.
- [16] Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N Engl J Med* 1994;331(10):637–42.
- [17] Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med* 1999;340(1):23–30.
- [18] Saiman L, Anstead M, Mayer-Hamblett N, Lands LC, Kloster M, Hocevar-Trnka J, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA* 2010;303(17):1707–15.
- [19] Saiman L, Marshall BC, Mayer-Hamblett N, Burns JL, Quittner AL, Cibene DA, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA* 2003;290(13):1749–56.
- [20] McCoy KS, Quittner AL, Oermann CM, Gibson RL, Retsch-Bogart GZ, Montgomery AB. Inhaled aztreonam lysine for chronic airway *Pseudomonas aeruginosa* in cystic fibrosis. *Am J Respir Crit Care Med* 2008;178(9):921–8.
- [21] Oermann CM, Retsch-Bogart GZ, Quittner AL, Gibson RL, McCoy KS, Montgomery AB, et al. An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr Pulmonol* 2010;45(11):1121–34.
- [22] Retsch-Bogart GZ, Burns JL, Otto KL, Liou TG, McCoy K, Oermann C, et al. A phase 2 study of aztreonam lysine for inhalation to treat patients with cystic fibrosis and *Pseudomonas aeruginosa* infection. *Pediatr Pulmonol* 2008;43(1):47–58.
- [23] Konstan MW, Byard PJ, Hoppel CL, Davis PB. Effect of high-dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995;332(13):848–54.
- [24] Lands LC, Milner R, Cantin AM, Manson D, Corey M. High-dose ibuprofen in cystic fibrosis: Canadian safety and effectiveness trial. *J Pediatr* 2007;151(3):249–54.
- [25] Wilschanski M, Dupuis A, Ellis L, Jarvi K, Zielenski J, Tullis E, et al. Mutations in the cystic fibrosis transmembrane regulator gene and in vivo transepithelial potentials. *Am J Respir Crit Care Med* 2006;174(7):787–94.
- [26] Accurso FJ, Van Goor F, Zha J, Stone AJ, Dong Q, Ordonez CL, et al. Sweat chloride as a biomarker of CFTR activity: proof of concept and ivacaftor clinical trial data. *J Cyst Fibros* 2014;13(2):139–47.
- [27] Barreto C, Mall M, Amaral MD. Assessment of CFTR function in native epithelia for the diagnosis of cystic fibrosis. *Pediatr Pulmonol Suppl* 2004;26:243.
- [28] Hirtz S, Gonska T, Seydewitz HH, Thomas J, Greiner P, Kuehr J, et al. CFTR Cl⁻ channel function in native human colon correlates with the genotype and phenotype in cystic fibrosis. *Gastroenterology* 2004;127(4):1085–95.
- [29] Cohn JA, Noone PG, Jowell PS. Idiopathic pancreatitis related to CFTR: complex inheritance and identification of a modifier gene. *J Investig Med* 2002;50(5):247S–55S.
- [30] Noone PG, Zhou Z, Silverman LM, Jowell PS, Knowles MR, Cohn JA. Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. *Gastroenterology* 2001;121(6):1310–9.
- [31] Seliger VI, Rodman D, Van Goor F, Schmelz A, Mueller P. The predictive potential of the sweat chloride test in cystic fibrosis patients with the G551D mutation. *J Cyst Fibros* 2013;12(6):706–13.
- [32] Davies JC, Wainwright CE, Canny GJ, Chilvers MA, Howenstine MS, Munck A, et al. Efficacy and safety of ivacaftor in patients aged 6 to 11 years with cystic fibrosis with a G551D mutation. *Am J Respir Crit Care Med* 2013;187(11):1219–25.
- [33] Rowe SM, Heltshe SL, Gonska T, Donaldson SH, Borowitz D, Gelfond D, et al. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. *Am J Respir Crit Care Med* 2014;190(2):175–84.
- [34] Davies JC, Cunningham S, Harris WT, Lapey A, Regelman WE, Sawicki GS, et al. Safety, pharmacokinetics, and pharmacodynamics of ivacaftor in patients aged 2–5 years with cystic fibrosis and a CFTR gating mutation (KIWI): an open-label, single-arm study. *Lancet Respir Med* 2016;4(2):107–15.
- [35] Moss RB, Flume PA, Elborn JS, Cooke J, Rowe SM, McColley SA, et al. Efficacy and safety of ivacaftor in patients with cystic fibrosis who have an Arg117His-CFTR mutation: a double-blind, randomised controlled trial. *Lancet Respir Med* 2015;3(7):524–33.
- [36] Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E, et al. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. *Lancet Respir Med* 2014;2(7):527–38.
- [37] Flume PA, Liou TG, Borowitz DS, Li H, Yen K, Ordonez CL, et al. Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation. *Chest* 2012.
- [38] Barry PJ, Jones AM, Webb AK, Horsley AR. Sweat chloride is not a useful marker of clinical response to Ivacaftor. *Thorax* 2014;69(6):586–7.
- [39] Durmowicz AG, Witzmann KA, Rosebraugh CJ, Chowdhury BA. Change in sweat chloride as a clinical end point in cystic fibrosis clinical trials: the ivacaftor experience. *Chest* 2013;143(1):14–8.
- [40] Knowles MR, Paradiso AM, Boucher RC. In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. *Hum Gene Ther* 1995;6(4):445–55.
- [41] Standaert TA, Boitano L, Emerson J, Milgram LJ, Konstan MW, Hunter J, et al. Standardized procedure for measurement of nasal potential difference: an outcome measure in multicenter cystic fibrosis clinical trials. *Pediatr Pulmonol* 2004;37(5):385–92.

- [42] Rowe SM, Liu B, Hill A, Hathorne H, Cohen M, Beamer JR, et al. Optimizing nasal potential difference analysis for CFTR modulator development: assessment of ivacaftor in CF subjects with the G551D-CFTR mutation. *PLoS One* 2013;8(7), e66955.
- [43] Solomon GM, Konstan MW, Wilschanski M, Billings J, Sernet-Gaudelus I, Accurso F, et al. An international randomized multicenter comparison of nasal potential difference techniques. *Chest* 2010;138(4): 919–28.
- [44] Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sernet-Gaudelus I, Wilschanski M, et al. Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Respir Med* 2014;2(7):539–47.
- [45] Accurso FJ, Moss RB, Wilmott RW, Anbar RD, Schaberg AE, Durham TA, et al. Denufosal tetrasodium in patients with cystic fibrosis and normal to mildly impaired lung function. *Am J Respir Crit Care Med* 2011;183(5):627–34.
- [46] Clancy JP, Rowe SM, Accurso FJ, Aitken ML, Amin RS, Ashlock MA, et al. Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. *Thorax* 2011.
- [47] Clancy JP, Rowe SM, Bekob Z, Aitken ML, Gibson R, Zeitlin P, et al. No detectable improvements in cystic fibrosis transmembrane conductance regulator by nasal aminoglycosides in patients with cystic fibrosis with stop mutations. *Am J Respir Cell Mol Biol* 2007;37(1):57–66.
- [48] Clancy JP, Bekob Z, Ruiz F, King C, Jones J, Walker L, et al. Evidence that systemic gentamicin suppresses premature stop mutations in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2001;163(7):1683–92.
- [49] Rubenstein RC, Zeitlin PL. A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in deltaF508-homozygous cystic fibrosis patients: partial restoration of nasal epithelial CFTR function. *Am J Respir Crit Care Med* 1998;157(2):484–90.
- [50] Wilschanski M, Yahav Y, Yaacov Y, Blau H, Bentur L, Rivlin J, et al. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N Engl J Med* 2003;349(15):1433–41.
- [51] Mall M, Hirtz S, Gonska T, Kunzelmann K. Assessment of CFTR function in rectal biopsies for the diagnosis of cystic fibrosis. *J Cyst Fibros* 2004;3(Suppl. 2):165–9.
- [52] Hug MJ, Derichs N, Bronsveld I, Clancy JP. Measurement of ion transport function in rectal biopsies. *Methods Mol Biol* 2011;741: 87–107.
- [53] Clancy JP, Szczesniak RD, Ashlock MA, Ernst SE, Fan L, Hornick DB, et al. Multicenter intestinal current measurements in rectal biopsies from CF and non-CF subjects to monitor CFTR function. *PLoS One* 2013; 8(9), e73905.
- [54] Bronsveld I, Mekus F, Bijman J, Ballmann M, Greipel J, Hundrieser J, et al. Residual chloride secretion in intestinal tissue of deltaF508 homozygous twins and siblings with cystic fibrosis. The European CF Twin and Sibling Study Consortium. *Gastroenterology* 2000;119(1): 32–40.
- [55] Derichs N, Sanz J, Von Kanel T, Stolpe C, Zapf A, Tummler B, et al. Intestinal current measurement for diagnostic classification of patients with questionable cystic fibrosis: validation and reference data. *Thorax* 2010;65(7):594–9.
- [56] Parad RB, Gerard CJ, Zurakowski D, Nichols DP, Pier GB. Pulmonary outcome in cystic fibrosis is influenced primarily by mucoid *Pseudomonas aeruginosa* infection and immune status and only modestly by genotype. *Infect Immun* 1999;67(9):4744–50.
- [57] Rosenfeld M, Ramsey BW, Gibson RL. *Pseudomonas* acquisition in young patients with cystic fibrosis: pathophysiology, diagnosis, and management. *Curr Opin Pulm Med* 2003;9(6):492–7.
- [58] Li Z, Kosorok MR, Farrell PM, Laxova A, West SE, Green CG, et al. Longitudinal development of mucoid *Pseudomonas aeruginosa* infection and lung disease progression in children with cystic fibrosis. *JAMA* 2005;293(5):581–8.
- [59] Navarro J, Rainisio M, Harms HK, Hodson ME, Koch C, Mastella G, et al. Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. European Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2001;18(2):298–305.
- [60] Dasenbrook EC, Checkley W, Merlo CA, Konstan MW, Lechtzin N, Boyle MP. Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in cystic fibrosis. *JAMA* 2010; 303(23):2386–92.
- [61] Hauser AR, Jain M, Bar-Meir M, McColley SA. Clinical significance of microbial infection and adaptation in cystic fibrosis. *Clin Microbiol Rev* 2011;24(1):29–70.
- [62] Moskowitz SM, Emerson JC, McNamara S, Shell RD, Orenstein DM, Rosenbluth D, et al. Randomized trial of biofilm testing to select antibiotics for cystic fibrosis airway infection. *Pediatr Pulmonol* 2011; 46(2):184–92.
- [63] Schuster A, Haliburn C, Doring G, Goldman MH. Freedom Study G. Safety, efficacy and convenience of colistimethate sodium dry powder for inhalation (Colobreathe DPI) in patients with cystic fibrosis: a randomised study. *Thorax* 2013;68(4):344–50.
- [64] Flume PA, Clancy JP, Retsch-Bogart GZ, Tullis DE, Bresnik M, Derchak PA, et al. Continuous alternating inhaled antibiotics for chronic pseudomonal infection in cystic fibrosis. *J Cyst Fibros* 2016.
- [65] Retsch-Bogart GZ, Quittner AL, Gibson RL, Oermann CM, McCoy KS, Montgomery AB, et al. Efficacy and safety of inhaled aztreonam lysine for airway pseudomonas in cystic fibrosis. *Chest* 2009;135(5): 1223–32.
- [66] Stuart Elborn J, Geller DE, Conrad D, Aaron SD, Smyth AR, Fischer R, et al. A phase 3, open-label, randomized trial to evaluate the safety and efficacy of levofloxacin inhalation solution (APT-1026) versus tobramycin inhalation solution in stable cystic fibrosis patients. *J Cyst Fibros* 2015;14(4):507–14.
- [67] Le Gall F, Le Berre R, Rosec S, Hardy J, Gouriou S, Boisrame-Gastrin S, et al. Proposal of a quantitative PCR-based protocol for an optimal *Pseudomonas aeruginosa* detection in patients with cystic fibrosis. *BMC Microbiol* 2013;13:143.
- [68] Zemanick ET, Wagner BD, Sagel SD, Stevens MJ, Accurso FJ, Harris JK. Reliability of quantitative real-time PCR for bacterial detection in cystic fibrosis airway specimens. *PLoS One* 2010;5(11), e15101.
- [69] Cox MJ, Allgaier M, Taylor B, Baek MS, Huang YJ, Daly RA, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. *PLoS One* 2010;5(6), e11044.
- [70] Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc Natl Acad Sci U S A* 2012;109(15):5809–14.
- [71] Carmody LA, Zhao J, Kalikin LM, LeBar W, Simon RH, Venkataraman A, et al. The daily dynamics of cystic fibrosis airway microbiota during clinical stability and at exacerbation. *Microbiome* 2015;3:12.
- [72] Lim YW, Evangelista III JS, Schmieder R, Bailey B, Haynes M, Furlan M, et al. Clinical insights from metagenomic analysis of sputum samples from patients with cystic fibrosis. *J Clin Microbiol* 2014;52(2):425–37.
- [73] Twomey KB, Alston M, An SQ, O'Connell OJ, McCarthy Y, Swarbrick D, et al. Microbiota and metabolite profiling reveal specific alterations in bacterial community structure and environment in the cystic fibrosis airway during exacerbation. *PLoS One* 2013;8(12), e82432.
- [74] Quinn RA, Lim YW, Maughan H, Conrad D, Rohwer F, Whiteson KL. Biogeochemical forces shape the composition and physiology of polymicrobial communities in the cystic fibrosis lung. *MBio* 2014;5(2): 1–13.
- [75] Jorgensen KM, Wassermann T, Johansen HK, Christiansen LE, Molin S, Hoiby N, et al. Diversity of metabolic profiles of cystic fibrosis *Pseudomonas aeruginosa* during the early stages of lung infection. *Microbiology* 2015;161(7):1447–62.
- [76] Whiteson KL, Meinardi S, Lim YW, Schmieder R, Maughan H, Quinn R, et al. Breath gas metabolites and bacterial metagenomes from cystic fibrosis airways indicate active pH neutral 2,3-butanedione fermentation. *ISME J* 2014;8(6):1247–58.
- [77] Fens N, van der Schee MP, Brinkman P, Sterk PJ. Exhaled breath analysis by electronic nose in airways disease. Established issues and key questions. *Clin Exp Allergy* 2013;43(7):705–15.
- [78] Sly PD, Brennan S, Gangel C, de Klerk N, Murray C, Mott L, et al. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. *Am J Respir Crit Care Med* 2009;180(2):146–52.

- [79] Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, et al. Risk factors for bronchiectasis in children with cystic fibrosis. *N Engl J Med* 2013;368(21):1963–70.
- [80] Gibson RL, Emerson J, McNamara S, Burns JL, Rosenfeld M, Yunker A, et al. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2003;167(6):841–9.
- [81] Paul K, Rietschel E, Ballmann M, Griese M, Worlitzsch D, Shute J, et al. Effect of treatment with dornase alpha on airway inflammation in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2004;169(6):719–25.
- [82] Fayon M, Kent L, Bui S, Dupont L, Sermet I. European Cystic Fibrosis Society Clinical Trial Network Standardisation C. Clinimetric properties of bronchoalveolar lavage inflammatory markers in cystic fibrosis. *Eur Respir J* 2014;43(2):610–26.
- [83] Sagel SD, Kapsner R, Osberg I, Sontag MK, Accurso FJ. Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. *Am J Respir Crit Care Med* 2001;164(8 Pt 1):1425–31.
- [84] Zemanick ET, Wagner BD, Robertson CE, Stevens MJ, Szeffler SJ, Accurso FJ, et al. Assessment of airway microbiota and inflammation in cystic fibrosis using multiple sampling methods. *Ann Am Thorac Soc* 2015;12(2):221–9.
- [85] Sagel SD, Chmiel JF, Konstan MW. Sputum biomarkers of inflammation in cystic fibrosis lung disease. *Proc Am Thorac Soc* 2007;4(4):406–17.
- [86] DeBoer EM, Swiercz W, Heltsh SL, Anthony MM, Szeffler P, Klein R, et al. Automated CT scan scores of bronchiectasis and air trapping in cystic fibrosis. *Chest* 2014;145(3):593–603.
- [87] Mayer-Hamblett N, Aitken ML, Accurso FJ, Kronmal RA, Konstan MW, Burns JL, et al. Association between pulmonary function and sputum biomarkers in cystic fibrosis. *Am J Respir Crit Care Med* 2007;175(8):822–8.
- [88] Sagel SD, Wagner BD, Anthony MM, Emmett P, Zemanick ET. Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. *Am J Respir Crit Care Med* 2012;186(9):857–65.
- [89] Ordonez CL, Henig NR, Mayer-Hamblett N, Accurso FJ, Burns JL, Chmiel JF, et al. Inflammatory and microbiologic markers in induced sputum after intravenous antibiotics in cystic fibrosis. *Am J Respir Crit Care Med* 2003;168(12):1471–5.
- [90] Waters VJ, Stanojevic S, Sonneveld N, Klingel M, Grasemann H, Yau YC, et al. Factors associated with response to treatment of pulmonary exacerbations in cystic fibrosis patients. *J Cyst Fibros* 2015;14(6):755–62.
- [91] Gray RD, Imrie M, Boyd AC, Porteous D, Innes JA, Greening AP. Sputum and serum calprotectin are useful biomarkers during CF exacerbation. *J Cyst Fibros* 2010;9(3):193–8.
- [92] Van Der Vliet A, Nguyen MN, Shigenaga MK, Eiserich JP, Marelich GP, Cross CE. Myeloperoxidase and protein oxidation in cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2000;279(3):L537–46.
- [93] Rowe SM, Jackson PL, Liu G, Hardison M, Livraghi A, Solomon GM, et al. Potential role of high-mobility group box 1 in cystic fibrosis airway disease. *Am J Respir Crit Care Med* 2008;178(8):822–31.
- [94] Liou TG, Adler FR, Keogh RH, Li Y, Jensen JL, Walsh W, et al. Sputum biomarkers and the prediction of clinical outcomes in patients with cystic fibrosis. *PLoS One* 2012;7(8), e42748.
- [95] Hector A, Kormann MS, Mack I, Latzin P, Casaulta C, Kieninger E, et al. The chitinase-like protein YKL-40 modulates cystic fibrosis lung disease. *PLoS One* 2011;6(9), e24399.
- [96] Horsley AR, Davies JC, Gray RD, Macleod KA, Donovan J, Aziz ZA, et al. Changes in physiological, functional and structural markers of cystic fibrosis lung disease with treatment of a pulmonary exacerbation. *Thorax* 2013;68(6):532–9.
- [97] Griese M, Kappler M, Eismann C, Ballmann M, Junge S, Rietschel E, et al. Inhalation treatment with glutathione in patients with cystic fibrosis. A randomized clinical trial. *Am J Respir Crit Care Med* 2013;188(1):83–9.
- [98] Conrad C, Lymp J, Thompson V, Dunn C, Davies Z, Chatfield B, et al. Long-term treatment with oral N-acetylcysteine: affects lung function but not sputum inflammation in cystic fibrosis subjects. A phase II randomized placebo-controlled trial. *J Cyst Fibros* 2015;14(2):219–27.
- [99] Chmiel JF, Konstan MW, Accurso FJ, Lymp J, Mayer-Hamblett N, VanDevanter DR, et al. Use of ibuprofen to assess inflammatory biomarkers in induced sputum: Implications for clinical trials in cystic fibrosis. *J Cyst Fibros* 2015;14(6):720–6.
- [100] Proesmans M, Els C, Vermeulen F, De Boeck K. Change in IgG and evolution of lung function in children with cystic fibrosis. *J Cyst Fibros* 2011;10(2):128–31.
- [101] Cantin AM, Bilodeau G, Larivee P, Richter MV. Plasma biomarkers and cystic fibrosis lung disease. *Clin Invest Med* 2012;35(4):E173–81.
- [102] Reid PA, McAllister DA, Boyd AC, Innes JA, Porteous D, Greening AP, et al. Measurement of serum calprotectin in stable patients predicts exacerbation and lung function decline in cystic fibrosis. *Am J Respir Crit Care Med* 2015;191(2):233–6.
- [103] Quon BS, Dai DL, Hollander Z, Ng RT, Tebbutt SJ, Man SF, et al. Discovery of novel plasma protein biomarkers to predict imminent cystic fibrosis pulmonary exacerbations using multiple reaction monitoring mass spectrometry. *Thorax* 2016;71(3):216–22.
- [104] Shoki AH, Mayer-Hamblett N, Wilcox PG, Sin DD, Quon BS. Systematic review of blood biomarkers in cystic fibrosis pulmonary exacerbations. *Chest* 2013;144(5):1659–70.
- [105] Downey DG, Brockbank S, Martin SL, Ennis M, Elborn JS. The effect of treatment of cystic fibrosis pulmonary exacerbations on airways and systemic inflammation. *Pediatr Pulmonol* 2007;42(8):729–35.
- [106] Sagel SD, Thompson V, Chmiel JF, Montgomery GS, Nasr SZ, Perkett E, et al. Effect of treatment of cystic fibrosis pulmonary exacerbations on systemic inflammation. *Ann Am Thorac Soc* 2015;12(5):708–17.
- [107] Ratjen F, Saiman L, Mayer-Hamblett N, Lands LC, Kloster M, Thompson V, et al. Effect of azithromycin on systemic markers of inflammation in cystic fibrosis patients uninfected with *Pseudomonas aeruginosa*. *Chest* 2012.
- [108] Nick JA, Sanders LA, Ickes B, Briones NJ, Caceres SM, Malcolm KC, et al. Blood mRNA biomarkers for detection of treatment response in acute pulmonary exacerbations of cystic fibrosis. *Thorax* 2013;68(10):929–37.
- [109] Wright JM, Merlo CA, Reynolds JB, Zeitlin PL, Garcia JG, Guggino WB, et al. Respiratory epithelial gene expression in patients with mild and severe cystic fibrosis lung disease. *Am J Respir Cell Mol Biol* 2006;35(3):327–36.
- [110] Joseloff E, Sha W, Bell SC, Wetmore DR, Lawton KA, Milburn MV, et al. Serum metabolomics indicate altered cellular energy metabolism in children with cystic fibrosis. *Pediatr Pulmonol* 2014;49(5):463–72.
- [111] Guerrero IC, Astarita G, Jais JP, Sands D, Nowakowska A, Colas J, et al. A novel lipidomic strategy reveals plasma phospholipid signatures associated with respiratory disease severity in cystic fibrosis patients. *PLoS One* 2009;4(11), e7735.
- [112] Yang J, Eiserich JP, Cross CE, Morrissey BM, Hammock BD. Metabolomic profiling of regulatory lipid mediators in sputum from adult cystic fibrosis patients. *Free Radic Biol Med* 2012;53(1):160–71.
- [113] Karp CL, Flick LM, Park KW, Softic S, Greer TM, Keledjian R, et al. Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. *Nat Immunol* 2004;5(4):388–92.
- [114] Sloane AJ, Lindner RA, Prasad SS, Sebastian LT, Pedersen SK, Robinson M, et al. Proteomic analysis of sputum from adults and children with cystic fibrosis and from control subjects. *Am J Respir Crit Care Med* 2005;172(11):1416–26.
- [115] Thomson E, Brennan S, Senthilmohan R, Gangell CL, Chapman AL, Sly PD, et al. Identifying peroxidases and their oxidants in the early pathology of cystic fibrosis. *Free Radic Biol Med* 2010;49(9):1354–60.
- [116] Roxo-Rosa M, da Costa G, Luider TM, Scholte BJ, Coelho AV, Amaral MD, et al. Proteomic analysis of nasal cells from cystic fibrosis patients and non-cystic fibrosis control individuals: search for novel biomarkers of cystic fibrosis lung disease. *Proteomics* 2006;6(7):2314–25.
- [117] Ziady AG, Sokolow A, Shank S, Corey D, Myers R, Plafker S, et al. Interaction with CREB binding protein modulates the activities of Nrf2 and NF-kappaB in cystic fibrosis airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2012;302(11):L1221–31.
- [118] Chen J, Kinter M, Shank S, Cotton C, Kelley TJ, Ziady AG. Dysfunction of Nrf-2 in CF epithelia leads to excess intracellular H2O2 and inflammatory cytokine production. *PLoS One* 2008;3(10), e3367.
- [119] Nichols DP, Ziady AG, Shank SL, Eastman JF, Davis PB. The triterpenoid CDDO limits inflammation in preclinical models of cystic fibrosis lung disease. *Am J Physiol Lung Cell Mol Physiol* 2009;297(5):L828–36.