Cystic Fibrosis Foundation Genetic Therapies Working Group Guidance Document

Proof of Concept and Early Trial Outcomes for Genetic Therapies to treat Cystic Fibrosis

The burgeoning field of nucleic acid therapies for the treatment of cystic fibrosis (CF) faces several challenges that must be addressed to optimally define safety, efficacy, and portability to the CF community. This position paper is issued with the goal of providing guiding principles on considerations of selecting study populations, developing trial designs and selecting clinical outcome measures. The diverse array of current nucleic acid therapy mechanisms presents unique considerations, as differing treatment types vary in their relative duration of transduction, risks and safety concerns. At present, the therapeutic development landscape for genetic therapies in CF can be broadly divided into 'transient' therapies that will require chronic repeated dosing (e.g., CFTR mRNA delivery, viral vector gene delivery modalities that do not target basal cells with transgene insertion or gene editing) and limited-dose durable, gene altering therapies (e.g., gene insertion or gene editing of a persistent cell type, i.e., airway basal and/or stem cells). To address the imminent needs of current drug development and clinical trials, this position paper focuses principally on transient therapeutic modalities that will require chronic repeated dosing.

Importantly, there are a number of unknown challenges in demonstrating bioactivity and, ultimately, efficacy for this new area of therapeutic development. It is the opinion of this working group that the guiding principles for advancing therapeutic development will more than likely be based on a body of evidence demonstrating CFTR expression, CFTR activity, and downstream physiological benefit rather than any singular biomarker. This concept parallels the preclinical data recommendations from this working group. Accordingly, this guidance document has been developed to provide expert input regarding the relative strength and utility of a number of novel and emerging outcome measures that are in current use or are actively being piloted in CF clinical trials. For the purposes of this document, routinely used and well-established efficacy outcome measures such as percentage of predicted forced expiratory volume in one second (ppFEV1) and sweat chloride concentrations will not be addressed here, as the former will necessarily be included as a safety assessment and the latter is not relevant unless systemic vector transmission is expected. With a lens toward early phase and proof-of-concept (POC) studies, the outcome measures weighed in this discussion are focused on earliest indications of bioactivity that reflect the potential for efficacy and safety in CFTR transduction, replacement, or restoration. Challenges with each technique are reviewed and weighed, and the relative strength of recommendation for each endpoint has been considered by the CF Genetic Therapies Working Group.

Under terms of confidentiality, independent experts serving on Cystic Fibrosis Foundation's Therapeutic Development Network (TDN) committees conduct several tiers of evaluation of CF trial protocols submitted by sponsors. The TDN Compound Review Committee reviews the mechanism of action of the study product, the preclinical data supporting efficacy and identifies safety considerations based on preclinical toxicity testing. The TDN Protocol Review Committee, which includes clinical investigators, clinical research coordinators, statisticians, and members of the CF community, evaluates the scientific soundness of the study rationale, design, and statistical plan, as well as any ethical or feasibility concerns for protocol conduct. These reviews are subsequently taken under consideration by the TDN's Clinical Research Executive Committee whose members provide additional protocol feedback and assign a strategic fit score based on the study's importance to the CF community and in consideration of other ongoing and upcoming trials. Finally, priority score ratings and associated comments are provided to the directors of TDN sites to aid site investigators in their decision-making regarding

study selection and prioritization for participation. Safety and ethical considerations remain the foremost priorities to guide study design and conduct, particularly in this nascent field where knowledge of potential risks and benefits for transient and permanent genetic therapies are evolving.

Population and Trial Design Considerations

As stated**,** for the purposes of this position paper, trial design issues related to early phase genetic therapy studies will be considered. These trials typically include both a first in CF trial aimed at establishing acute safety and tolerability through careful dose escalation and a proof-of-concept study aimed at establishing bioactivity and the potential for clinical effect. These trials may variably be categorized as Phase 1 through Phase 2A, and in some cases a single staged protocol may be developed to incorporate multiple early phase aims.

Patient Population

Inclusion of Non-CF Populations for Early Phase Development
It is important to note that regulatory guidance based on the type of genetic therapy and supporting safety and bioactivity data will dominate decisions regarding the target patient population and inclusion eligibility. In particular, genetic therapies may or may not require initial testing for safety in healthy (non-CF) volunteers prior to testing in CF individuals, depending on the risk and of the potential impact of the study product on the participant's genome; therapies with permanent genetic modification or that have greater risk to the participant will likely forgo non-CF volunteer studies.

The ability to study acute safety and tolerability of genetic therapies in healthy individuals (i.e. those without CF) and/or CF carriers differs from the approach used for the development of symptom-based therapies in CF. However, the ability to detect evidence of transduction in healthy or CF carrier volunteers will depend upon the development and precision of the outcome measures utilized and the nature of the genetic technology. Notably, there is prior experience with viral vector delivery to healthy volunteers in genetic therapy development. Importantly, the personal risks of participation without benefit for healthy volunteers or CF carriers in early clinical studies designed to assess safety must be carefully considered in trial design and informed consent.

CFTR mutations and eligibility for/ use of CFTR modulators
Genetic therapy trial designs in CF are impacted by CFTR modulators (e.g., ivacaftor, lumacaftor-ivacaftor, tezacaftor-ivacaftor, elexacaftor-tezacaftor-ivacaftor) given the widespread use of these therapies, their effect on baseline CFTR function prior to genetic therapy exposure, and their potential to modulate the activity of the genetic study product itself. At present, approximately 90% of the US CF population is eligible for CFTR modulator therapy based on genotype, and uptake of use is increasing in other countries. Whether CF patients receiving CFTR modulators can or should be considered in clinical trials of CFTR genetic therapies will ultimately be based on guidance from regulatory bodies, driven principally by risk:benefit ratio derived from both the potential product efficacy and safety profiles and subsequently the risk tolerance of the individual patient. The rationale to consider conducting early safety and tolerability studies in modulator-treated individuals is similar to that described for studies in non-CF individuals, in the setting of minimal long-term risks. Enrolling these patient populations in early studies that characterize acute safety, tolerability and potentially proof of transduction may preserve those who are not eligible for CFTR modulators for studies that offer more potential for direct benefit and enhance pivotal efficacy studies. Further, this strategy may mitigate concerns

about developing deleterious antibody responses to vectors that could impact the potential to benefit from vector redosing regimens in subsequent studies involving the target population. The role of people chronically taking CFTR modulators in future genetic therapy trials will depend on the specific type of genetic therapy proposed (permanent vs transient), supportive safety data, and regulatory guidance. Finally, it should also be noted that CFTR modulators may augment CFTR activity of experimental CFTR constructs, given the propensity of certain CFTR modulators (and in particular CFTR potentiators) to activate wild type CFTR. This can potentially be beneficial in proof of concept (POC) studies where concurrent CFTR modulator use would be expected to augment function of the genetic construct.

Eligibility Considerations in the CF Population

Lung function

The severity of underlying lung disease is an important consideration for eligibility in early phase studies in people with CF (pwCF). Reevaluating the lower and upper boundaries of FEV1 inclusion criteria for these studies is useful to optimize the number of eligible participants while maintaining a focus on protecting vulnerable patients with low lung function. Furthermore, the well-established FEV1 range of ppFEV1>40% or <90% used often in clinical trials, may not be as applicable in early phase studies of genetic therapies. In particular, the need to ensure safety for a therapy that may induce bronchospasm or other unanticipated effects may necessitate a higher ppFEV1 lower boundary of inclusion (e.g. ppFEV1>50%). However, reducing this lower boundary after characterization of acute safety and tolerability (particularly the risk of bronchoreactivity or hypersensitivity reactions) may be warranted, given that several studies have reported that patients with FEV1 <40% can demonstrate large improvements in ppFEV1 following treatment with highly effective modulators. Additionally, the population level improvement in lung function of US CF population may warrant using a higher ppFEV1 upper boundary in inclusion criteria, particularly if alternative measures of functional improvement are used to complement spirometry, which may be subject to ceiling effects. The degree to which incremental gains in FEV1 may be observed in patients taking HEMT remains under study, however increases in FEV1 have been documented with baseline values of 95-100% in recent CFTR modulator studies. One consideration in examining cohorts with mild lung disease is that incorporation of emerging outcome measures such as lung clearance index (LCI) via multiple breath washout (MBW) may serve as a more sensitive physiologic biomarker of transduction and downstream efficacy. LCI/MBW could complement FEV1 since substantial and clinically relevant improvements have been documented for HEMT even in those with normal FEV1 at baseline. In contrast to patients with mild disease, patients with advanced lung disease may have variable or poor drug deposition with aerosolized therapies thereby limiting exposure and the potential for efficacy.

Age

Adults (of age 18 years and older) are expected be the initial study population for early phase trials due to anticipated safety concerns for first in human or first in disease testing. Sponsors will require guidance on safety, toxicity, and the potential for clinical benefit prior to initiating studies in younger individuals (<18 years of age). Given the increased survival of the CF population and unique risks of these therapies, special consideration may be warranted for elderly individuals with CF who enroll in these trials.

Participation in prior genetic based therapy trials

Exclusion criteria that involve prior participation in genetic therapy trials should be based both on safety risks posed by prior dosing and ability (or potential lack thereof) to detect a molecular response to transduction given prior interventions. Ultimately, given the relatively small number of individuals with CF who are in critical need of CFTR-based therapies (including those who are ineligible, non-responsive, or intolerant to CFTR modulators), there would ideally be minimal limitations for patients with eligible mutations to participate in multiple different clinical trials. This extends to individuals in long-term safety follow up periods. However, experience will dictate these parameters as data emerge. The impact of prior study participation on the development of anti-vector antibodies, anti-CFTR antibodies, modifications of the native genome, or intolerance to repeated administration of a particular vector represent factors that could impact participation in a subsequent study, including safety or detection of bioactivity. Using screening tests, such as vector-specific antibody screening in inclusion:exclusion criteria may help to maximize the enrollment pool by allowing inclusion of those with prior genetic therapy study experience but without evidence of immunity to the vector. To reduce risks and optimize benefits, genetic therapy strategies may also consider the relative risk:benefit of immunosuppression at the time of dosing to mitigate the development of neutralizing antibodies (or other immune responses) to vectors,. Thorough patient education and informed consent will be of paramount importance due to undefined risks.

Trial Design and Outcomes

The use of modular designs and/or a common 'master protocol' could allow use of a single common control group across studies and may optimize early phase study efficiency. However, these trial designs require both regulatory approval of a standardized protocol to be shared across sponsors and reasonable concurrency in the timing of trial conduct. Another concern will be implementation of data sharing agreements within common study protocols that also preserve proprietary information. Efficiencies gained, especially given the number of appropriate patients available for enrollment, may outweigh the increased contractual or regulatory burden associated with such an effort. Clinical trial network coordination and inter-institutional agreements regarding common agreed upon protocol elements could also facilitate such conduct and provide a more expeditious development path.

An alternative to a master protocol approach is the use by one or more sponsors of a synthetic or external control arm to spare the number of patients necessary for a study design requiring a control while maximizing the small available patient population available to the active therapy arm. The external control arm can be derived using real world data such as from the CFF Patient Registry (or other national registries), or from archived clinical trial data acquired through the TDN or CTN clinical trial networks. Sponsors should be encouraged to contribute non-proprietary data to a central repository for continued access to an accumulating external control resource.

First in CF studies

While healthy volunteer studies may be permissible for some therapeutic modalities, others may necessitate first in CF trials aimed at demonstrating acute safety and dose ranging to inform subsequent studies of a proposed therapy. An important guiding principle for these studies includes limiting the number of subjects exposed to experimental therapy, particularly for those strategies in which early exposure may impact later efficacy. Trials of genetic therapies may require a shift in paradigm depending on the nature of the technology and the number of patients appropriate for enrollment, given the limited population with the appropriate genotype available as compared to supportive CF therapies that are more broadly applicable. For example, early studies using a dose-ascending with 3 subjects per group in an open label

design (commonly used in early phase cancer trials) rather than larger cohort studies (historically used for other CF therapies) may help to more efficiently define dose-limiting toxicities and minimize enrollment requirements. This approach may also be particularly relevant for programs that seek to limit the number of participants exposed to vector doses that are unlikely to provide clinical benefit and may limit future dosing efficacy. Staggered approaches (i.e., sentinel dosing) may warrant consideration. Defining dose limiting toxicities will be provided through regulatory discussions and may be specific to different strategies/agents. The maximum proposed dose will be initially determined by nonclinical toxicology data and/or feasibility. The established clinical outcome of ppFEV1 will likely serve as a safety outcome measure in these early phase studies and is not expected to be an efficacy outcome within such small studies of this type.

Proof of concept studies
Proof of concept (POC) studies would ideally demonstrate molecular and/or physiologic evidence of biological activity following the genetic intervention. Early phase POC studies should examine sensitive biomarkers and translational endpoints discussed in this document (Table 1). At present, the primary drivers for advancing transient therapies (e.g.: mRNA:LNPs) from phase 1 to phase 2 are likely to be based on acute safety and tolerability since longer term risks may be lower (e.g.: generation of neutralizing antibodies). In contrast, POC evidence for a permanent genetic therapy will likely require more robust evidence of target engagement and measurable improvements in physiological outcome measurements compared with transient therapies. It is recommended that phase 1 study results provide a clear rationale for POC studies that also limit enrollment requirements. Since POC studies may not be part of the critical path to regulatory approval, re-enrollment of prior phase 1 study participants may be appropriate. These include strategies where prior exposure does not predict limitations to future dosing, such as some transient therapeutic strategies. Furthermore, this may help avoid dis-incentivization of participation in initial trials. The recommended cohort sizes for POC studies will be dependent on effect sizes of expected POC outcomes and the inherent variance of the selected measures. The CF community continues to conduct research on the variance of certain outcome measures under development (e.g., lower airway potential difference (LAPD)) to inform adequate design of clinical trials.

For phase 1 and POC endpoints to inform future trials, the duration of follow up will be driven by pharmacodynamic effect and regulatory requirements. The impact of ongoing study participation on eligibility to enroll in future studies should be clearly outlined and discussed during the consent process.

Early Phase Study Outcome Measures: Clinical and Translational Endpoints

Tables 1 and 2 provides a summary of study outcome measures relevant to early phase trials of genetic therapies. Their relative importance depends on the nature of the genetic therapy approach, the phase in drug development and the target study population. In addition, considerations that may be of particular interest in genetic therapy development are included below.

The role of nasal dosing in the development of pulmonary genetic therapies
In prior CF genetic therapy trials, dosing of the nasal epithelium and bronchial epithelium have been conducted to varying degrees of emphasis as the initial point of evaluation. Testing of genetic therapies in the nasal epithelium as a precursor to dosing of the lower airways is attractive for several reasons, including the ease of sampling, flexibility in assays to detect transgene expression and function, and the potential to identify unanticipated adverse effects without exposure to the entire respiratory tree (e.g.: off target effects for integrating vectors or

gene editing, etc.). Dosing of the nasal epithelium in POC studies may also simplify study conduct as performance of these studies may be portable to numerous study sites compared with studies of transduction that incorporate lower airway testing and sampling (and thus require bronchoscopy and additional expertise). Existing data indicate that human nasal epithelial cells (HNEs) and human bronchial epithelial cells (HBEs) derived from the same individual and cultured in vitro have similar growth characteristics, CFTR function and response to CFTR modulators, although it should be noted that differences in cell type distribution or other factors could alter tropism of a vector as compared to intact tissues. Side-by-side in vitro transduction studies via gene or mRNA vectors in polarized HNEs and HBEs have not been compared, These findings provide some support to consider dosing the nasal epithelium prior to pulmonary dosing to confirm epithelial transduction of a given vector. However, it is not clear whether these observations from in vitro studies centered on CFTR modulation translate to genetic therapies and to in vivo dosing/transduction. Furthermore, the relative distribution of cell types in the nasal vs bronchial epithelium is unknown and similar dosing of the genetic study agent to both epithelia may be difficult to accomplish (and include additional regulatory requirements). Due to these uncertainties, there is not a firm recommendation regarding the utility of using the nasal epithelium as an initial testing ground for genetic therapies in POC studies.

CFTR replacement/editing and risks of CFTR immunogenicity

The majority of CF subjects who are ineligible for CFTR modulators have *CFTR* with null mutations that interrupt CFTR biosynthesis. Thus, there is a theoretical risk that anti-CFTR antibodies and/or T-cell responses could result from the expression of CFTR secondary to successful transduction, particularly if the transduction strategy incorporates unique CFTR epitopes. Thus, sponsors may need to develop assays to monitor CFTR antibody responses as well as the potential of these antibodies to inhibit CFTR channel function, as well as T-cell responses (e.g.: cytotoxic T cell responses), as has been required for some recent genetic therapy efforts in CF. For gene editing that uses viral vectors (1) as well as foreign protein such as Cas9 (2, 3), the immunogenicity of these reagents is well documented. Thus, sponsors should have validated assays to assess both humoral and T-cell responses to these editing components.

Assessments of Bioactivity

Selection of efficacy measures to assess bioactivity of a proposed genetic therapy must also consider any special procedures or measures and take into consideration both the availability of sites capable of performing an assessment and experience of study site investigators respective to these measures. These considerations are addressed in the “Caveats” column of the summary table below including the use of standard CF clinical endpoints.

Table 1: Comparison of outcome measures specific to CFTR-based therapies

Tool Assessment:

Outcome	Assay	Notes	Caveats	Recommendation Level (+ to +++)
Expression (by bronchoscopy) Overall limitation: sampling error, regionality issues, do assessments align with delivery Brush vs. EBBx	mRNA RT-PCR	• More useful for DNA therapies rather than mRNA replacement, unless intracellular mRNA can be assured • Likely derived from brushing via bronchoscopy, but nasal sampling also possible and more established • Can be batched and centralized • Primer specificity could be used to discriminate native from non-native CFTR if introduced sequence unique (e.g.: codon optimized)	• mRNA expression does not necessarily reflect intact protein expression • Reproducibility within subject not yet established and could be contribute to regional variability	+++
	IHC/IF or ISH	• Likely requires EBBx (or cryobiopsy), possibly brush • Best opportunity to demonstrate cell type • Can be batched and centralized • Cryobiopsy could improve yields, particularly of surface epithelium • ISH technologies can reveal cell type with increased sensitivity if mRNA appropriate marker	• Lower end of sensitivity unknown, likely inferior to PCR. • May be problematic background for some missense mutations • Antibody selection crucial and evolving	++
	Western blot	• In vitro gold standard for processed protein	• Poor low-end sensitivity	++

		• Probably requires EBBx (or cryobiopsy), not just brush, although yields may remain limiting • Could incorporate epithelial marker as control for biopsy depth, as in rectal western blot • Can be batched and centralized	• May be problematic background for some missense mutations due to antibody specificity • Semi- quantitative • May not distinguish surface localized CFTR vs. sub-surface CFTR • Antibody selection crucial	
	KEY QUESTIONS or GAPS	• Determine most appropriate sampling method, including number of samples and regions, of lower airways to demonstrate CFTR expression and cell type of expression (e.g., endobronchial biopsy, cryobiopsy, brushing) for various cell-based outcome measures (RT-PCR, ICH, ISH, IF, WB, MS, etc.). • Development of SOPs for various assays • Opportunity to examine banked airway specimens from established biorepositories (e.g.: Severe Asthma Research Program, Lung Transplant Consortium, others) for validation		
CFTR function (by bronchoscopy, potentially nasal potential difference – NPD - for nasal POC studies)	Lower Airway Potential Difference (LAPD)	• PD is highly related to CFTR function in target tissue • Need to establish expected ranges, currently in progress • Centralized scoring possible via established method for NPD • Preclinical evaluation possible • New, standardized equipment under development	• Non-standardized equipment at present for LAPD; standardization is currently under investigation • Requires ongoing training and QA, and limited to select centers • Subject to accuracy issues if not conducted rigorously; temporal and/or regional variation must be considered • Risks associated with bronchoscopy and sedation	++
	KEY QUESTIONS or GAPS	• Firmly establish normative and CF data for LAPD and correlations to NPD values • Evaluation of standardized and integrated PD equipment capable of image guided measures in both nose and lower airways		

		• Train sites in LAPD; pilot novel LAPD and other lower airway assessment tools (e.g.: simplified LAPD probe, topical sedation protocols, μOCT combined with cryobiopsy)		
Physiology	MCC	• Tool will be available at 6 MCC centers[1] in U.S.; 3 European Centers also have participated in joint efforts[2] • Demonstrated link between restored CFTR function and MCC for HEM-treated subjects in GOAL and PROMISE studies[3,4]; sensitivity adequate to detect sustained effect of HTS on MCC[5,6] • Expected to detect relevant physiologic response at early timepoints, and before FEV1 changes achieved • May be well suited for inhaled therapies since assay is most sensitive to changes in large airways where therapeutic deposition will be greatest • Established performance to inform study size for POC (ranging from N <10 sufficient to see change from baseline similar to HEMT, to ~15-20/group for parallel group design with HTS sized effect)	• Sensitivity limit established by negative studies with LUM/IVA and TEZ/IVA[7] • Very small site numbers	++
	Structural imaging	• HRCT well established and amenable for multicenter studies, although need to assure standardized protocols	• Utility of HRCT for short term changes in POC less well established	++

		and uniform scoring system applied • Low dose HRCT protocols available and approach dose associated with chest radiography[8] • Existing scoring systems have been validated • HRCT detects HEM treatment effects – mainly via reduced mucus plugging and airway wall thickening (rather than bronchiectasis); Sensitivity to small effects or shorter time frames uncertain • 1H-UTE MRI results correlated with HRCT, avoids radiation exposure, can be combined with functional imaging[9]	• Emerging scoring systems may be more quantitative, as in CF-PRAGMA • CT has radiation exposure but is manageable with low dose protocols. Concerns may be most prevalent in pediatric population • 1H-UTE MRI less established and less sensitive to structural changes than HRCT, but rapidly improving	
	Functional imaging	• Functional assessments of ventilation (129Xe, 19F, 3He, oxygen enhanced, etc.) can be conducted in concert with structural 1H-UTE MRI, and are perhaps most sensitive endpoint to detect changes in disease status • Ventilation imaging is most advanced with 129Xe-MRI; multisite standardization underway at 13 sites (129Xe MRI Clinical Trials Consortium) across US, Canada and UK • 129Xe submitted for FDA approval	• Functional MRI is rapidly evolving[11,12]; additional multisite standardization required before used as a primary endpoint in a POC study • Published data largely limited to single center studies • Clinical correlates and treatment effects not well established	++

		• Demonstrated link between restored CFTR function and improved 3He-MRI ventilation[10] imaging with HEM (ivacaftor) within 4 weeks of treatment		
	Sputum Solids and rheology	• % solids measurement with spontaneous or induced sputum collection are straightforward; rheology measurements require significant expertise • Macrorheology validated for HEM, but may be insensitive to less efficacious treatments (e.g.: HTS, ENaC inhibitor, surfactant) • Sputum rheology more complicated, though rapid/simple macro-rheology assays are becoming available[13]	• Sputum mucus endpoints may lack sensitivity due to variability of expectorated sputum[14], especially if correction is partial or patchy • Rheology is amenable to centralized analysis, but susceptible to effects of processing and freeze thaw if not performed rigorously • Non-production of sputum may be more likely after an IMP with effective restoration of CFTR activity, but can be mitigated with sputum induction.	+
	RECOMMENDATION	• Continue standardization of HRCT methodology and scoring • Continue standardization of multi-center 129Xe functional imaging • Continue standardization of multisite MCC capabilities • Explore conduct of multi-center micro/macro-rheology studies and standardization		
Safety:	Anti-CFTR and/or anti-Cas detection by antibody or cell-based immunity assay	• Antibody detection likely required by regulators, although unclear if concern is beyond theoretical given that CFTR is a membrane protein • Both vector and Cas9 antibodies can be measured in serum and BAL/induced sputum	• Assays could be centralized to an academic lab or CRO • Current standards not established	+++

		• T cell assays to both vector and Cas9 are published focused on IL-2 and IFNg Elispots which can be run in PBMCs in clinical trials and blood and lung in pre-clinical studies.		
	RECOMMENDATION	• Establish SOPs for detection of anti-CFTR antibodies, T cell responses to CFTR antigen • Include anti-capsid antibody testing into viral-based genetic trials		

Table 2: Outcome measures under development for consideration:

Outcome	Assay	Notes	Caveats	Recommendation Level (+ to +++)
Expression (by bronchoscopy) Overall limitation: sampling error, regionality issues, do assessments align with delivery Brush vs. EBBx	Other protein detection technologies (proteomics)	• In development thus lower end sensitivity not established yet in humans (e.g.: MS, others)		+
	mRNA scSeq	• Has potential to demonstrate cell type(s) transduced, which is presently a major biological question • Relative value in clinical trials and in human tissues unknown	• Requires specialized centers and advanced informatics • Live cells processing protocols needed • Largely theoretical (at this time) for human use	+
CFTR function (by bronchoscopy, potentially nasal potential difference – NPD - for nasal POC studies)	Micro Optical Coherence Tomography (μOCT)	• measures physiologic products of CFTR function (PCL, ASL, MCC, percent of active cilia at epithelial level) • Proof of concept data with CFTR modulators for human nasal imaging and in vitro/animal study applications in data review	• Available at single center at present for nasal use • Development needed for lung probe • Need POC and normative data in lower airways, already established in nares	+

		• Lung probe conceived but not implemented • Primarily a research tool at present		
	OTHER	• Fluorescence-based CFTR function of isolated airway cells ex vivo		
Physiology	Quantitative Microbiology	• May require sputum induction or BAL sampling • Validated for HEMT • Could include traditional and molecular (e.g. 16sRNA or qPCR) techniques • Can/should be centralized	• May require homogenous CFTR correction or sufficient durability; may be more appropriate for later phase studies • May be too far downstream to CFTR restoration to serve as viable endpoint • Extent of CFTR restoration necessary to impart benefit unknown, but likely greater than that of lumacaftor/ivacaftor or tezacaftor/ivacaftor • Biologic variance may limit use to larger studies	+

Table 3: Outcome measures commonly included in the development of CF pulmonary therapies:

Outcome	Assay	Notes	Caveats	Recommendation Level (+ to +++)
Clinical	FEV1	• Gold standard surrogate endpoint for majority of approved CF pulmonary therapies • Large effects can be measured with reasonable sample sizes within subject	• Consider use of absolute FEV1 volumes (vs ppFEV1) for studies in adults to reduce measurement errors in ppFEV1 calculations	

	Other traditional pulmonary function tests	• Measurement of lung volumes (e.g.: FVC, RV, IC) and small airway flows (FEF25%-75%) established in all CF care center PFT labs	• These additional PFT measures are typically secondary outcome measures relative to FEV1 • Plethysmography and DLCO rarely included in CF clinical trials	
	MBW/LCI	• Improved sensitivity for those with retained lung function • Well-validated measure; used in POC studies for several CF pulmonary therapies (CFTR modulators, HTS, rhDNAse, inhaled antibiotics)	• Limited utility in low lung function (i.e., advanced lung disease) due to practical features • MCID not established	
	PROs	• CFQ-R well validated for several pulmonary therapies • MCID generally established for respiratory scale • Daily symptom diaries (CRISS) established although not validated for approval	• CFQ-R has 2-week recall • Ceiling effects observed with HEM therapies	
	Pulmonary Exacerbation (PEx)	• Accepted measure for how a patient feels, functions, or survives (not a surrogate endpoint) • Well-validated measure with clinical, quality of life, and cost of healthcare impacts studied	• Classic signs and symptoms characterizing a PEx have been routinely captured in clinical trials but these may differ for patients now taking HEMT • Requires large sample sizes and lengthy duration studies to detect clinically meaningful effects • PEx frequency has been diminished in CF population taking HEMT in current form	

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