Study Protocol

Title: iPS Cell Response to CFTR Modulators: Study of Trikafta in CF Patients Carrying Partial Function Mutations or N1303K CFTR

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Full study title: iPS cell response to CFTR modulators: Study of Trikafta™ in CF patients carrying partial function mutations or N1303K CFTR

Short study title: Partial function and N1303K mutation iPS response

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2. Precis/Abstract:

Cystic Fibrosis (CF) is a life threatening genetic disorder resulting from mutations found in the gene known as the cystic fibrosis transmembrane conductance regulator (CFTR). Defects in this gene prevent correct chloride and bicarbonate transport in and out of cells. It has become increasingly important to develop new *in vitro* model systems capable of predicting *in vivo* clinical effectiveness of modulator therapy among patients with CF. This objective represents a significant and unmet need for advancing personalized therapeutics in the disease. The current trial is intended to show for the first time that primary iPS cells differentiated to an airway epithelial phenotype can be used to predict *in vivo* clinical response for rare CF patient populations – with the long-term goal of facilitating drug access for individuals with unusual (or even private) CF variants. Trikafta™ is currently approved for patients with CF carrying at least one copy of the common F508del variant and over 170 other CFTR abnormalities. In this protocol, we describe a clinical study of 42 subjects without the F508del mutation, carrying partial function or N1303K mutations not approved for Trikafta™, and who are not expected to be approved for CFTR modulator treatment in the immediate future. Each patient will have clinical and/or preclinical evidence that Trikafta™ should offer benefit, and each will be given Trikafta™ for approximately four weeks. We will monitor clinical endpoints that include FEV_1 and sweat chloride. We will differentiate iPS cells from each subject to generate airway epithelial monolayers that can be tested for response to Trikafta™. In this way, this study will evaluate an emerging and readily accessible *in vitro* surrogate endpoint as a predictor of clinical response. Our trial will also serve as a pilot/test case for other clinical protocols relevant to patients with rare CFTR variants and evidence of modulator responsiveness who do not have an approved modulator therapy—due to rarity of their mutation. We hypothesize that a robust correlation will be established between *in vitro* Trikafta™ responsiveness of iPS cells and *in vivo* benefit (FEV_1,) in patients, and providing a powerful tool for utilizing iPS cells to identify rare CF patient populations most suitable for cystic fibrosis modulator therapy.

3. Introduction and Background: A summary of the primary hypothesis, purpose, scholarly rationale, and prior literature.

CFTR variants have classically been assigned to one of six mechanistic categories, depending on the underlying protein defect (Rowe et al., 2005; Elborn et al., 2017; De Boeck and Amaral, 2016). Mutations such as G542X or W1282X are representative of class I abnormalities, in which a premature stop codon leads to destabilization of messenger RNA (by virtue of nonsense-mediated decay) in addition to markedly diminished production of foreshortened and nonfunctional polypeptide (Rowe et al., 2005). The common F508del CFTR variant (deletion of a phenylalanine occurring at CFTR position 508) is found

in at least one CFTR allele among ~90% of CF patients in the United States. This mutation is categorized as class II, indicating failure of proper biogenesis, maturational arrest in the endoplasmic reticulum, trafficking away from the plasma membrane, and premature degradation by the proteasome. N1303K is another distinct class II variant. Class III mutations disrupt ability of the CFTR ion channel to open properly (i.e., 'gate'), impairing transmembrane flow of chloride and/or bicarbonate. The best described variant in this category is G551D, in which glycine at CFTR position 551 is replaced by a charged residue (aspartic acid), disrupting a normal interface between two CFTR nucleotide binding domains necessary for channel function. Other CFTR molecular defects confer distortion of the anion selective pore (class IV), interrupt normal protein biosynthesis (class V), or destabilize CFTR at the plasma membrane (class VI).

For many years, the CF research field pursued a conceptual approach to treatment in which mutation subclass was intended to match tailored, small molecule interventions. Among certain class III defects, for example, CFTR modulators that activate channel gating (termed 'potentiators') have now become standard of care (Accurso et al., 2010). For the common class II (maturational processing) variant, F508del, 'corrector' molecules that overcome improper biogenesis (lumacaftor, tezacaftor, elexacaftor) have advanced through FDA registration (Wainwright et al., 2015; Taylor-Cousar et al., 2017; Keating et al., 2018; Middleton et al., 2019). Nonsense mutations (class I) have been viewed as requiring a completely distinct family of therapeutic agents, capable of enhancing translational read through to generate full-length CFTR and stabilize mRNA by virtue of a successful (sentinel) round of translation (Howard et al., 1996).

Current status of cystic fibrosis personalized medicine includes the emerging concept of theratype (an empiric evaluation of modulator responsiveness). The high cost of CF (and other orphan) pharmaceuticals and the influence of cost on commercial incentive, third party reimbursement, FDA registration, and patient access are acutely felt in CF patient care, although rarely described in scientific or medical literature.

Advances in cystic fibrosis drug discovery

A 'personalized' approach has underpinned remarkable progress in CF therapy. The potentiator ivacaftor, for example, is viewed as a safe and effective compound suitable for enhancement of CFTR gating and forestalling pulmonary decline. The drug is now FDA approved for numerous distinct CFTR variants, or ~12-13% of the cystic fibrosis population. Lumacaftor, in combination with ivacaftor (under the trade name Orkambi™), is registered for therapy of F508del homozygous individuals, or ~46% of those with the disease[8]. These two drugs, therefore, account for over 50% of CF patients being 'on label' for an FDA-approved modulator. Tezacaftor, a rescue molecule similar to lumacaftor but with optimized pharmacodynamic and safety profile, was approved in combination with ivacaftor for F508del homozygous individuals, as well as those who carry an F508del allele and one of several partial function variants at the second CFTR locus. 'Next generation' correctors under development by pharmaceutical, academic, or other groups act by mechanisms distinct from lumacaftor or tezacaftor and are designed to provide additional benefit when given in combination. Lung function improvement among patients with a single copy of F508del, for example, has been established following combined drug treatment with next-generation agents, irrespective of the CFTR mutation at the second locus (Herper, 2017). The magnitude of response for patients encoding one copy of F508del is comparable to that obtained with ivacaftor in G551D-associated CF. Elexacaftor is approved together with tezacaftor and ivacaftor for patients with at least one copy of F508del, as well as > 170 rare CFTR polymorphisms (Middleton et al., 2019; Keating et al., 2019; CFF News, 2020).

Patients who may be "left behind"

Because ~90% of CF patients in the United States carry at least one copy of F508del, pharmacotherapies (Trikafta™ in particular) are now available to a sizable majority of those with the disease. However, thousands of patients harboring relatively common variants will remain without effective drug therapy (e.g., those with premature truncation alleles such as G542X, R553X, W1282X, etc., or refractory CFTR folding defects such as N1303K). Others with ultra-rare or private CFTR mutations have forms of the disease that are very likely to benefit from available drugs, but no access to these therapies due to inherent challenges associated with obtaining data for registration and third-party reimbursement (see below). It has been estimated that over 1,000 CFTR mutations are represented by less than 5 patients each (Thomas and Boyle, 2017). Establishing processes so that individuals with very rare and/or poorly characterized alleles can gain access to effective modulator treatment remains one of the predominant challenges in the field.

Complexity of CF pathogenesis

Cystic fibrosis personalized treatment—like other precision-type therapies—was originally envisioned as a strategy for directing tailored small molecules to specific protein defects. G551D CFTR, for example, represents an essentially pure gating problem that ivacaftor rescues robustly. However, it has become increasingly clear that most CFTR mutations exhibit multiple abnormalities (instability, limited membrane targeting, defective ion transport, etc.). A case in point, the common F508del variant has been shown to exhibit not only inadequate biogenesis (class II defect), but also defective gating (class III) and diminished cell surface stability (class VI) (Veit et al., 2016). Another example, the rare P67L CFTR variant (reported in ~238 individuals worldwide), has been described as a class IV abnormality (physical disruption of the ion conductive pore). From a 'personalized' medicine perspective, therefore, P67L might not be expected to benefit from correctors such as lumacaftor or tezacaftor. Findings from several groups, however, indicate P67L responds dramatically to both molecules, and the variant should be viewed as exhibiting characteristics of both class II (processing) and class III (gating) pathophysiology (Sabusap et al., 2016; Ren et al., 2013). Both ivacaftor and corrector molecules have been FDA approved for treatment of the P67L variant.

Nuances that underlie the molecular disease process have become increasingly difficult to simplify using only a few mechanistic subcategories. Even mutations at the same position within CFTR show substantial difference in cellular phenotype. S549R and S549N, for example, both exhibit partial function and are FDA approved for gating activation with ivacaftor. In model systems, however, S549N leads to more abundant CFTR at the cell surface than S549R, and may be more responsive to ivacaftor in human subjects (Vertex Pharmaceuticals, Kalydeco full prescribing information). Preclinical and clinical data suggest a corrector molecule might differentially benefit patients carrying S549R versus S549N (Rab et al., 2017).

Complexity of molecular mechanism is also demonstrated by variants such as W1282X (class I), which retains significant ion channel function even in truncated form, and is modestly responsive to stabilization by CFTR correctors (Wang et al., 2016; Haggie et al., 2017). Other premature stop codons such as E831X might be predicted to have no response whatsoever to currently available modulators (based on both nonsense mediated decay (attributable to the premature stop codon) and a severely foreshortened polypeptide). However, in part because the mutation occurs near a splice junction, a fraction of E831X CFTR mRNA produces a full-length protein lacking only amino acid 831 (i.e., generating E831del) (Haggie et al., 2017; Hinzpeter et al., 2010). The E831del variant maintains significant residual activity, improved clinical phenotype, and is FDA-approved for ivacaftor. Numerous CFTR class IV (splice) defects (e.g., 2789+5 G→A, 3272-26 A→G, 3849+10KbC→T) also retain residual activity and have recently become registered for modulator therapy. Overall, it has become increasingly clear that

modulators shown to impact one CF mutation are commonly found to benefit numerous other disease categories.

Challenges in concept and practice

Based on considerations such as these, two essential questions have recently emerged regarding cystic fibrosis therapeutics: First, how precisely can (or should) molecular mechanism dictate personalized treatment? In other words, if a modulator exhibits a surprising benefit against a mutation for which it was not designed, how should this be approached to optimize patient access? Second, as summarized below, how will CF patients with ultra-rare or even private variants gain access to highly effective but costly modulator treatments, when 'off label' prescribing is largely proscribed? Use of *in vitro* systems stably expressing unusual CFTR variants (e.g., the FRT model) can be used to provide data in support of FDA registration (Durmowicz et al., 2018), and feasibility of testing rare CFTR mutations in this fashion has recently been utilized for certain molecular defects which can be adequately represented by studies of CFTR cDNA.

Among the thousands of cystic fibrosis patients carrying low prevalence variants, it is expected that many would benefit from a compound such as elexacaftor, ivacaftor or tezacaftor. As with any drug, physicians caring for a CF patient (irrespective of genotype) may recommend these modulator therapies, in the same way a panoply of non-registered agents are safely and routinely given in most diseases without medical restriction. A difficulty arises in the case of CF modulator treatment, however, due to very high cost (upwards of $250,000 - $300,000 per year, which represents an economic burden for third-party payers). As a consequence, and in contrast to less expensive pharmaceuticals in CF and the vast majority of other diseases, insurance reimbursement for modulators has been limited to patients with an FDA-approved ('on label') indication. 'On label' variants currently comprise ~180 CFTR mutations from among approximately 2,000 associated with clinical disease. Therefore, although many patients who are 'off label' would likely benefit, the cost of drug vitiates patient access. On the other hand, an attempt to broaden label to include a new CF genotype can present a formidable challenge. Numbers of patients with a particular mutation are often so small that phase 3, double blind placebo-controlled trials to promote registration are not possible. The issue is more than academic—failure to expand the label or otherwise augment drug access means thousands of CF patients must forego a potentially life-saving intervention.

Facilitating modulator availability

A series of innovative strategies have been devised by pharmaceutical and/or academic groups to address the issue of inadequate CF drug access. In part because a dramatic clinical benefit from modulator treatment can often be obtained within a matter of weeks, N-of-1 trials have been evaluated for a possible role during FDA registration (McGarry et al., 2017; Nick et al., 2014). Such studies are designed so that individual patients serve as their own controls, and a robust drug response can provide data relevant to label expansion. Variability among CF patients with the same genotype, inconsistent clinical findings from a single study subject, preexisting (irreversible) lung injury, disease trajectory (including presence of respiratory exacerbation), and practical barriers to drug approval on a case-by-case basis have limited usefulness of this approach. As an alternative strategy, patients with similar molecular phenotype (e.g., residual CFTR function as judged by *in vitro* testing), and/or evidence of mild clinical disease (pancreatic sufficiency or intermediate sweat chloride values) have been evaluated as a group (independent of underlying gene defect or mechanistic subcategory) (Herper, 2017). In a cohort of patients with rare variants that can be meaningfully binned or designated based on strong scientific rationale, a robust clinical response to CFTR modulators can help promote label expansion to an entire

group of patients with the disease – despite differences in CF genotype for the cohort. While admittedly less 'precise,' the situation can be viewed as similar to conventional pharmacotherapies—where registration is based on compelling clinical evidence of benefit to the overall group or enriched study population (for further background, see Schuck et al., 2018).

Emerging approaches

In a recent, landmark series of approvals from FDA, ivacaftor and tezacaftor labels were extended to include rare variants based on clinical and/or *in vitro* assay data (Durmowicz et al., 2018). Trikafta™ approvals for new genotypes have been accomplished in the same fashion (CFF News, 2020). The notion that compelling *in vitro* data could constitute criteria for label expansion represents a paradigm shift in CF personalized treatment. In the same fashion that a highly predictive surrogate such as lowering blood cholesterol can support approval of drugs that prevent heart disease or stroke, *in vitro* systems capable of predicting CF clinical improvement (e.g., increased FEV1, fewer pulmonary exacerbations) represent a compelling means to rationally expand drug access among patients with ultra-rare forms of the disease. The nature and extent of *in vitro* data necessary to support registration is being evaluated for a number of model systems, including primary airway epithelium, organoid-type preparations, iPS cells differentiated to airway epithelial phenotype, and recombinant cell types, among others. The polarizing FRT cell line encoding heterologously expressed mutant CFTR cDNAs has been utilized by FDA as suitable for this purpose (Durmowicz et al., 2018; see also above). An example showing surrogate testing systems for the rare N1303K variant is shown in **Figure 1**. Although not clinically approved for Trikafta™ or other modulator treatments, N1303K expressed in FRT cells exhibits activity in a range compatible with clinical benefit for the elexacaftor/tezacaftor/ivacaftor (Trikafta™) combination therapy. Studies of FRT cells encoding N1303K, as well as iPSC derived from a patient with an N1303K/W1282X genotype (in which airway epithelial monolayers differentiated from these cells would be viewed as essentially "null" for the W1282X allele) and human nasal primary airway epithelial monolayers all showed a moderate short circuit current (ISC) response to pharmacomodulation. The findings support the possibility of clinical benefit using Trikafta™ in patients encoding N1303K. In this context, it should be noted that not all research groups have reported this level of N1303K activity following modulator therapy. For example, at a recent meeting of the CFF RARE Consortium, several laboratories (Lexington, Cincinnati, Birmingham) viewed N1303K as a variant meriting clinical study with Trikafta™. On the other hand, Vertex did not include N1303K in their recent label expansion (presumably because of inadequate modulator responsiveness). Findings such as these indicate the importance of a formal clinical trial to determine whether patients with an N1303K genotype (the 5th most common cystic fibrosis mutation worldwide) might benefit from drugs such as Trikafta™.

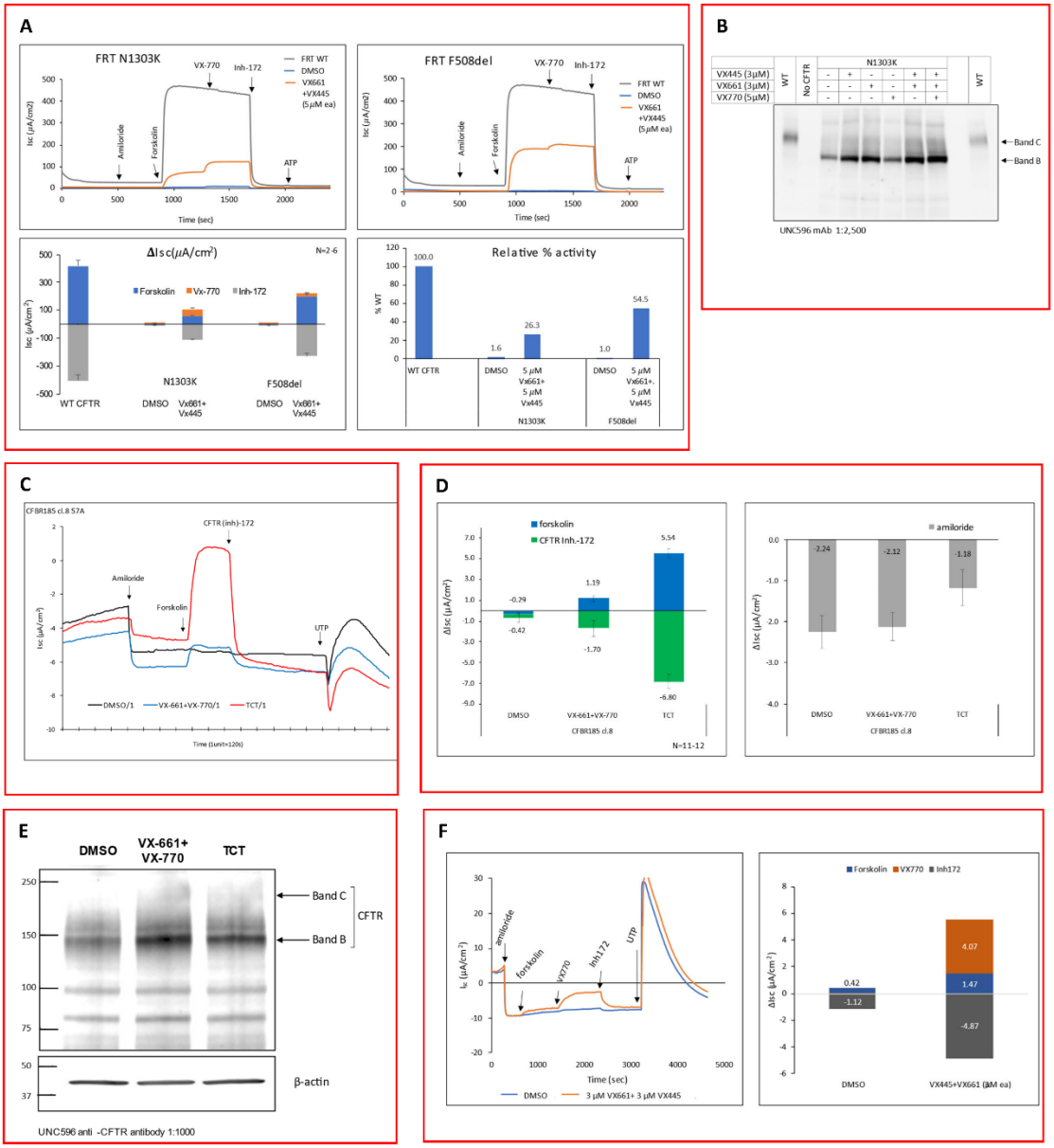
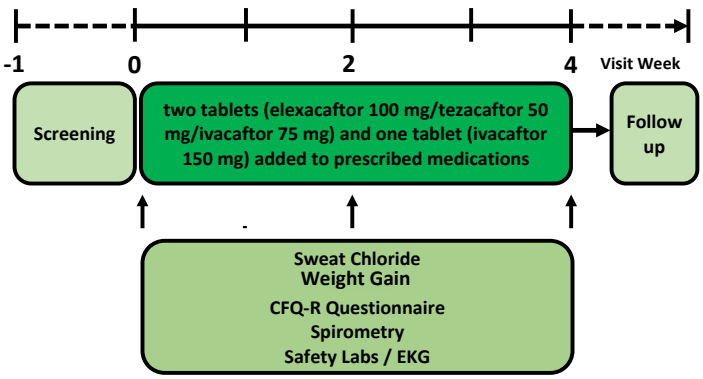


Figure 1. Activity of N1303K CFTR in FRT, iPSC, and primary nasal cells. **Panel A**: depicts short circuit current response to elexacaftor/tezacaftor/ivacaftor (ETI) in FRT cells stably expressing the N1303K variant. Note that activation of ~ 10% wildtype level has been viewed as predictive of clinical benefit in the FRT cell system advanced by Vertex Pharmaceuticals. N1303K activity shown here is > 25% wildtype levels. **Panel B**: Western blot indicating enhancement of N1303K bands B and C by ETI. **Panel C**: Transepithelial chloride transport on skin fibroblast derived iPS cells with airway epithelial differentiation. **Panel D**: summary data using the same protocol. Triple combination therapy (TCT) using ETI indicates 5-7 μA/cm^2 short circuit current, which is similar to the level of activity obtained using ivacaftor in G551D airway epithelial monolayers derived from iPSCs. **Panel E**: Western blot from an iPSC/N1303K experiment of this sort. **Panel F**: indicates ~ 5 μA/cm^2 in primary nasal airway epithelial monolayers following ETI therapy. This level is in a range that might be predictive of clinical benefit in some settings.

As above, poor access comprises a commonplace barrier to optimal care throughout CF clinics in the United States and abroad. The same issue is a major impediment to personalized treatments in diseases such as cancer and HIV infection (Menon et al., 2015; Evans et al., 2015; Nardini, 2014; Jabbour et al., 2015; Lammers et al., 2014). As a result, FDA has sought *in vitro* model systems that could serve as surrogates for clinical benefit among patients with very rare (or even private) alleles and for whom traditional, late-phase clinical testing would be difficult. Preliminary evidence suggests differentiated iPS cells may be an excellent candidate in this regard (Crane et al., 2015; Mou et al., 2015).

As part of an evaluation to test the relationship between CFTR activity and modulator effectiveness, we worked with 43 rare CFTR variants stably expressed from a single site in the genome of the human CF bronchial epithelia cell line (CFBE41o-) (Han et al., 2018). The magnitude of drug response in these cell models was highly correlated with residual CFTR function for the potentiator ivacaftor, the corrector lumacaftor, and ivacaftor-lumacaftor combination therapy. Response of a second set of 16 variants expressed stably in FRT cells showed similar correlation (although certain variants were identified that demonstrate significantly higher response to modulator treatment than predicted by *in vitro* analysis). In addition, nearly all variants studied in CFBE (40/43) and FRT cells (13/16) demonstrated greater response to ivacaftor-lumacaftor combination therapy than either drug alone. Together, the variants studied here represent 87% of individuals in the CFTR2 database with at least one missense defect. Our results therefore indicate that most patients with CF carrying missense variants are likely to: 1) respond modestly to current potentiator and/or corrector therapy, while a small fraction will exhibit more pronounced CFTR rescue and 2) derive the greatest benefit from combination treatment.

Figure 2. Summary of trial design.



Overview FDA has indicated new *in vitro* model systems capable of predicting *in vivo* clinical benefit may represent a significant and unmet need for advancing personalized CF therapeutics. The present study is designed to specifically and prospectively test iPS cells taken from patients encoding rare CF variants as a means to determine *in vitro* thresholds for CFTR rescue. Using iPS cells differentiated to exhibit a respiratory epithelial phenotype, we will determine whether the cells can be used to predict FEV_1 response among individuals carrying rare, partial function mutations who receive Trikafta™. Substudy 1 will comprise an open-label, two center trial of orally administered elexacaftor, tezacaftor and ivacaftor (Trikafta™) that will enroll 22 patients (11 at Emory) with rare/orphan genotypes (Figure 2 summarizes trial endpoints); a detailed schedule of events is provided under "Subject Involvement and Characteristics". We will also enroll subjects who encode the N1303K variant as emblematic of a mutation not approved for Trikafta™, but likely to respond (Substudy 2). As described above, N1303K CFTR is relatively common, not on the Trikafta™ label, and has been shown by our laboratory and others to exhibit activation following Trikafta™ in a number of cell-based systems. We plan to enroll 20 subjects (10 at Emory) that carry N1303K and test these individuals for lung function, iPSC/airway epithelial CFTR current, and other clinically important endpoints when the drug is administered *in vivo*.

4. Objectives: Primary and secondary aims and outcome measures.

Primary Objective: We propose a clinical study of subjects carrying either partial function mutations, or encoding N1303K CFTR. Each patient will be given Trikafta™ for approximately four weeks. We will monitor clinical endpoints that include FEV_1, sweat chloride, quality of life questionnaire, and weight. We will also evaluate cutaneous punch biopsy material and/or blood samples from each subject so that iPS cells can be differentiated into airway epithelial monolayers and tested for response to Trikafta™.

Substudy 1 (Partial Function)
Primary Endpoints: $FEV_{1,}$ response of iPS cells to Trikafta™ treatment

Secondary Endpoints: Sweat chloride, CFQ-R (quality of life questionnaire), weight gain, safety laboratory evaluation/EKG

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine whether iPS cells differentiated to primary airway epithelia -- and treated with ivacaftor/tezacaftor/elexacaftor *in vitro* -- can predict clinical responsiveness to Trikafta™ *in vivo*.	• Functional and biological correction of CFTR expressed in iPS cells as judged by short circuit current measurements in monolayers, and western blot analysis • Forced expiratory volume in one second (FEV_1)	• These *in vitro* tests represent standard functional and biochemical analysis that address CFTR rescue, with direct relevance to the *in vivo* situation. • FEV_1 is a conventional endpoint and provides a direct measurement of patient health benefit among individuals with cystic fibrosis
Secondary		
To verify safety and establish efficacy of Trikafta™ in enrolled patients.	• Sweat chloride testQuality of life survey • Standard laboratory tests and other measurements, as well as history and physical examination	• Provide supportive information regarding study intervention effect using *in vivo* measures known to indicate CFTR rescue • A well-established tool for monitoring patient benefit in cystic fibrosis • Confirm safety of the FDA-approved drug

<u>Substudy 2 (N1303K)</u>
Identical to Substudy 1, except that sweat chloride will serve as primary endpoint with other endpoints (shown in the table above) designated as secondary.

5. Study design and methods:

Human Subjects Involvement

Procedures	Screening/ Enrollment Day -28 to -1	Baseline Visit 1, Day 1	Study Visit 2 (phone call) Day 7 +/-2 days	Study Visit 3 Day 14 +/- 2 days	Study Visit 4 Day 28 +1/-2 days	Final Study Visit Day 56 +/-2 days
Informed consent	X					
Medical history	X	X	X	X	X	X
Physical exam	X	X		X	X	X
Cutaneous punch biopsy and/or blood sample[A]		X				X[E]
CFTR genotype	X					
Spirometry	X	X		X	X	X
Oxygen saturation	X	X		X	X	X
Sweat chloride testing	X	X		X	X	X
Safety laboratory assessment[B]	X	X		X	X	X
Pregnancy Test[C]	X	X				X
EKG	X			X		
Ophthalmologic exam[D]	X					
Drug accountability		X		X	X	
Questionnaire (CFQ-R)	X	X		X	X	X
Administer study drug		X			X	
Concomitant medication	X	X	X	X	X	X
Adverse event assessment		X	X	X	X	X

[A] Substudy 1: cutaneous punch biopsy. Substudy 2: blood sample with optional cutaneous punch biopsy.
[B] Renal and liver panels, CBC/Diff urinalysis
[C] Females of reproductive age
[D] Slit lamp ophthalmologic exam if not done within 3 months before screening for those ages 12-17 at the time of screening
[E] If necessary to produce additional iPS cells

Minor protocol changes for Substudy 2 are described in text.

The study is an open-label clinical trial of orally-administered Trikafta™ in subjects with CF and carrying certain mutations that are without FDA approved modulator treatment. Subjects will be given the study drug elexacaftor 100 mg/tezacaftor 50 mg/ivacaftor 75 mg (2 pills once daily in the morning) and ivacaftor (150 mg) once daily in the evening, as the FDA-registered agent, Trikafta™. Dose and schedule will be identical to what has already been FDA-approved for the F508del CF genotype. Enrollment is planned at two centers, Emory University and University of Alabama at Birmingham. In Substudy 1, patients with residual CFTR function (based on sweat chloride < 80 meq/L or pancreatic sufficiency) will be tested. In Substudy 2, patients with CF encoding the N1303K variant will be enrolled as a "case in point" – emblematic of variants with *in vitro* evidence of Trikafta™ responsiveness who are not approved (i.e., not "on label" for the drug). iPSC-derived airway epithelial monolayers from these patients will be examined for their ability to predict FEV_1 and sweat chloride response and other clinical endpoints. Our study will not significantly increase the risks (or decrease the acceptability of risks) associated with the use of Trikafta™ in CF.

Human subject participation will consist of a screening day (-28), day 1 (+/-2), day 7 (+/- 2) [phone call visit], day 14(+/-2), day 28 (+1/- 2) and day 56 (+/-2) follow up (wash-out). The visits will include patient history with concomitant medication review, physical exam, safety laboratory assessments (renal and liver panels, CBC/Diff, urinalysis), serum and urine pregnancy testing, CFTR genotype, spirometry, O_2 saturation, sweat chloride test, ophthalmology exam in children, cutaneous punch biopsy and/or blood sample for iPSC derivation, EKG, drug accountability and questionnaire (CFQ-R assessment), and adverse event assessment.

We anticipate the screening and enrollment visit will require 4-6 hours to complete the informed consent, medical history, O_2 saturation, concomitant medication review, physical exam, safety laboratory assessments (CBC/Diff, RENAL/LFT chemistry panel, and urinalysis), EKG, CFTR genotyping, questionnaire, serum pregnancy test for females, sweat chloride test, and spirometry.

On day 1 (when subject returns), the visit will require approximately 6 hours to complete the concomitant medication review, adverse event assessment, interval medical history, physical exam, O_2 saturation, safety laboratory assessments (CBC/Diff, RENAL/LFT chemistry panel, and urinalysis), questionnaire, sweat chloride, urine pregnancy test for females, spirometry, cutaneous punch biopsy (3mm) and/or blood sample, drug dispensing, and drug administration. The cutaneous punch biopsy may occur at any time between the Screening Visit and Day 1 based on dermatology clinic schedule availability.

- 1 Cutaneous 3 mm punch biopsy (performed here (and on day 56 if necessary to produce additional iPS cells)): In this procedure, a circular blade is rotated from epidermis to subcutaneous fat, yielding a ~ 3 mm cylindrical core of tissue. Extending the skin perpendicular to the incision line allows for facile closure, which can typically be accomplished with a single suture. Pain is well-managed by local subcutaneous anesthetic. A small fibrotic scar can be minimized by linear closure of the biopsy. Significant pain, bleeding, or infection are very rare.

The subject will complete a phone call visit on day 7 (+/-2) that will require approximately 30 minutes to complete concomitant medication review, interval medical history, and adverse event assessment.

On day 14 (+/-2) the subject will return for a visit that will require approximately 5-6 hours to complete concomitant medication review, interval medical history, adverse event assessment, physical exam, drug dispensing and drug return, questionnaire, safety laboratory assessments (CBC/Diff, RENAL/LFT chemistry panel, and urinalysis), sweat chloride test, O_2 saturation and spirometry. EKG will be performed on day 14.

On day 28 (+1/-2) we will conduct interval medical history, adverse event assessment, concomitant medications, physical exam, sweat chloride test, spirometry, O_2 saturation, safety laboratory assessments (CBC/Diff, RENAL/LFT chemistry panel, and urinalysis), questionnaire, and drug return. This visit should require 4-6 hours to complete. This visit should occur no later than Day 29 since a 28-day supply of Trikafta™ will be provided.

The 'wash-out' visit will be conducted on day 56 (+/-2) which will require approximately 4-6 hours to complete concomitant medication review, interval medical history, adverse event assessment, physical exam, safety laboratory assessments (CBC/Diff, RENAL/LFT chemistry panel, and urinalysis),

questionnaire, sweat chloride, urine pregnancy test for females, cutaneous punch biopsy (3mm) and/or blood sample, O_2 saturation, and spirometry. The cutaneous punch biopsy may occur within ± 14 days of the day 56 visit based on dermatology clinic schedule availability.

Spirometry may be performed either pre- or post-bronchodilator at Screening. At all other visits, spirometry should be performed pre-bronchodilator. However, if patient is unable to withhold bronchodilator use at the Day 1 visit, then the spirometry assessment will be considered 'post-bronchodilator' and attempts should be made to collect spirometry post-bronchodilator at remaining visits.

In order for a spirometry assessment to be considered pre-bronchodilator, subjects should adhere to the following:
• Subjects should refrain from using short-acting bronchodilators (e.g., albuterol) or anticholinergic (e.g., ipratropium bromide [Atrovent®]) for more than 4 hours before the spirometry assessment.
• Subjects should refrain from using long-acting bronchodilator (e.g., salmeterol) for more than 12 hours before the spirometry assessment; and
• Subjects should refrain from using once-daily, long-acting bronchodilator (e.g., tiotropium bromide [Spiriva®]) for more than 24 hours before the spirometry assessment.

Sources of Patient-Derived Material

- Review of patient medical records and demographic information
- History and physical exam
- Blood and urine for safety studies (<6 tablespoons (depending on weight) of blood per visit)
- Sweat chloride results
- Questionnaire data
- Spirometry (FEV_1, FVC, and FEF25-75%) calculated per Hankinson equation
- Cutaneous punch biopsy (3 mm) and/or blood sample /iPS cell derivation
- O_2 saturation
- EKG
- Ophthalmologic exam
- CFTR genotype
- Pregnancy test
- Drug accountability

6. Participant selection:

Inclusion Criteria:

- Provision of signed and dated informed consent form, and when appropriate, assent form
- Stated willingness to comply with all study procedures and availability for the duration of the study
- Male or Female age ≥12
- A clinical diagnosis of CF or CFTR-related disease* and either: 1) evidence for a partial function mutation not currently covered or likely to be covered for FDA treatment with a CFTR modulator (Substudy 1), or 2) N1303K CFTR and a minimal function mutation (Substudy 2).
- Sweat Chloride < 80 mmol/L and/or pancreatic sufficiency (no exogenous pancreatic enzyme supplement therapy) or carrying the N1303K CFTR variant

- Able to perform spirometry meeting ATS criteria for acceptability and repeatability
- Clinically stable in the past 4 weeks with no evidence of CF exacerbation (prior to Screening AND Day 1)
- Willingness to use at least one form of acceptable birth control including abstinence or condom with spermicide. This will include birth control for at least one month prior to screening and agreement to use such a method during study participation for an additional four weeks after the last administration of study drug.
- Ability to take Trikafta™.
- Agreement to adhere to all current medical therapies as designated by the CF care center physician.

* A diagnosis of CF as defined by current guidelines: one or more clinical features consistent with the CF phenotype or positive CF newborn screen AND one or more of the following criteria: sweat chloride ≥ 60 mmol/L by quantitative pilocarpine iontophoresis or 2 well characterized disease-causing CFTR gene mutations

OR

With the consensus of two study co-investigator physicians, a clinical diagnosis of CF or CFTR-related disorder with documented evidence of chronic pulmonary disease (chronic cough with sputum production, persistent pulmonary radiographic abnormalities (e.g bronchiectasis), chronic abnormality in ppFEV1, and/or persistent colonization with a typical CF pathogen)

Exclusion Criteria

- Documented history of drug or alcohol abuse within the last year
- Subjects should not have a pulmonary exacerbation or changes in therapy for pulmonary disease in the 4 weeks prior to screening
- Listed for lung or liver transplant at the time of screening
- Cirrhosis or elevated liver transaminases > 3X ULN
- Pregnant or breastfeeding
- Inhibitors or inducers of CYP3A4, including certain herbal medications and grapefruit/grapefruit juice, or other medicines known to negatively influence Trikafta™ administration
- History of solid organ transplant
- Active therapy for non-tuberculosis mycobacterial infection or any plan to initiate non-tuberculosis mycobacterial therapies during the study period
- Known allergy to Trikafta™
- Treatment in the last 6 months with an approved CFTR modulator
- Any other condition that in the opinion of the lead investigators might confound results of the study or pose an additional risk from administering study drug
- Treatment with another investigational drug or other intervention within one month prior to enrollment, throughout the duration of study participation, and for an additional four weeks following final drug administration.
- Evidence of cataract/lens opacity determined to be clinically significant by an ophthalmologist at or within 3 months prior to the Screening Visit

Subject recruitment plan

Under Substudy 1, we plan to recruit 22 subjects (11 subjects per site at Emory and the University of Alabama at Birmingham) with cystic fibrosis carrying a partial function mutation genotype not covered or expected to be covered by an FDA approved treatment with a CFTR modulator. Under Substudy 2, we plan to recruit 20 subjects encoding N1303K CFTR to Emory and UAB (10 subjects per site). In both substudies we intend to include ~50% female and ~50% male subjects age $\geq$ 12. Subjects will first be recruited from the cities participating in the trial (Atlanta and Birmingham), and CF physicians have previously expressed interest in discussing the proposed clinical trial with eligible individuals. Study investigators also have immediate access to a robust national patient registry through the Cystic Fibrosis Foundation therapeutic development network, and (following IRB approval) will contact cystic fibrosis physicians caring for individuals with the appropriate genotypes to ask whether these patients might be interested and/or willing to discuss possible study participation (including consent) with one of the lead investigators on the proposed trial (Sorscher, Linnemann, Solomon, Stecenko).

All individuals who consent to participation will be evaluated at one of two clinical CF care centers (Emory, University of Alabama in Birmingham). Travel, meals, accommodations, etc. for study visits will be covered by funds available through the project, and an incentive payment of $150 per visit will be provided as a gift card or check. For minors who participate in the study, travel expenses, etc. will also be provided for one parent and the gift card or check for each visit provided to that parent (i.e., not the minor). For adult subjects accompanied by a caregiver, travel expenses, etc. will also be provided for the caregiver.

7. Informed Consent Process:

Informed consent will be obtained by well-trained study coordinators or investigators using an Institutional Review Board approved document. Parents/guardians/participants will be asked to sign the informed consent documents only after the study has been fully described to the subject and all questions have been answered. Participants will be offered at least 24 hours to review the consent/assent before the research is to begin, although consent may be obtained on the day of screening if preferred by study subjects (eg those traveling from significant distance).

Assent for minors will be obtained by experienced study personnel with many years expertise in this area. Minors will be informed about the study using age appropriate, and easily understood language. Care will be taken to assure minors: 1) Are very comfortable with the study, 2) Are under no pressure to enroll or participate, and 3) Understand they can withdraw at any time without penalty or change in their medical care. The investigator obtaining consent/assent will provide a written summary of the interaction for inclusion with the patient's research file.

The participant's parent/guardian will be asked to read and review the document. The investigator will explain the research study to the participant and parent/guardian and answer any questions that may arise. A verbal explanation will be provided in terms suited to the parent/guardian and participant's comprehension of the purposes, procedures, and potential risks of the study and of a subject's rights as a participant. Participants and parent/guardian will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants and parent/guardian will have the opportunity to discuss the study with their family and/or think about the consent document prior to agreeing to participate. Participants and parent/guardian will be informed that participation is voluntary

and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent/assent document will be given to the parent/guardian/participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. Parents of subjects under the age 18 will sign the consent form with separate assent signed by children participating in the study, as applicable.

CF is a life long illness, and patients/families are typically very sophisticated regarding the nature of CF care -- including clinical trial participation. The disease is not associated with cognitive or other intellectual limitations. No vulnerable adult subjects are intended for enrolment in the trial. If, in the opinion of the study investigator, a prospective participant lacks sufficient understanding and is therefore not able to provide informed consent, that subject will not be enrolled. The ability to obtain consent represents criteria for inclusion in the trial.

8. Compensation for time and effort:

Travel, meals, accommodations, etc. for study visits will be covered by institutional or other funds and an incentive payment of $150 per in-person visit and $30 at the Day 7 telephone call will be provided as a gift card or check. All payments will be pro-rated. For minors who participate in the study, travel expenses, etc. will also be provided for one parent and the gift card or check for each visit provided to that parent (i.e., not the minor). For adult subjects accompanied by a caregiver, travel expenses, etc. will also be provided for the caregiver. An incentive payment of $150 will also be provided if an unscheduled visit is needed (for multiple repeat assessments or cutaneous punch biopsy occurring on another day). If the unscheduled visit is needed just for a repeat laboratory sample collection, an incentive payment of $30 will be provided.

9. Study Discontinuation

Criteria for discontinuing the study intervention (e.g., halting rules), may include any monitoring test(s) and associated clinical decision point(s) which in the opinion of the clinical investigator, qualified designee, or Data Safety Monitoring Board are viewed as placing the patient at significant risk if Trikafta™ administration is to continue. This could include, for example (but is not limited to) worsening liver function or abnormalities associated with Trikafta™ administration. Discontinuation from Trikafta™ does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee, and/or Data Monitoring Committee (DMC) (also referred to as Data Safety Monitoring Board, DSMB) will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include:

- All measurements described for the day 56 follow-up/washout visit

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- If any clinical adverse event, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant is unable to receive Trikafta™ for 3 days.
- Pregnancy
- Significant study intervention non-compliance

The reason for participant discontinuation or withdrawal from the study will be recorded. An attempt will be made to replace subjects who sign the informed consent form and receive Trikafta™, and subsequently withdraw, or are withdrawn or discontinued from the study.

A participant will be considered lost to follow-up if he or she fails to return for any scheduled visit and is unable to be contacted by the study site staff.

The following actions will be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2-5 days, and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

10. Data Safety Monitoring Plan

Oversight for this trial will be performed by a DSM Chair. A Data Safety Monitoring Board (DSMB) will be selected in consultation with the Chair to specifically oversee the clinical study. The DSMB will consist of at least two physicians experienced in treating patients with cystic fibrosis, a DSMB coordinator and a biostatistician. The DSMB Chair will convene a protocol conference call with committee members so that the DSMB can approve the monitoring plan and review any concerns regarding the current protocol prior to initiation of study enrollment.

The DSMB is primarily responsible for reviewing safety data which will be provided on a continual basis throughout the performance of the study as well as reviewing interim analyses report after four patients have completed the 28-day study drug administration, and again following 50% enrollment (completion of 11 subjects in Substudy 1 and 10 subjects in Substudy 2). The DSMB may recommend early termination or modification of either trial substudy for reasons of study subject safety. Subjects will be monitored for adverse events (AEs) and serious adverse experiences (SAEs) by the Investigators, the study sites' Institutional Review Boards, and the DSMB. The DSMB will also provide recommendations needed to assist the investigative team.

One goal of the present study is to provide clinical data that will support a correlation between IPS CFTR activity and clinical benefit of a CFTR modulator. In the present study, the highest level of stringency will be applied. This will include: 1) DSMB, as described above, 2) detailed interim evaluations, 3) generation of case report forms for all study subjects, 4) detailed statistical analysis, tabular summaries, and leading edge evaluation to test clinical benefit, 5) compiling data in SAS or other well-accepted formatting, 6) discussions with interested parties (e.g. Vertex and CF Foundation) to provide updates regarding clinical trial progress, and 7) timely publication of data, etc. Logistical support and resources of the type intended here are provided by the Institution and the Department of Pediatrics for physician-sponsored clinical trials at Emory. Our intent will be to furnish a clinical profile that provides high stringency and rigor. We recognize that for the present clinical trial data will not be used for submission to FDA or towards label expansion of Trikafta™. However, by providing a compelling data set and offering this information early and openly to Vertex, interested third-party insurers, CFF, as well as CF patients and their families, we will furnish information that will help guide more extensive studies of IPS cell analysis among patients with rare forms of the disease.

Study Monitoring: The Emory University self-monitoring tool will be customized to fit the needs of the current study. This tool will be utilized by the study team for monitoring the progress of the trial, and assuring data accuracy and protocol compliance. Study monitoring will take place at least once a year.

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s). The self-monitoring process and study team will visually inspect study records collected since each previous monitoring visit. The scope of the monitoring shall include the informed consent process, eligibility, CRFs, biological specimen tracking and AE reporting.

Quality control (QC) procedures will be implemented beginning with the data entry system. Data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

A designated member of the study team will provide the completed self-monitoring tool to the study PI and team within 7 days of the monitoring visit. The study PI will document receipt and review of the monitoring report, resolutions and/or corrective actions to findings on the Site Monitoring Log. The monitoring reports will be shared between sites, with the Emory Clinical Trials Audit and Compliance (CTAC) office, and with the DSMB. Protocol deviations/violations will be reported to the Emory IRB per reporting guidelines and will be reviewed by the DSMB in a timely fashion or during regular meetings, as needed.

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, NIH, and any other regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

11. Statistical Methods For a proposed open-label prospective 4-week trial of Trikafta™.

Substudy 1: Patients with CF or CFTR-dependent illness who do not have access to modulator therapy but exhibit a clinical phenotype suggestive of partial/residual CFTR activity.
We will serially evaluate clinical outcomes of interest prior to and after the start of therapy. Change in clinical measurements (spirometry, weight/BMI, quality of life, sweat chloride) will be monitored. Our main goals are to determine the clinical response to Trikafta™ and to determine if *in vitro* responsiveness of iPS cells predicts clinical response. The primary outcome is response to Trikafta™ after 4 weeks of treatment. In this study a clinical response to Trikafta™ at week 4 of treatment will be defined as an improvement, from baseline, in FEV_1 > 5% predicted and each subject will be classified a responder or non-responder. In this study, if at least 40% of subjects meet the definition of responder, the drug will be considered efficacious (i.e., comparable to the benefit which led to Orkambi™ approval by FDA following trials in F508del homozygotes). Overall response rate will be accompanied by an approximate binomial 95% confidence interval. To determine the role of iPS *in-vitro* response to Trikafta™, we will compare the Δ lsc (change in lsc) among Trikafta™ responders and Trikafta™ non-responders using a two-sample t-test or a Wilcoxon rank sum test if distributions are skewed. Additionally, we will correlate the Δ lsc obtained from *in vitro* experiments with the change in FEV_1% predicted (post minus pre) using Spearman's rank-order correlation with an associated 95% confidence interval. We will also test whether the iPS response reaches a CFTR functional threshold of 40% wild-type levels (a magnitude of activity in primary airway epithelial cells viewed as potentially relevant to clinical benefit). In a sensitivity analysis, we will replicate the FEV_1 analysis with sweat chloride responses, and determine the *in vivo* response rate.

For other outcomes of interest including sweat chloride levels, quality of life, and weight/BMI, we will apply a mixed effect model to examine the change in these measures over the study duration. Models will include a subject-specific intercept, to account for variability among subjects prior to the start of the treatment, and a categorical variable for time (baseline, day 14, day 28, day 56). A Dunnet's post-hoc comparisons procedure will be used to determine which time points demonstrated a significant change from baseline. Using the same mixed effects models, we will include Δ lsc as predictors of *in vivo* improvement. We will also test the interaction between time and Δ lsc to determine when *in-vitro* response is most associated with change in clinical response. Adverse events and other safety endpoints will be tabulated and reported using counts and percentages or means and standard deviations, as appropriate.

In addition, because clinical parameters may vary among patients prior to the start of therapy, we will also approach the analysis using a modified N of 1 design configured for the purpose of comparing iPS studies in contrast to the aggregate analysis described above. Using the framework of single subject research, we will evaluate therapeutic response in individual subjects by an interrupted time series (ITS) approach. Briefly, in the absence of an independent control group, ITS analysis is a quasi-experimental design in which each subject acts as their own control and is followed serially in time prior to and after an interruption (in this case, the start of treatment). If the treatment has causal

impact, the post-intervention time series will have a different level or slope than the pre-intervention series. Time series models also have the power to test and correct for possible cyclical patterns and outliers. Using segmented regression analysis, we will conduct ITS models for each of the subjects enrolled in the trial. A significant change in slope from the pre-treatment period will indicate a significant treatment effect. ITS models will be constructed for each outcome of interest where historical and pre-treatment data are available. The change in slope will also be correlated with the *in-vitro* response using Spearman's rank-order correlation coefficient.

All analyses will be conducted using the intention to treat principal, where all subjects will be included in analysis regardless of how long they took the study medication. Statistical significance will be assessed at the 0.05 level and analyses conducted using SAS v. 9.4 (SAS Institute; Cary, NC).

Sample size

The FDA has suggested that scientifically meaningful 'binning' of patients based on clinical characteristics can help guide future approval of specific patient groups for drug treatment. The present study is not intended for FDA submission or label expansion of Trikafta™, but as a means to determine whether IPS cells differentiated to respiratory phenotype can be used to predict clinical response to CFTR modulators. For the purposes of this protocol, the subjects enrolled, although potentially of different genotypes, will represent a scientifically meaningful cohort or 'bin' of individuals with partial function mutations, sweat chloride < 80 mmol/L, and/or pancreatic sufficiency. This protocol is designed to compare *in vitro* responsiveness of differentiated iPS cells to *in vivo* clinical activity of Trikafta™, and will test the null hypothesis that the true Trikafta™ response rate of CFTR individuals with partial function mutation genotypes is ≤ 10% vs. the alternative hypothesis that the true Trikafta™ response rate is ≥ 40%. We will initially enroll four subjects, and expect all of these individuals will show a clinical response (e.g., > 5% improvement of % predicted FEV_1 from baseline). If none of the first four subjects exhibit a response, the study will be terminated. Otherwise, we intend to enroll a total sample size of 22 individuals. Subjects who do not complete the study will be replaced. In this design, if Trikafta™ is ineffective, the probability of early termination after four subjects is 66% and only a 5% chance of concluding the drug is effective (type I error). If Trikafta™ is effective, there is an 86.4% chance of reaching this conclusion. If fewer than four patients show a clinical effect, Trikafta™ will be considered ineffective. To demonstrate the ability of iPS cells to predict clinical benefit, 22 patients will provide at least 80% power to detect a significant correlation of at least r = 0.55 between the changes in clinical outcomes with changes in short circuit current as measured in iPS cells. Tabular details of this analysis are provided below. Power calculations were performed using PASS v. 14 (Kaysville, UT) with a two-sided Z-test and a significance level of 0.05.

	Cumulative # of responses	**Decision**	**95% Confidence Interval (CI)**
Stage 1: Enter 4 subjects	0	Terminate the trial because the agent is ineffective. **Response rate is ≤10%**	LB of 95% CI is < 10%
	At least 1	Inconclusive result, continue trial (proceed to stage 2).	95% CI contains 10% and the UB is < 40%

Stage 2: Enter 18 additional subjects	4 or less (stage I + stage II)	Consider that the agent is ineffective. Response rate is ≤10%	LB of 95% CI is less than 10%
	5 or more (stage I + stage II)	Drug may be effective Response rate is greater than 10% and not different from 40%	95% CI contains 40% and LB is > 10%
	14 or more (stage I + stage II)	Drug is effective Response rate is at least 40%	95% CI LB ≥ 40%
LB = Lower Bound, UB = Upper Bound			

Substudy 2: **Patients with N1303K CFTR.**

The primary analysis will be to estimate the mean absolute change in sweat chloride concentration from baseline through day 28 among the adolescents and adults with at least one copy of an N1303K mutation and a minimal function mutation who are not currently modulator eligible. A one-sided 0.025 alpha level single sample t-test comparing this estimate to no change will be reported, along with corresponding confidence intervals. A complementary analysis evaluating the percentage of participants with ≥15 mmol/L decline will also be reported. With a previously estimated 9.7 mmol/L standard deviation of the change in sweat chloride for successful modulator trials previously (Boyle et al., 2014; Vermeulen et al., 2017), an anticipated 6% of responders would be expected by chance alone. Therefore, the treatment response prevalence will be compared to a reference rate of 6%. For longitudinal sweat chloride through day 28 and for secondary endpoints (absolute change in ppFEV1, CFQ-R respiratory domain, and weight/BMI from baseline through day 28), means at each time point and changes over time will be summarized along with corresponding confidence intervals estimated by mixed effects models to account for within-subject correlation.

Changes in sweat chloride following treatment with ETI will be correlated with iPS cell responses in vitro, in addition to secondary clinical outcomes measures. A non-linear Spearman's correlation coefficient will be estimated for each measure paired with iPS cell responses in vitro, with corresponding fitted curves displayed graphically along with coordinates for participant's paired in vivo and in vitro responses. We expect variation both in sweat chloride and in iPS cell short circuit current measurements (Isc) due to heterogeneity in genotypic profiles among participants in the study. Sample selection for iPS cell experiments will be coordinated across studies to optimize power and unbiasedness as specified in a sample selection plan.

Sample Size

With a sample size of 20 participants, we anticipate 99% power to detect a mean sweat chloride decrease of 10 mmol/L assuming a standard deviation of 9.7 mmol/L (Boyle et al., 2014; Vermeulen et al., 2017) under a one-sided 0.025 alpha-level t-test. With a higher standard deviation of 15 mmol/L, the power decreases to 81% to detect a mean decrease of 10, but we retain 98.9% power to detect the hypothesized mean decrease of 15 mmol/L.

SAEs and AEs will be tabulated using standard coding terms sorted by System Organ Class (SOC). The incidence of AEs will be tabulated by seriousness and severity. The number of SAEs and AEs will be

summarized as follows: (i) The proportion of subjects with at least one (S)AE, (ii) The average number of (S)AEs per subject, and (iii) The rate of (S)AEs per subject week of follow-up.

Expected Outcomes Subjects will be recruited by study investigators as above, including through the CF Foundation Therapeutic Development Network resource. Please note that based on considerable past experience conducting clinical trials involving CF subjects at both participating institutions, we do not have concerns regarding our ability to perform the patient-oriented study described here, or to propagate and characterize iPS cells (Crane et al., 2015; Mou et al., 2015; Hawkins et al., 2017; Jacob et al., 2017). The protocol will furnish one test of our hypothesis that thresholds of CFTR activity in differentiated iPS cells encoding partial function mutations *in vitro* or *in vivo* predict benefit following Trikafta™ *in vivo*. If a strong correlation can be established between Trikafta™ response *in vitro* and *in vivo* (e.g., FEV_1) in both substudies, the clinical results will provide early evidence for possible usefulness of *in vitro* surrogate models in this setting.

Scientific Rigor All *in vitro* and *in vivo* studies will be conducted with appropriate "n" so that therapeutically relevant conclusions can be drawn. Multiple biologic or technical replicates will be performed and evaluated in blinded fashion by an experienced statistician. CFTR mutations are equally prevalent in both sexes, and we expect iPS cells studied in human subjects carrying partial function mutations will reflect male and female individuals equally.

Summary Many CF patients who might substantially benefit from new and precise modulator treatments do not have access to these drugs. Off-label prescribing of expensive new agents is often not reimbursed by third-party payers. This project will test new *in vitro* predictive thresholds using iPS cells for CFTR rescue *in vivo*, and furnish a body of knowledge relevant to such issues among individuals with rare or even private CF alleles. We will also specifically evaluate patients with CF encoding N1303K for evidence of clinical benefit attributable to Trikafta™.

12. Confidentiality:
    - Participant confidentiality and privacy will be strictly held in trust by the participating investigators, their staff, and others responsible for the intended interventions. This confidentiality is extended to cover testing of biological samples and other tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval.
    - All research activities will be conducted in as private a setting as possible.
    - The clinical investigators, other authorized representatives, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.
    - The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or other requirements.
    - Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored securely. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be

identified by a unique study identification number. The study data entry and study management systems used by clinical sites research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived by the PI. With regard to other patient related aspects of confidentiality, all interviews and procedures will be held in a private room.

- Identifiers will be destroyed 3 years from the date of the Federal Financial Report (FFR).

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