II. SIGNIFICANCE. New therapies are needed for the treatment of COPD, which accounts for over $40 billion in annual healthcare costs[10] and recently surpassed stroke as the 3rd leading cause of death in the U.S.[11] Though smoking cessation is essential to slow the progression of disease, no current pharmaceuticals alter the natural history of the disease or improve mucus retention that is characteristic of COPD, which persists even in ex-smokers, and is independently associated with FEV_1 decline and death.[12-14] The need for agents that combat mucus stasis has been highlighted in the *New England Journal of Medicine*,[1] highlighting the global significance of the problem.[15] Published data from our group and multiple other laboratories strongly indicate that exposure to cigarette smoke inhibits anion transport by the cystic fibrosis transmembrane conductance regulator (CFTR), the causative protein in CF,[2,16-19] leading to delayed mucociliary transport (MCT) and mucus stasis.[2] Recent *in vivo* studies in mice[4,20] and humans[2-5,21] further demonstrate the presence of acquired CFTR dysfunction in COPD patients and that the defect can persist in both the lung and periphery despite smoking cessation and is associated with chronic bronchitis severity. The development of efficacious modulators of CFTR anion transport has raised the exciting possibility that pharmacologic enhancement of CFTR activity may confer clinical benefit to this population, even in the absence of congenital CFTR mutations.[2,3,22] We have shown that the CFTR potentiator ivacaftor robustly enhances anion secretion by wild-type CFTR by potentiating CFTR channel gating[2,23] pointing to a novel treatment strategy for airway diseases characterized by mucus stasis.[2,3,22] Our comprehensive *in vitro* studies further establish that CFTR dysfunction caused by smoke exposure can be reversed by ivacaftor, thereby augmenting airway surface liquid (ASL) depth and MCT, two important contributors to the regulation of mucociliary clearance (MCC).[2] These findings indicate that CFTR potentiators offer a new treatment opportunity in COPD[2,3,22,24] that could overcome previous limitations with inhaled or weak mucolytics.[1] *The primary purpose of this project is to test the hypothesis that CFTR activity in COPD patients can be augmented by administration of the CFTR potentiator ivacaftor, conferring meaningful improvements in airway physiology and clinical outcome, by conducting a Phase 2 trial that will inform future efforts.* **If our hypothesis is found to be correct, the results will justify development of an entirely new treatment paradigm for COPD that could benefit over 8 million patients with COPD and chronic bronchitis in the U.S. alone.**[9] Further, this study will provide an initial test as to whether augmenting CFTR confers benefit in those without genetic CF mutations, opening new treatment opportunities for several common disorders in which CFTR may have a role, including those that affect the airway (e.g. asthma[25], ABPA[26], or sinusitis[27]), pancreas (e.g. recurrent pancreatitis[28,29]), and GI tract (e.g. chronic constipation[30]).

III. INNOVATION. Our study is designed to provide the first test as to whether augmentation of CFTR function confers clinical benefit in patients with COPD. If proven, this could establish an entirely new therapeutic approach to improve mucus retention in the disease, an area of intense interest to academic and commercial laboratories.[1,31] The relatively limited benefit of mucolytic therapy in COPD to date has been attributed to lack of efficacy, poor bioavailability, and failure to deliver the drug to the distal airways where mucus obstruction is observed.[32-35] An improved approach that is highly efficacious and targets the small airways, such as oral ivacaftor, represents an innovative strategy to overcome these limitations and thus ameliorate mucus stasis, an important cause of residual airway obstruction that cannot be addressed by bronchodilators alone and is closely linked to mortality and pulmonary function decline.[12,14,36] Further, the concept that CFTR activation may have clinical benefits in individuals without congenital mutations of the CFTR gene is completely new, and has the potential to elucidate an entirely novel treatment approach for diseases of mucus clearance, even beyond COPD. The protocol and extensive preclinical basis for evaluating ivacaftor in COPD was developed using novel and cutting edge assays of ion transport and MCC (some of which is not presented here due to page limitations, see Protocol in Human Subjects Section); the proposed clinical trial will extend these assays to human subjects, allowing us to ask innovative questions regarding the mechanistic basis of CFTR activation in non-CF individuals, and the downstream effects on pulmonary physiology. The proposed PK studies in Aim 3 capitalize on simulation based population models to determine treatment response and optimum dosing. We will also evaluate predictors of treatment response, allowing us to select the appropriate population for subsequent studies and representing a personalized sub-phenotypic approach emblematic of state of the art COPD drug development.[37] The protocol has been approved by the FDA, we hold an investigator initiated IND for the use of ivacaftor in COPD patients, and have already begun pilot testing (single dose, 2 weeks) to establish feasibility and optimize the protocol via an Investigator Initiated Study Program (IIS) supported by Vertex (8 of 12 patients have been enrolled); this same mechanism will provide ivacaftor and identically matched placebo (see letter of support and UAB-Vertex IIS Contract), significantly reducing costs of the study.

IV. APPROACH. Background. A. Disease states associated with CFTR Dysfunction. An improved understanding of the role of CFTR in the maintenance of normal epithelial function has revealed that reduced CFTR activity plays a causative role in a number of diseases in addition to CF. For example, CFTR mutations

that confer mild abnormalities are present in ~30% of individuals with recurrent idiopathic pancreatitis,[28,29] and similar associations have been established in congenital bilateral absence of the vas deferens,[38] allergic bronchopulmonary aspergillosis,[39] chronic sinusitis,[40] and idiopathic bronchiectasis.[41,42] The genetic basis of these diseases illustrates that mild CFTR dysfunction can contribute to substantial pathology.[43] With the recent discovery and clinical validation of potent modulators of CFTR ion channel activity, there is considerable scientific interest from academic and commercial laboratories to examine the effects of CFTR stimulation for diseases in which CFTR plays pathogenic role, including COPD.[2,16-19,25]

B. Pathologic Resemblance of CF and COPD. Like CF, the defining feature of COPD is airflow limitation, although it is recognized that the disease exhibits heterogeneous pathologic features in the lung.[44-48] Of the two classically defined COPD phenotypes, emphysema and chronic bronchitis,[46,49] the latter exhibits pathologic features similar to CF, including mucin hyperexpression, mucus accumulation, and goblet cell hyperplasia.[45,49-51] Like CF, a relatively high incidence of bronchiectasis has also been reported in COPD.[52] These abnormalities lead to impaired airway clearance, chronic bacterial colonization and persistent neutrophilic airway inflammation similar to CF lung disease.[44,47,50,53-57] Though these changes are usually less pronounced in patients with COPD, mucus obstruction is observed in the lower airways and is accompanied by delayed mucus clearance as judged by impaired tracheal mucus velocity and delayed elimination of inhaled radionuclear particles.[58-60] Furthermore, mucus obstruction also occurs in the small airways of COPD patients and is associated with excess morbidity and mortality.[12,14,36] Based on the pronounced CFTR suppression caused by tobacco smoke exposure,[2,16-19] neutrophilic inflammation,[61,62] and hypoxia[63] recently reported by several laboratories,[2-4,16,17,19,22,62-64] there is now a large body of evidence strongly indicating that CFTR dysfunction contributes to COPD pathogenesis, particularly among individuals with chronic bronchitis. Our laboratory has defined a robust association between smoking and CFTR functional decrements in four independent study populations each of which evaluated distinct CFTR readouts (e.g. nasal potential difference (NPD)[2], lower airway potential difference (LAPD)[3], sweat chloride[4,5], and sweat rate[5]) as described below.

C. State of Present COPD Therapeutics. Like lung cancer and coronary artery disease, the need for new COPD therapies is well accepted despite evidence that cigarette smoking is the major contributing and modifiable risk factor.[65,66] Even after smoking cessation, COPD continues to progress and airflow obstruction is present in a substantial subset of ex-smokers, of whom up to half exhibit chronic bronchitis.[67] The shared pathophysiologic features between CF and COPD have led to a number of prior investigations examining the potential role of therapeutics developed for patients with one of these airway diseases for use in the other. Although not all efforts have been successful,[68-71] randomized trials have demonstrated the shared benefits of intravenous corticosteroids during exacerbations,[72,73] chest physiotherapy,[74-76] and chronic administration of azithromycin to reduce acute exacerbations.[77,78] Roflumilast, a PDE inhibitor recently approved for the treatment of COPD specifically in patients with chronic bronchitis and frequent exacerbations,[79] is a well known CFTR activator via cAMP stimulation[80]; based on this, our laboratory has demonstrated that the bioactivity of roflumilast in the COPD sub-phenotype with bronchitis and frequent exacerbations is a consequence of its function as a CFTR activator,[64] strongly suggesting CFTR activation is worthy of further investigation in COPD.

D. Rescue of CFTR Function: A new Paradigm in CF Care. High-throughput drug screening programs in both industrial and academic settings have resulted in the discovery of novel compounds that strongly potentiate chloride transport through both wild type (WT) and mutant CFTR.[81-83] These 'CFTR potentiators' are a class of agents developed to correct the underlying cause of CF, and principally target surface mutations such as Class III (severe gating mutants, e.g., G551D) CFTR defects.[82-84] Ivacaftor (formerly VX-770) robustly activates multiple CFTR forms, including WT, G551D, and other missense mutations in primary airway epithelial monolayers, but only if they are located at the cell surface in sufficient number.[81,85,86] This explains why ivacaftor therapy is not effective in F508del homozygous individuals as a single agent,[87] and provides proof that ivacaftor is specific for residual surface CFTR.[85,86] The underlying mechanism of the compound is through augmentation of the open probability (P_o), as judged by single channel membrane patch analysis of CFTR[82] and lipid bilayer reconstitution.[88] Findings from Phase 2 clinical trials reported in the *New England Journal of Medicine* demonstrated significant and dose-dependent improvements of sweat chloride, correction of CFTR-dependent chloride transport in the airways as measured by NPD, and a significant increase in lung function.[89] Dr. Rowe helped plan this landmark study, treated the first subject, directed the top enrolling site, and contributed to the design of subsequent studies, such as an ongoing 30-center post-approval study evaluating mechanistic consequences of CFTR activation, including the effects of ivacaftor on MCC (See Fig. 13).[8] Phase 3 testing demonstrated that ivacaftor produced a marked, rapid, and sustained improvement in spirometry (absolute improvement in FEV_1% of > 10% at 24 and 48 weeks), while also conferring a substantial reduction in pulmonary exacerbations (by 55%), normalizing sweat chloride (a 48 mEq/L reduction), and

improving quality of life as assessed by the CFQ-R (8 point treatment effect at 24 weeks, well above the MCID of 4); this degree of benefit has never been seen in prior CF clinical studies with any other intervention and was highlighted in the *New England Journal*.[7] Moreover, no significant adverse events or dose-limiting toxicities were observed either in this study, the earlier Phase 1 and 2 evaluations that included CF subjects and healthy volunteers, or during preclinical testing in laboratory animals. *These results are significant, since they represent the first evidence that drug treatment can improve CFTR activity in the airways, correct the sweat chloride abnormality, and ameliorate pulmonary obstruction, firmly establishing CFTR as a viable therapeutic target*. These remarkable results formed the basis for the FDA approval of ivacaftor in CF patients with the G551D-CFTR mutation, and ivacaftor was also efficacious in a variety of other CFTR mutations localized to the cell surface (e.g. non-G551D gating, and mutations associated with pancreatic sufficiency).[85,86] In addition to these robust effects on mutant CFTR, evidence from our laboratory[2,64] (see also below) and others[23,90,91] indicate ivacaftor strongly augments wild type CFTR channel activity and mucus transport in normal and smoke exposed epithelia, suggesting a treatment strategy for patients with *acquired* defects of mucus clearance, including those with COPD in whom surface expression of CFTR is sufficiently preserved even if partially reduced.[2,3,18]

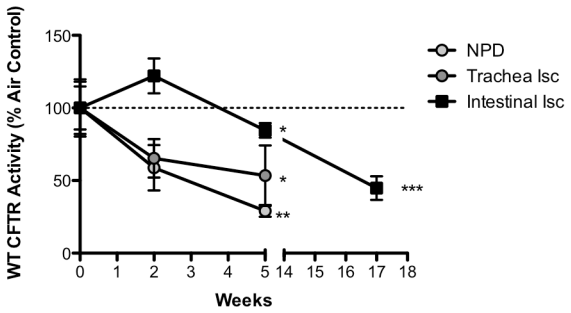

Fig. 1. Time-dependent inhibition of pulmonary and systemic CFTR function by cigarette smoke *in vivo*. CFTR dysfunction (% decrement) caused by cigarette smoke are plotted by duration of cigarette smoke exposure. Mice were exposed to whole cigarette smoke twice daily for 2 and 5 weeks and once daily for 17 weeks. CFTR function measured in nose (NPD), trachea and intestine (Ussing chamber electrophysiology). n=8-15, *P<0.05, **P<0.005, ***P<0.0005.

<u>Preliminary Data</u>. A. Cigarette Smoke Blockade of CFTR Function. Data generated both *in vitro* and *in vivo* from multiple laboratories have indicated that cigarette smoke exposure results in CFTR dysfunction among individuals with normal CFTR genotype. Cantin et al. has shown that healthy cigarette smokers (confirmed to be WT CFTR homozygotes) exhibit decreased CFTR activity *in vivo* by NPD.[16] This complemented *in vitro* studies in normal epithelial monolayers by Kreindler et al. who showed that cigarette smoke or cigarette smoke extract (CSE) reduced CFTR mRNA levels, protein expression, and ion channel function in airway epithelial cells grown in culture, a finding that led to ion transport discovery programs led by Novartis.[16,17,19] R Tarran's group has confirmed these findings, and demonstrated that cigarette smoke inhibited maintenance of ASL depth by stimulating some CFTR internalization (see ref[18] and also Figs. 6 & 11), and that this defect could be overcome by hypertonic saline administration *in vitro*, supporting clinical studies.

To prepare for advancing a pharmacologic approach, our laboratory defined the magnitude and dose dependence of these effects using well-differentiated primary epithelial cells derived from non-CF (CFTR WT) donors. Incubation of cigarette smoke extract (CSE) on the apical surfaces of primary airway cell monolayers resulted in dose-dependent reductions in CFTR mediated Cl^- transport (Isc)[2]; we also confirmed this with various whole cigarette smoke (WCS) exposure intensities.[64] Because short-circuit current observed *in vitro* with human bronchial epithelial (HBE) cell monolayers was very predictive of ion transport results by NPD in CF patients treated with ivacaftor, this model is recognized as highly relevant to *in vivo* effects.[6,23] Single channel conductance studies further demonstrate the deleterious effect of CSE on CFTR single channel open probability (Po),[92] the component of CFTR activity enhanced by ivacaftor.[23] We confirmed CFTR inhibition *in vivo* using a murine model by demonstrating that WCS exposure causes a decrement in CFTR activity as determined by NPD, trachea Isc, and intestinal current measurements (Fig. 1).[4] Together with results from multiple labs investigating WCS,[18-20] CSE,[16,17] and cigarette smoke *in vivo* (see refs[16,20] and also below) these data establish specific and clinically meaningful reductions in CFTR dependent ion transport due to cigarette smoke exposure. For example, reduced CFTR activity shown above is similar to the decrement observed among individuals with CFTR related metabolic disorders (e.g. non-classic CF),[93] a phenotype associated with adult onset chronic bronchitis or bronchiectasis.[43]

Given the deleterious effects of cigarette smoke on CFTR-dependent anion conductance, we also examined the impact of smoke exposure on mucus expression and transport, pathways severly affected in COPD.[94] CSE generated a pronounced increase in mucus expression measured by PAS staining.[2] The changes in CFTR activity and mucus expression induced by smoke also led to prominent reductions in MCT rates,[2] a finding confirmed using functional anatomic imaging (Fig. 2). *The collective results demonstrate that reduced CFTR-mediated anion secretion, together with excessive mucus expression, severely inhibits MCC*. These data also indicate the exciting possibility that CFTR potentiators could have therapeutic effects in COPD, combatting delayed MCC[95-98] and increased mucus expression[99] in the disease.

B. COPD patients exhibit reduced CFTR activity and expression in the upper and lower airways. Given the effects of cigarette smoke on CFTR-dependent ion transport and mucus transport in respiratory epithelial cells and *in vivo* using congenic mice, we also assessed whether this defect is detectable among individuals with smoking related COPD. As predicted by results in primary respiratory epithelial cells[6,23,100] and demonstrated in healthy smokers without CFTR mutations,[16] we established that individuals with smoking-related COPD exhibit reduced CFTR expression and activity measured by NPD.[2] Smokers with COPD had a severe reduction in CFTR-dependent ion transport (Fig. 3). The defect in chloride conductance in smokers with and without COPD was not attributable to changes in mucosal integrity and appeared specific to CFTR, as no significant difference in PD was observed following adenosine triphosphate (ATP) perfusion,[2] which stimulates activity of Cl^- channels other than CFTR. Reduced CFTR activity measured by NPD was also predictive of the severity of bronchitis symptoms (r=0.30, P<0.05), as determined by the Breathless Cough and Sputum Score[101], even when controlled for cigarette smoking ($r_{adjusted}$=0.31, P<0.05), indicating a strong association with the chronic bronchitis phenotype. Furthermore, individuals with a history of COPD exacerbation during the past year exhibited 35% less CFTR function than those without a history of exacerbation (P<0.005), a result that points to important clinical consequences associated with acquired defects in CFTR activity.[2]

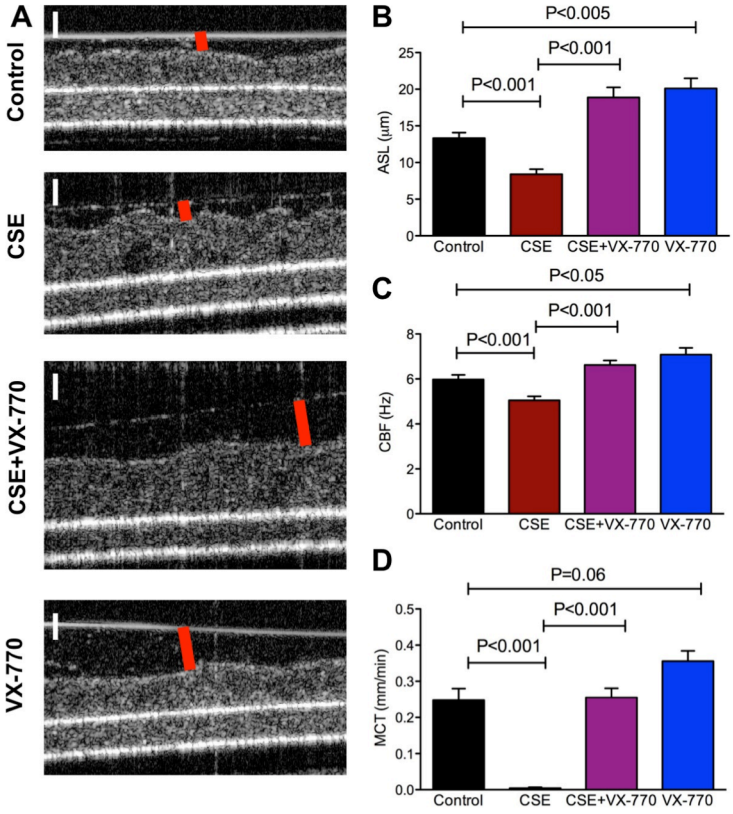

Fig. 2. Ivacaftor augments epithelial function of CSE exposed cells as revealed by one micron resolution optical coherence tomography (μOCT) imaging. (A) Representative μOCT images of well-differentiated HBE cells were exposed to CSE (2%) or vehicle (media with 2% DMSO) apically and ivacaftor (VX-770, 10 μM) or vehicle (0.1% DMSO) basolaterally for 24 hrs. ASL depth is shown as red bar. White scale bar = 10 μm. **(B-D)** ASL depth (B), cilia beat frequency (C), and mucociliary transport (MCT) rate (D) are quantified. Data are derived from 5 measures per well, and 8 wells per condition. See also Liu et al. *PLoS ONE* 2013 for detailed methods.

To confirm if these findings were also present in the lung, we conducted the first lower airway PD measurements performed under conscious sedation in COPD patients. Results demonstrated marked reductions in CFTR activity among affected subjects compared to controls (Fig. 4).[3] Like NPD, CFTR decrements in LAPD were associated with chronic bronchitis (r=0.38, P<0.05) as well as dyspnea (r=0.41, P<0.05), indicating clinical relevance. NPD correctly characterized 18 of 25 (72%) of patients with CFTR dysfunction in the lung, supporting the use of NPD as a proxy for lower airway CFTR activity. NPD is not impacted by the pro- inflammatory environment of the lung that likely explains disproportionately reduced CFTR measured by LAPD in some patients,[3,61,62,102] which could interfere with an accurate assessment of treatment effect, and allows us to avoid the cost and risk associated with LAPD bronchoscopy. Combined with data demonstrating similar decrements following smoke exposure to wild type AJ mice (see Fig. 1),[4] these results indicate that clinically relevant acquired CFTR dysfunction is present in the lung, and is potentially amenable to therapeutic intervention.

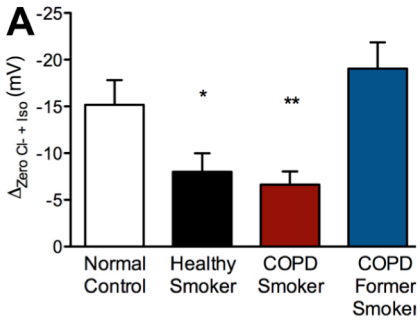

Fig. 3. Reduced CFTR activity in smokers with COPD measured by nasal potential difference (NPD). CFTR-dependent Cl^- conductance measured by NPD (change in PD following Cl^--free plus isoproterenol (10 μM) perfusion) in the four patient groups shown. *P<0.05, **P<0.005. See Sloane et al. *PLoS ONE* 2012 for details.

C. Evidence of a Sustained Systemic CFTR Defect in COPD and its Physiologic Significance. Our results strongly indicate that smoking causes a readily detectable and clinically relevant decrement in CFTR function in the airways. Since COPD is now known as a systemic disease with a number of non-pulmonary manifestations,[103,104] we studied patients using sweat chloride analysis (the hallmark diagnostic test for CF) as well as genetic analysis for CFTR mutations (as in **all** of our other studies, patients who had any CFTR mutation were excluded from the analysis *a priori*). Sweat chloride levels demonstrated that the

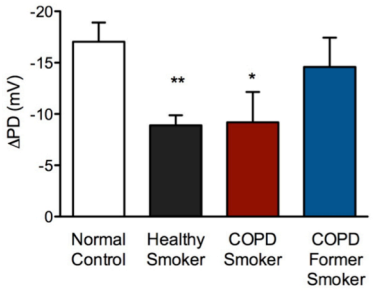

Fig. 4. Reduced CFTR activity in smokers with COPD measured by lower airway potential difference. CFTR measured by change in PD of the lingual outlet with perfusion of Cl^--free plus isoproterenol (10 μM). **P<0.005, *P<0.05, N=10-12/group. See Dransfield et al., *Chest* 2013.

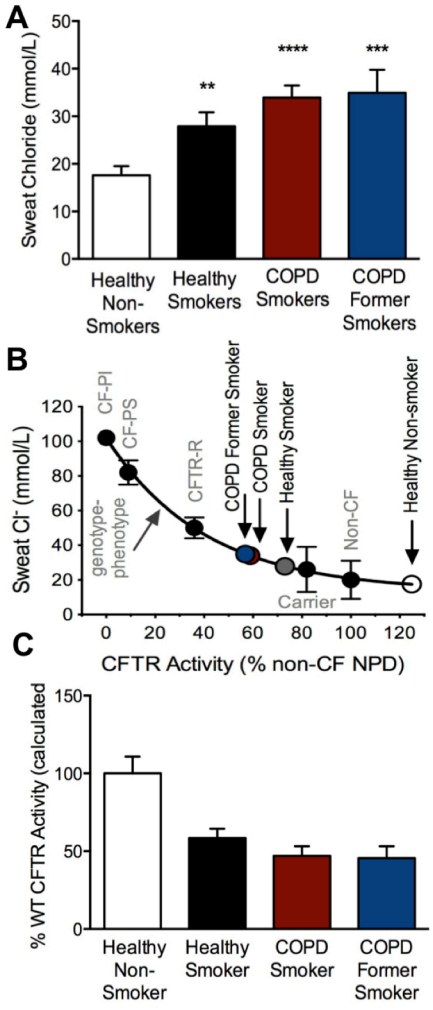

Fig. 5. Evidence of systemic CFTR dysfunction in COPD (A) Sweat chloride, a measure of CFTR activity, from healthy non-smokers (n=28), healthy smokers (n=31), COPD smokers (n=36) and COPD former smokers (n=14). **P<0.01, ***P<0.001, ****P<0.0001. No differences in serum sodium, chloride, or aldosterone were observed. **(B)** Relationship between CFTR activity measured by NPD (x-axis) and sweat chloride (y-axis) based on genotype-phenotype correlations in CF by Wilshanski, *AJRCCM* 2006 (R^2=0.99, P<0.001). Colored circles represent the 4 disease groups tested in *A* and their corresponding location on the curve based on mean sweat chloride. **(C)** Normalized CFTR activity estimated using sweat chloride values determined in *A* corrected for the non-linear relationship between sweat chloride and CFTR function shown in *B*. See Raju et al., *AJRCCM* 2013.

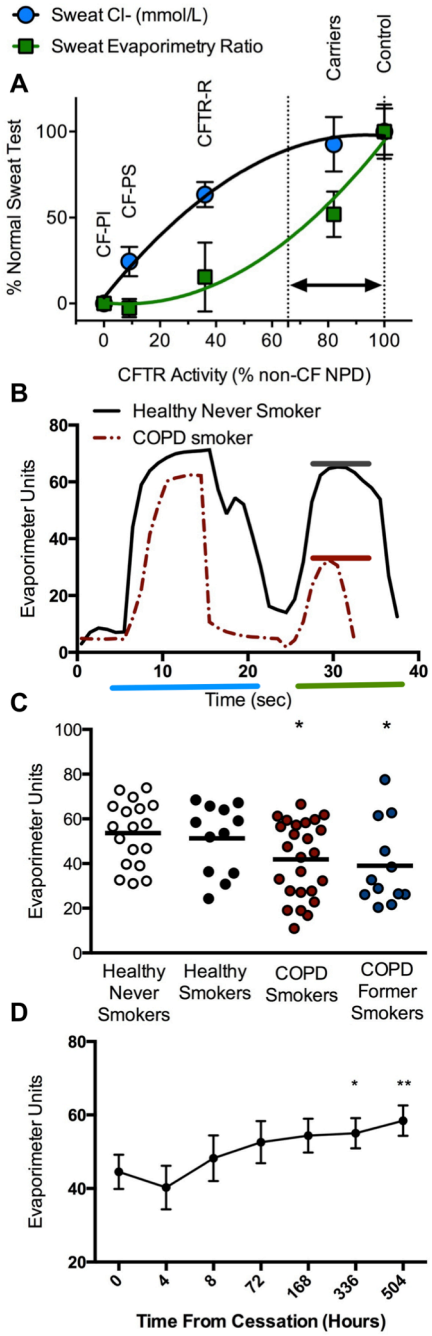

Fig. 6. Sweat secretion rate confirms systemic CFTR Dysfunction in COPD. (A) Normalized values for sweat chloride and sweat evaporimetry plotted against CFTR activity assayed by NPD for CF genotype-phenotype groups. CF-PI=CF with pancreatic insufficiency; CF-PS=CF with pancreatic sufficiency; CFTR-R=CFTR related disorder; Carrier=obligate heterozygotes. Arrows indicate difference between normals and COPD. **(B)** Sweat rate tracing measured by evaporative water loss of a normal control compared to a COPD smoker. β-adrenergic sweat rate (green bar) as opposed to cholinergic sweating (blue bar) is used as a marker of CFTR activity (black and red bars designate peak rate). **(C)** Summary data of *B*. **(D)** Sweat rate serially measured in healthy smokers following a monitored smoking cessation program (n=7). *P<0.05, **P<0.005. See Courville et al, *Resp Res* 2014.

sweat gland, a site sensitive to CFTR dysfunction and representative of peripheral CFTR activity, was also affected by cigarette smoking and COPD, including evidence of a sustained decrement after smoking cessation (Fig. 5A).[4] In light of the established non-linear relationship between sweat chloride and CFTR activity (Fig. 5B and 6A),[93] the severity of CFTR dysfunction in the sweat duct was similar to that observed in the airway in individuals with CFTR related disorders and other clinical manifestations of CFTR deficiency (i.e. ~50% decrement; Fig. 5C). Once again, CFTR dysfunction in the sweat gland was associated with chronic bronchitis (as measured by BCSS score, β=0.37, P<0.001), an effect that persisted even when smoking, COPD status, and BMI were included in a multivariate regression model (β=0.21, P<0.001)[4]; furthermore, sweat chloride elevation ≥ 35 mEq/L, was associated with more severe airflow limitation (i.e. COPD GOLD Stage).[4] Remarkably, confirmatory studies in human intestine were consistent with an acquired extra-pulmonary CFTR deficit in smokers (60% decrement in CFTR activity, P<0.01, N=15, see ref[4] and Fig. 16 in Study Protocol). To confirm these important findings, and investigate the most suitable biomarkers to track CFTR in COPD patients, we next examined β adrenergic sweat rate, an assay well suited to detect modest CFTR abnormalities (see Fig 6A and ref[105]). Sweat rate demonstrated clearly reduced CFTR function in COPD (Fig. 6B,C) and was associated with dyspnea severity.[5] In addition, sweat rate steadily improved following smoking cessation (within subject differences were demonstrated in just 14 days, see Fig. 6D); the assay also exhibits excellent within subject reproducibility.[21] *These results have been highlighted in recent editorials[106,107], and indicate that CFTR abnormalities*

in smoking related COPD can be detected in sites remote fr om direct inhalation even in those who no longer smoke, correlate with symptoms of bronchitis and COPD severity, and are dynamic. The data also suggest that CFTR dysfunction may be transmitted systemically, and point to a potential mechanism underlying the increased incidence of systemic disorders attributed to smoking that are also strongly associated with CFTR abnormality, including idiopathic pancreatitis,[108] osteopenia,[109-112] diabetes mellitus,[113-115] and male infertility.[116] In complementary studies[4], we have identified a mechanistic basis underlying these observations by demonstrating that acrolein, a cigarette smoke constituent and endogenous inflammatory byproduct detectable in the serum of COPD smokers, acts as mediator in the blood of individuals with COPD to cause systemic CFTR dysfunction[4] (see Figs. 17-20 in the Study Protocol); this will be studied as a serum biomarker by assessing serum acrolein using an innovative LC/MS/MS assay (see ref[4] and Study Protocol for methods).

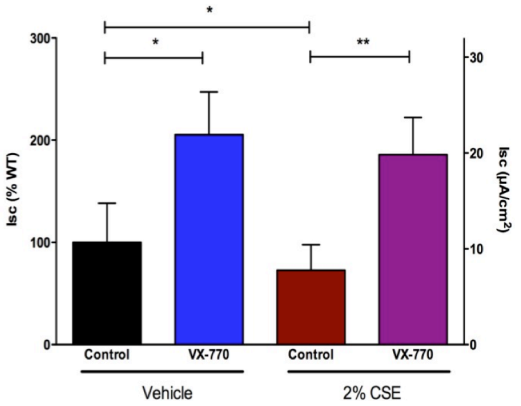

Fig. 7. Ivacaftor augments CFTR-dependent Isc following CSE exposure. Primary HBE cells were treated with 2% CSE or vehicle control for 24 hrs. Forskolin (100 nM) stimulated Isc followed by ivacaftor (VX-770, 10 μM) or vehicle control is shown as the percentage of wild-type Isc with forskolin alone. ±SEM, N=6 per condition. See Sloane et. al *PLoS ONE* 2012 for additional details and raw tracings.

D. Effect of CFTR potentiators on wild type CFTR. While CFTR potentiators were originally developed to restore activity to mutant CFTR localized to the cell surface, some CFTR potentiators, including ivacaftor, also augment wild type (WT) CFTR function in human airway epithelia[63,68] (as opposed to F508del CFTR that does not reside at the cell surface and does not respond to ivacaftor[117]). As depicted in Fig. 7, ivacaftor increased WT CFTR dependent Isc, compatible with its known effects on open channel probability of WT CFTR.[23,90] Activation by ivacaftor was also observed in CFBE41o- cells (which lack detectable CFTR) following recombinant WT CFTR expression, but as expected, not in parental (non-CFTR complemented) airway cells.[2] The mechanism of ivacaftor is specific to human CFTR (no activity on murine CFTR).

Because CFTR regulates ASL depth,[118] which in turn determines MCC,[119-121] it follows that potentiation of CFTR anion secretion by ivacaftor in WT epithelia should result in augmented mucus transport compared to resting conditions.[122,123] We have shown that ivacaftor enhanced ASL depth in WT HBE monolayers (Fig. 8), causing a marked increase in MCT (Fig. 9), indicative of the highly responsive nature of the MCC apparatus even in healthy WT monolayers (see also ref[2]). This result is further supported by reports that inhaled hypertonic saline augments MCC among healthy individuals[123] and indicates that MCC can be augmented even when CFTR is not abnormal, which could be beneficial in a disease such as COPD. These changes are readily apparent by μOCT imaging and indicate ivacaftor causes a robust enhancement of MCT in WT epithelia (see Fig. 2). Ivacaftor also potentiated CFTR-dependent current (I_{sc}) in normal explanted human trachea, regardless of the smoking status of the donor.[2]

E. Ivacaftor reverses acquired CFTR abnormalities and augments MCT in vitro. Given the robust effects of ivacaftor in non-CF epithelia, including potentiation of wild type CFTR-mediated ion transport, ASL depth, and MCT, we next examined whether smoke mediated CFTR inhibition could be overcome by addition of ivacaftor. Ivacaftor potentiated CFTR-dependent I_{sc}, regardless of prior administration of CSE, to levels that

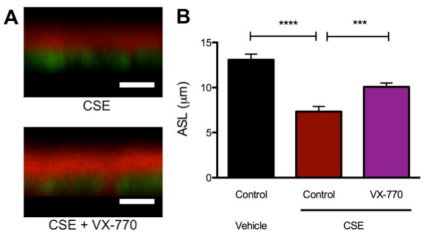

Fig. 8. Ivacaftor augments airway surface liquid depth following CSE exposure (A) Representative Z-scan confocal images derived from HBE cells following exposure to CSE (2%, apical),) or CSE + ivacaftor (VX-770, 10 μM, basolateral) for 24 h prior to assay. White scale bars designate 10 μm. **(B)** Summary data from experiments shown in A. ***P < 0.0005, ****P < 0.0001, *n* = 9-11.

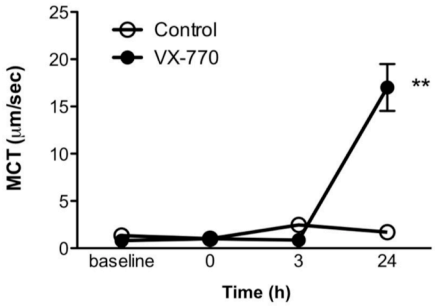

Fig. 9. Ivacaftor augments mucociliary transport of non-CF HBE monolayers. Ivacaftor (VX-770; 10 μM) or vehicle control was added to basolateral compartment of monolayers co-stimulated with the cAMP agonist VIP (30 nM) immediately after the time = 0 h measurement. **P < 0.001 vs. vehicle control, *n* = 10 /condition. MCT measured using movement of fluorescent beads; see Sloane et al. *PLoS ONE* for detailed methods.

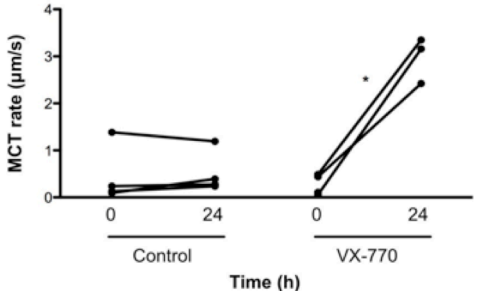

Fig. 10. Ivacaftor augments mucociliary transport following CSE exposure. Mucociliary transport studies were performed in HBE cells as shown in Fig 11. Beads were added 24 h prior to time 0 h measurements, followed by CSE (2%) in the apical compartment together with ivacaftor (VX-770; 10 μM) or vehicle control in the basolateral compartment. *P < 0.05. See also Sloane et al. *PLoS ONE* 2012.

exceeded WT-CFTR activity (Fig. 7).[2] Ivacaftor also robustly increased ASL depth in CSE-exposed cells (Fig. 8) and caused a large increase in MCT (Fig. 10).[2] Functional anatomic imaging using μOCT[124] established similar findings while also confirming health of the monolayer (See Fig. 2). Our data strongly indicate that the CFTR potentiator ivacaftor reverses acquired CFTR dysfunction induced by cigarette smoke, resulting in increased MCT and augmenting ASL depth in airway epithelia.[2] These data demonstrate that CFTR represents an attractive therapeutic target to address mucus stasis in COPD as demonstrated using models predictive of *in vivo* results in CF with ivacaftor in addition to the electrophysiological phenotype of COPD patients.

F. Ivacaftor may ameliorate acquired CFTR dysfunction in COPD patients. Based on the Vertex-UAB IIS already in place, we have begun abbreviated pilot testing of our proposed study in a small number of patients with COPD and chronic bronchitis to demonstrate feasibility, confirm adequate absorption, and validate CFTR endpoints. Single blind ivacaftor in the first subject analyzed achieved adequate levels after the initial dose (T_{max} 490 ng/mL at 6 hrs post dose, with food). The subject also demonstrated markedly improved NPD (Fig. 11; mean $\Delta_{Cl\ free\ +\ Iso}$ changed from -8.2 mV baseline to -18.2 mV post–dose) and sweat chloride (from 35.7 to 28.3 mEq/L) after 14 days of ivacaftor treatment indicating sustained response to ivacaftor and strongly supporting the notion that ivacaftor can potentiate CFTR in COPD patients with chronic bronchitis.. This early experience demonstrates feasibility, emphasizes the need to address the questions posed in this application, and provides confidence the effort will be worthwhile, ultimately informing subsequent trials, including defining the target population.

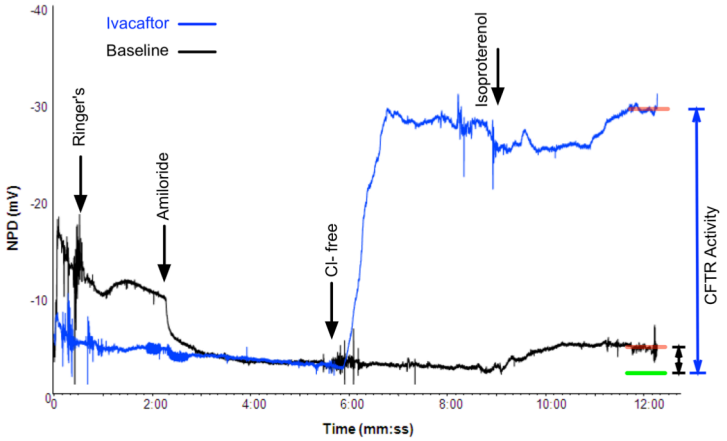

Fig. 11. NPD tracings of a COPD patient with chronic bronchitis, before (black) and after (blue) ivacaftor treatment (150 mg q12 hr x 3 days). CFTR activity is the ΔPD between the green and red bars (right margin). Right nostril shown for each procedure. Similar effect was seen after 14 days.

EXPERIMENTAL DESIGN. A. Overall Hypothesis and Research Questions. The central hypothesis of this project is that CFTR activity in patients with COPD can be stimulated by administration of the CFTR potentiator ivacaftor, conferring meaningful improvements in CFTR activity, epithelial function of the airways, lung function, and pulmonary symptoms. Based on the expectation that ivacaftor will be particularly efficacious in individuals with chronic bronchitis, we are focusing our initial studies in this population, but will also analyze results based on CFTR dysfunction at baseline (i.e. sweat chloride or NPD), a crucial step necessary to define the target population of interest for future studies. While we are confident that changes in CFTR function in humans can be readily detected and accurately measured (Aim 1), potentiation of wild type CFTR in humans has never been attempted and may exhibit a different dose-response relationship than mutant forms of the channel, hence our initial study explores various dose groups, and will include extensive PK/PD analysis (Aim 3). Moreover, we will perform exploratory analysis of pulmonary physiology and clinical outcome (Aim 2) so that subsequent studies can be designed (See future plans).

B. Pilot Trial Design. The study is a pilot randomized, double-blind, placebo-controlled, dose-ranging, single center study of orally-administered ivacaftor in subjects with COPD and chronic bronchitis (Fig. 12). Subjects will be administered ivacaftor or placebo twice daily (q 12 hr) for

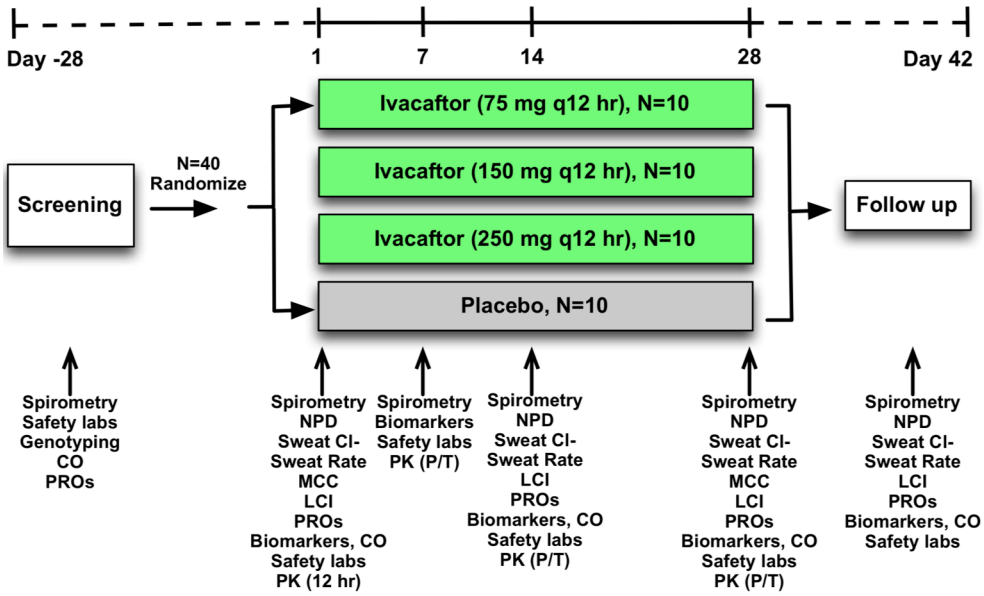

Fig. 12. Schematic of the Pilot TOPIC Study. PRO: patient reported outcome questionnaires. CO: carbon monoxide monitoring. P/T: peak/trough pharmacokinetics (PK).

28 days, with a 4 week screening period (sufficient to ensure stability and confirm normal CFTR genetics) and a 2 week follow-up period (for safety and to detect efficacy changes upon washout). The primary endpoints are safety and CFTR activity as measured by NPD (within group). Secondary endpoints include change in sweat chloride and sweat rate; measures of COPD clinical outcome (spirometry, San Diego Shortness of Breath Questionnaire, Breathlessness, Cough and Sputum Scale (BCSS), Cough and Sputum Assessment Questionnaire (CASA-Q), and the COPD assessment test (CAT)); changes in respiratory physiology and mucus obstruction (LCI and MCC as measures of small and large airway function downstream of CFTR); pharmacokinetic/ pharmacodynamic (PK/PD) relationships; and exploratory biomarkers, such as serum acrolein (see ref[4] and Figs. 17-20 in Study Protocol). Enrollment will include 40 subjects and patients will be randomized 3:1 to active drug (n=30) to one of three doses (75 mg, 150 mg, and 250 mg Q 12 hr; 10 each) used in Phase 2 and 3 CF trials, or placebo (n=10). Since the primary analysis is the within subject change in all patients assigned to active treatment (an analysis used previously with ivacaftor[6]), this will provide sufficient power to discern a meaningful change in CFTR activity while also preserving the blind. All patients must exhibit chronic bronchitis; a key secondary analysis will investigate the impact of baseline CFTR function on drug efficacy using NPD and sweat chloride so that the target population can be defined; minimum enrollment (N≥15) for those with and without CFTR dysfunction (as defined by sweat chloride elevation) is defined to enable this analysis; we will also analyze smoking status as a covariate. The prevalence of CFTR dysfunction in COPD is reasonably high and correlated with COPD severity (see Table 2-2 in the Protocol). Subjects will be instructed to continue their standard medication regimen and must meet the inclusion and exclusion criteria summarized in Table 1. The schedule of assessments is shown in Table 2 and the safety and efficacy endpoints are described below. A more detailed description of the study design, rationale, and preliminary data are included in the Clinical Protocol and FDA IND Submission, found in the Protection of Human Subjects Section.

Inclusion Criteria:	**Exclusion Criteria**
• Male or Female age 40-65	• Current diagnosis of asthma
• A clinical diagnosis of COPD as defined by GOLD	• Requirement for daytime use of chronic O_2 therapy
• At least a 10 pack year smoking history	• Documented history of drug abuse within the last year
• Exhibit symptoms of chronic bronchitis defined by Medical Research Council (MRC); see ref[125]	• Subjects should not have a pulmonary exacerbation or changes in therapy for pulmonary disease in the 4 weeks prior to screening
• FEV_1% predicted ≥ 35 and ≤70 Post Bronchodilator	• Cirrhosis or elevated liver transaminases > 3X ULN
• Clinically stable in the last 4 weeks with no evidence of COPD exacerbation	• GFR<50 estimated by Cockroft-Gault
• Weight of 40-120 kg	• Pregnant or breastfeeding
• Willingness to use at least one form of acceptable birth control including abstinence, condom with spermicide, or hormonal contraceptives	• Inhibitors or inducers of CYP3A4, including certain herbal medications and grapefruit/grapefruit juice
• Willingness to monitor blood glucose if known history of diabetes mellitus requiring insulin or medical therapy	• Presence of clinically relevant CFTR mutation
	• Clinically significant cardiac condition (see Protocol for details)
	• Type 1 diabetes mellitus or uncontrolled diabetes mellitus type 2
	• History of stroke/CVA or non-skin cancer < 5 years prior
	• Any other condition that might confound the results of the study or pose an additional risk in administering study drug

Table 1. Summary of Inclusion and Exclusion Criteria (please see TOPIC Protocol for complete list and additional detail)

C. Study Recruitment and General Feasibility. Since its inception, the UAB Lung Health Center (LHC) has been very successful in the recruitment and retention of study participants and has an established Recruitment Core. The LHC is among the top recruiting centers in the U.S. for many NIH trials including the clinical trials of the COPD Clinical Research Network (CCRN), the COPDGene study, and the Long Term Oxygen Treatment Trial (LOTT). In addition, the LHC has extensive experience in recruiting for complex clinical studies, including those that rely on innovative clinical endpoints[126-130]; this is exemplified by over 150 COPD patients studied for acquired CFTR dysfunction in the last 2 years[2,3,5,20,21], a pace adequate to achieve our recruitment goals. Successful enrollment in our pilot study (despite being unfunded) also allowed us to refine the order of procedures, optimize biomarker conduct, confirm utility of sweat evaporimetry, and identify a large number of patients interested in the proposed study, while clearly establishing our relationship with Vertex. To facilitate initial and end of study procedures, Day 1 and Day 28 assessments can be spread over a 3 day period (in addition to the 2 day study window); the UAB CCTS will provide support for overnight stays as required.

D. Safety monitoring: Details regarding safety monitoring planned for this study are provided in the Human Subjects Section and the TOPIC Protocol. The design of the data and safety monitoring plan (DSMP) are based on substantial experience in COPD trials including first in class medications directed against mucus retention and expression, and interventional trials in COPD conducted previously at our center.[129,131-136] Key features of the DSMP include use of the independent Data Safety and Monitoring Committee (see DMC

Charter provided in the Human Subjects Section) staffed with independent experts; interim safety analyses planned at 1/3 and 2/3 enrollment,in addition to FDA reporting requirements.

Assessment	Screening (Day -28 to -1)	Day 1 (Pre-dose) (Day -2 to +1)	Day 7 (± 2)	Day 14 (± 2)	Day 26-28 (± 2)	Follow Up (Day 42 ± 2)
Informed Consent	X					
Medical History	X	X	X	X	X	X
Physical Examination	X	X	X	X	X	X
Safety Labs	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X
Urine Smoking Biomarkers		X	X	X	X	X
CFTR Genotyping	X					
Serum/Urine Pregnancy Test[d]	X	X	X	X	X	X
Blood Biomarker Collection		X	X	X	X	X
EKG	X	X[a]	X	X	X	X
Spirometry	X	X	X	X	X	X
NPD		X		X	X	X
Sweat Chloride		X		X	X	X
Sweat Evaporimetry (rate)		X		X	X	X
PROs	X	X		X	X	X
Mucociliary clearance (MCC)		X			X	
Lung clearance Index (LCI)		X		X	X	X
Pharmacokinetics		X[b]	X[c]	X[c]	X[c]	
CO Monitoring	X	X		X	X	X

Table 2. Schedule of Assessments. PRO=patient reported outcome questionnaires. [a]EKGs at 30 min, 1 hr, 2 hr, 3 hr, and 4 hr post dose. [b]12 hr PK (1,2,4,8,12 hr timepoints). [c]peak and trough PK only. [d]serum at visit 1 only

E. Ivacaftor Concentrations and Pharmacokinetics (PK): Ivacaftor concentrations in CF have been stable and consistent across a large breadth of ages tested (2-53 years) across several dose groups. Dose-ranging studies in CF demonstrated statistically significant and clinically meaningful improvements in FEV_1 at the 75 mg, 150 mg, and 250 mg dose groups.[6] Based on this, we are confident that ivacaftor concentrations will be within the wide therapeutic range for activating CFTR among COPD subjects, but will be expressly quantified using sophisticated PK modeling. Due to the metabolism of ivacaftor, we will restrict individuals from participating who are taking medications known to alter CYP3A4, as performed during previous ivacaftor trials. We have demonstrated the feasibility of detecting ivacaftor in plasma using LC/MS/MS (see Protocol Fig. 34).

F. Biostatistics: The within subject change in CFTR activity as measured by NPD is the primary efficacy outcome measure; sweat chloride and evaporimetry, spirometry, MCC imaging, LCI, and other clinical outcomes relevant to COPD will serve as secondary measures. Based on the proof of concept design, the primary analysis will be within subject changes in outcome measures from day 1 to day 28, grouping all patients on active treatment. These data will be used to test the null hypothesis of no change using a paired t-test unless the distributions are notably skewed, in which case the non-parametric Wilcoxon signed-rank test will be implemented. Between group comparisons and composite time-dependent changes based on multiple measures (e.g. NPD, sweat chloride, LCI, etc.) will be conducted as secondary analyses using the two-group t-test (or the non-parametric Wilcoxon rank-sum test if needed) and analysis of covariance models. The dose groups will be compared simultaneously using analysis of variance (or the nonparametric Kruskal-Wallis test if needed). Longitudinal analyses will consist primarily of mixed models repeated measures analyses. An appropriate structure for the covariance matrix will be selected for these models using the final data. When a model term is statistically significant, the Tukey-Kramer multiple comparisons test will be used to determine which specific pairs of means are significantly different. Interaction terms may be included in some models. All statistical tests will be two-sided and will use $\alpha=0.05$. Our group has previous experience analyzing and interpreting changes in NPD and other CFTR outcome measures relevant to pulmonary outcome, and will benefit from CTSA resources from the Biostatistics Unit (see letter of support).[137-139]

G. Power and Sample Size Calculations: The size of the study was chosen based on the ability to detect a meaningful within subject change in NPD,[139-142] while also providing a study population suitable to detect safety signals and determine clinical and physiologic outcome measures for future studies. The sample size was calculated for the target population (e.g. those with chronic bronchitis, which is associated with CFTR dysfunction[2-5]) based on prior experience with the CFTR endpoints and our knowledge of anticipated activity of ivacaftor in CF airway epithelia, which is substantiated based on the close relationship between CFTR activity *in vitro* and potential difference findings *in vivo* in CF subjects.[6,23,100,142-147] Based on experience with ivacaftor using MCC imaging[8] and LCI[148], and our preliminary data demonstrating sweat test abnormalities among

COPD smokers (including repeated measures), our study also has sufficient power to detect changes in these parameters.[148,149] Sample size calculations for key primary and secondary endpoints are shown in Table 3.

Assay (unit)	Detectable difference		Power based on response magnitude of 50% of the difference between COPD and normal		Within-subject reproducibility (SD)
	80% Power	90% Power	Efficacy	Power	
NPD (mV) [Primary]	2.3	2.6	2.4 mV	85%	[c]4 mV[137,138]
Sweat Chloride (mmol/L)	2.3	2.6	9.9 mmol/L	>99%[a]	4 mmol/L[6,87]
MCC ($AUC_{60\ min}$)	3.9%	4.5%			7%[122,150]
Spirometry (FEV_1)[d]	2.8%[b]	3.2%			5%[151]

Table 3. Power and sample size of the proposed study. All calculations are based on within subject changes observed with prior experience (as indicated by references in Table) or preliminary data, n=27 active treatment patients among all doses assignments and accounting for 10% drop-out rate, a two-sided paired t-test, and α=0.05. [a]Power=80% between-groups at SD=12.8 mmol/L and 90% at SD=11.1 mmol/L. [b]Power=80% to detect 3.9% change in FEV_1% between groups; this corresponds to 5.1% relative improvement in FEV_1 assuming ~55% FEV_1% predicted upon enrollment, a typical mean value for COPD studies at our center with similar enrollment criteria. These two between-group calculations (a and b) assume a two-sided two-group t test. [c]This is a conservative estimate based on extensive experience in NPD; within group SD is < 3 at our center. [d]Based on experience testing ivacaftor in CF patients, LCI exhibits even greater power than spirometry, so we have an excellent sample size to detect a meaningful effect.[148]

H. Data Management Plan (DMP). The comprehensive DMP is described in the revised Study Protocol. We will conduct internal and external monitoring visits by independent research coordinators employed by the CCTS to assure data quality and stringency. We have done this already for the currently enrolling protocol and discovered only trivial deviations that did not impact the study; REDCap programming was also completed and is supported by the UAB CTSA (see Letter). We employ Dr. B. Liu, an experienced Data Manger for the CCD who is familiar with data entry, query resolution, and data cleaning. Our research pharmacist will conduct drug reconciliation and calculate adherence, which will be analyzed as a covariate for treatment response.

RESEARCH QUESTIONS. Specific Aim 1: Test whether CFTR potentiators augment CFTR activity in COPD patients. *We hypothesize that ivacaftor will improve CFTR activity in patients with COPD and chronic bronchitis*. CFTR activity will be measured in the airway by NPD, a methodology optimized by our laboratory[138,139,141] including for the characterization of CFTR abnormality among COPD subjects.[2,3] In addition, we will assess sweat tests as peripheral measures of CFTR activity since sweat chloride[4,5] and sweat rate[5] are highly sensitive to acquired CFTR dysfunction (Fig. 5,6) and the effect of CFTR-based therapeutics.[6,7,87]

1.1. Does ivacaftor increase CFTR activity in the airway? NPD measurements are sensitive and reliable for detecting changes in ion transport with efficacious CFTR directed-therapies and have been used successfully with ivacaftor treatment in CF.[6] The equipment and conditions for measuring bioelectric changes across the nasal mucosa capitalize on expertise in the UAB Center for CFTR Detection (see Figs. 3,11).[2,3,138,139,141] *NPD will provide a biologic readout (e.g. mechanistic biomarker) in the airway epithelia of COPD patients.*

1.2. Does ivacaftor augment CFTR activity in the periphery? Sweat chloride abnormality is correlated with COPD severity and symptoms[4], and is a highly sensitive outcome measure for CFTR-directed therapeutics.[10,11,81117] We have shown sweat chloride is sensitive to the presence of cigarette smoking and COPD (Fig. 5),[4,5] and the test has been successfully used as an endpoint in multiple CF trials, including studies to detect the efficacy of ivacaftor therapy.[6,7,87] Sweat rate is also abnormal in COPD, and was shown to improve following smoking cessation, suggesting it is a dynamic assay.[5,21] *Sweat tests will measure clinically relevant CFTR dysfunction and confirm bioactivity of ivacaftor in a non-respiratory organ.*

1.3 Does CFTR activity at baseline impact response to ivacaftor treatment? We hypothesize that CFTR potentiator therapy will be most effective among individuals with abnormal CFTR function at baseline. To test this, we will stratify patients by baseline CFTR activity levels using NPD, sweat chloride, and sweat rate, and evaluate relative efficacy of ivacaftor in these specific sub-phenotypes. The correlation between baseline CFTR activity (NPD, sweat tests) and clinical response will also be examined as a continuous variable. Based on our preliminary data phenotyping over 150 COPD subjects, ~32% of Gold II/III COPD patients who would be eligible for our study exhibit both chronic bronchitis *and* elevated sweat chloride, indicative of the large prevalence of the specific COPD sub-phenotype most likely to respond to ivacaftor (representing over 4 million patients in the U.S. alone[9]). Nevertheless, it is also possible that augmenting CFTR to super-normal levels could improve MCC and clear obstructed airways in individuals with chronic mucus hypersecretion despite near-normal CFTR function, widening applicability to over 8 million in the U.S.

Specific Aim 2: Establish the most informative clinical endpoints and mechanistic biomarkers for ivacaftor therapy in COPD patients with chronic bronchitis. *We hypothesize that CFTR activation by ivacaftor will improve mucociliary clearance and small airway occlusion, leading to improved lung function in patients with COPD and chronic bronchitis*. To test this hypothesis, and determine the biomarkers most informative for subsequent trials, we will conduct traditional measures of clinical outcome, including lung function (e.g. FEV_1) and patient symptoms. Results will test the initial proof of concept that CFTR potentiators are efficacious in COPD, potentially establishing an entirely new treatment paradigm while also providing data to determine suitable biomarkers for physiologic mechanism of action and future power analyses.

2.1. Does Ivacaftor Confer Clinical Benefit in COPD? A) Spirometry: Spirometry is a standard outcome measure in COPD and a major indicator of efficacy and safety in COPD trials. Post-bronchodilator spirometry will be performed by ATS criteria and percent predicted FEV_1, FVC, and FEF_{25-75} will be calculated based on Hankinson prediction equations. *Results will test the therapeutic potential of ivacaftor in COPD, indicate the clinical significance of our findings, and provide data necessary for a sample size estimate to base the design of follow-up clinical trials to definitively measure clinical efficacy*.

B) Patient Reported Outcomes: Questionnaires to detect the burden of cough and sputum symptoms will be used to monitor clinical outcome before and after study drug. This includes the San Diego Shortness of Breath Questionnaire[152], the Breathlessness, Cough, and Sputum Scale (BCSS)[101,153], the Cough and Sputum Assessment Questionnaire (CASA-Q)[154,155], and the COPD assessment test (CAT).[156,157] *Results will identify whether ivacaftor therapy improves symptoms of bronchitis, as seen with ivacaftor in prior CF studies.*[6,7]

2.2. Does Ivacaftor Augment Mucociliary Clearance and Relieve Small Airway Obstruction? To establish the mechanistic basis by which clinical benefit is observed, we will determine the effect of ivacaftor on the small and large airways using mucociliary clearance (MCC) imaging; lung clearance index (LCI) measurements will be used to assess mucus occlusion within the small airways. Results will provide the first test of whether activation of CFTR in individuals without CF can ameliorate aberrant lung physiology.

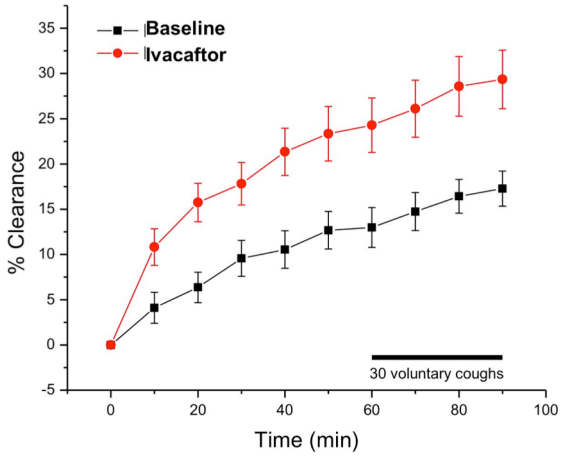

Fig. 13. Effect of ivacaftor on MCC in CF patients with the G551D mutation. MCC measured by clearance of Tc99 radiolabeled particles using proposed protocol N=19/group, P<0.001 for change in AUC_{60}. Magnitude of MCC improvement is larger than any therapeutic tested in CF, and was active in both the small (8-fold improvement vs. pretreatment) and large (2-fold improvement) airways.

A) Mucociliary Clearance by clearance of Tc^{99} Radiolabeled Particles: Clearance of Tc^{99} sulfur colloid is a measure of MCC of the lungs, and is calculated by a standard protocol developed by the Cystic Fibrosis Therapeutics Development Network and in use at our center.[122,158] The method provides a robust measure of MCC, and has been sensitive to the effects of inhaled pharmacologic agents in CF and COPD,[122,150,159] including improvements of an unprecedentedly large magnitude in CF patients with the G551D-CFTR mutation treated with ivacaftor measured in a multicenter study led by Dr. Rowe (Fig. 13).[8] The technique allows estimates of MCC in both the small and large airway compartments.[122] *Since the effect of ivacaftor was greater in the small airways in CF, and small airway lesions severely affect patients with COPD,*[14,160] *we hypothesize ivacaftor will cause robust improvement in MCC in the peripheral lung.* Both 1 hr (i.e. AUC_{60}) and 24 hr clearance rates will be calculated using 2D planar imaging, including estimates of both the central and peripheral airway clearance. *It has been demonstrated that this technique is sensitive to the MCC decrement in COPD*[59,60,159] *and exhibits a robust response to efficacious therapeutics in CF, including ivacaftor.*[8,122,150] *Results will test physiologic mechanism of action in COPD patients*.

B) Lung Clearance Index: LCI is a non-invasive, effort independent test that measures pulmonary function, and is particularly sensitive to small airway physiology and mucus obstruction.[161-163] The test recently demonstrated highly significant improvements in CF patients treated with ivacaftor, and exhibited power to demonstrate clinically meaningful changes with relatively small sample sizes.[148] *Use of nitrogen washout LCI method has been widely adopted by the CF research community*[164] *and will provide significant evidence that ivacaftor is active in the small airways of patients with COPD, providing independent validation of MCC studies and test our hypotheses regarding ivacaftor mechanism using a simple and effort independent assay.*

Specific Aim 3: Identify the optimum ivacaftor dose by establishing the PK/PD relationships in COPD patients. Our approach for assessing ivacaftor PK and establishing concentration-response relationships will

be four-fold. (1) First, we will determine the drug's PK parameters using a non-compartmental approach. Calculated PK parameters include: area-under-the-curve (AUC_{12}), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), oral clearance (CL/F), terminal apparent distribution volume (V_z/F), and elimination half-life ($t_{1/2}$). These results will be summarized in tabular format for each dose group and overall and will serve as a reference for subsequent modeled parameters. (2) Second, a population PK model will be developed and fit to the concentration-time data. One- and two-compartment models will be evaluated and covariates effects will be examined. Model validation will be assessed by diagnostic plots and corrected visual predictive checks (cVPC). (3) Our third step will be to apply a model to the treatment response data from Aims 1 and 2. In this regard, changes in CFTR function, mucus clearance, LCI, and spirometry will be modeled as continuous variables, similar to what our group has done for other disease biomarkers.[165] (4) Our final step is to link the overall drug-disease model to perform wide dose-ranging simulation studies. Population PK and biomarker parameter estimates (and their variability) are fixed and doses ranging from very low (~ 5 mg) to very high (~1000 mg) are input into the model. The resulting dynamic output (changes in spirometry and CFTR markers) and drug exposure are quantitated using maximum effect (E_{max}) models. Further details are in the Protocol.

Methods. We have developed a simple, rapid, and sensitive liquid chromatography–mass spectrometry (LC/MS/MS) method for quantitating ivacaftor in human plasma. A liquid–liquid extraction with ethyl acetate is used for sample preparation. Ivacaftor is separated from interferences using a C18 column and quantitated using a triple-quadrupole mass spectrometer operating in positive ion electrospray multiple reaction monitoring mode with a total run time of 3.5 min. The peak area of the m/z 393.0 →172.1 transition of ivacaftor was used to measure the compound in human plasma (Protocol Fig. 34). The standard curve is linear over a concentration range of 2.5-1000 ng/mL with a correlation coefficient of 0.997, providing 85-115% accuracy.

Anticipated Results, Caveats, and Alternative Approaches. We expect to establish the proof of concept that activation of wild type CFTR confers improvement in COPD, establishing a new paradigm for the treatment of acquired diseases of mucociliary clearance. We also will determine the appropriate dose and endpoints needed for subsequent clinical testing, including the mechanistic biomarkers that will advance our understanding of the importance of small airway obstruction in COPD and the pathologic role of CFTR. While CFTR is not decreased in all individuals with COPD, we have enriched our population for clinically relevant CFTR dysfunction by focusing on those with chronic bronchitis. While we expect those with CFTR dysfunction may exhibit the greatest response to ivacaftor, we may also find that supernormal CFTR function can be beneficial, a potential explanation for the treatment benefit conferred by roflumilast in patients with chronic bronchitis.[64] Although recent cigarette smoking can impact measures of ion transport, we will monitor the extent and timing of smoking by history and quantitative analysis (CO monitoring, cotinine), and examine this as a covariate of CFTR related endpoints. Our primary analysis combines dose groups into a single cohort, but we may find there are significant differences between ivacaftor dose groups, and will adjust our analysis plan accordingly. The pathways involved in muco-occlusion in COPD may be more complex than in CF, and include squamous metaplasia and reduced ciliary numbers and height, parameters that may not respond to activation of CFTR.[22,95,166-168] By examining both LCI and MCC in addition to CFTR activity, we should be able to understand whether small airway obstruction can be addressed by augmenting CFTR function.

Plans for a Subsequent Trial. The present study will inform the design of a subsequent confirmatory trial that will test the hypothesis that the CFTR potentiator ivacaftor provides meaningful benefit to patients with COPD and chronic bronchitis. From the present work, we expect to determine 1) the best dose of ivacaftor for potentiating wild-type CFTR; 2) the target treatment population (degree of CFTR dysfunction at baseline, the impact of smoking on treatment response, the degree of FEV_1 or MCC impairment); 3) the most informative biomarkers to monitor drug efficacy on CFTR and pulmonary physiology (i.e. will sweat chloride be sufficient, or will NPD and/or sweat rate be needed?; Is FEV_1 sufficiently sensitive, or will MCC or LCI be required?); and 4) sample size estimates. We plan to conduct a Phase 3 trial (6 months) to definitively demonstrate clinical efficacy (FEV_1, exacerbation frequency, cough); however, if clinical efficacy is more modest in the present study, we may need to conduct a Phase 2b design (3 month study) to explore alternative clinical endpoints that cannot be tested in the short-term pilot study (i.e. exacerbation frequency, exercise tolerance, functional status; or to continue to refine the target population) – this is a common approach for COPD drug development. We expect the study to target patients with COPD and chronic bronchitis in a randomized, double-blind, placebo controlled format with a standard 1:1 randomization scheme. Simplified endpoints would be selected to monitor CFTR function and airway physiology, informed by the present results; these could also be used to select patients with certain degrees of CFTR dysfunction if sub-phenotyping analysis is revealing. These results are necessary and sufficient to justify the commitment resources for a trial of this magnitude, since it would likely recruit at least 200-500 patients in a multi-center clinical trial design.