Early View

Research letter

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Cystic fibrosis airway inflammation enables elexacaftor/tezacaftor/ivacaftor-mediated rescue of N1303K CFTR mutation

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To the Editor:

Cystic fibrosis transmembrane conductance regulator (CFTR) modulators promote robust clinical improvements in people with cystic fibrosis (pwCF) with qualifying genotypes [1, 2]. A triple combination therapy consisting of two CFTR folding correctors (elexacaftor and tezacaftor) plus CFTR potentiator ivacaftor, referred to as ETI, has been approved by the FDA and European agencies (https://www.fda.gov/news-events/press-announcements/fda-approves-new-breakthrough-therapy-cystic-fibrosis,
https://www.ema.europa.eu/en/medicines/human/EPAR/kaftrio; https://ec.europa.eu/health/documents/community-register/html/h1468.htm) as highly effective modulatory therapy (HEMT) that improves lung function, decreases pulmonary exacerbation rates, increases body mass index (BMI), and lowers sweat chloride concentrations in pwCF with at least one copy of F508del CFTR mutation [1-3]. However, ~10% of pwCF do not have the F508del mutation or other genotypes that are currently approved for HEMT.

N1303K is one of the most common rare CFTR mutations, found in 2.4% of pwCF (https://www.cff.org/medical-professionals/patient-registry). Like F508del, N1303K is a class II mutation that causes CFTR misfolding, but its response to currently approved correctors is variable and less robust. The folding and functional defects of N1303K did not improve with the corrector lumacaftor in human bronchial epithelia (HBE; [4]) or rectal organoids [5], and modest and varying CFTR responses were observed in ETI-treated N1303K nasal epithelial cells [6, 7]. Studies in rectal organoids suggest that ETI needs to be combined with the novel potentiator apigenin to approach the levels of F508del rescued with lumacaftor/ivacaftor [8]). However, although N1303K is not an FDA-approved indication for ETI, ETI has been reported to restore N1303K CFTR expression to nearly 40% of wild-type function in vitro and led to clinical improvement in an 11-year-old female (genotype N1303K/E193X) [9]).

Inflammation is commonly found in the airways of pwCF. Using supernatant of mucopurulent material (SMM) – a pro-inflammatory material derived from CF lungs that includes bacterial products, neutrophil factors, cytokines, purines, mucins, and peptides [10] – we have shown that inflammation enhances the efficacy of CFTR modulator-promoted folding and activity of F508del CFTR in HBE [11-13]. These findings have been subsequently supported by a study from other investigators [14], and suggest that inflammation may also promote efficacy of modulators in other folding mutations such as N1303K.

To test the impact of inflammation on N1303K rescue, we exposed primary HBE cultures exhibiting the genotype N1303K/394delTT to SMM. Because 394delTT introduces a
frameshift mutation that leads to the absence of CFTR protein [15] any measured CFTR activity exclusively reflects the function of N1303K. CFTR activity was evaluated in Ussing chambers with and without ETI treatment in the presence and absence of SMM-induced inflammation (Fig.1). SMM exposure increased N1303K activity (Fig. 1A, B), based on quantification of maximal CFTR responses (Fig. 1C) and CFTR inhibition by CFTRinh-172 (Fig. 1D). Notably, SMM-enhanced rescue of N1303K CFTR by ETI (Fig. 1 A-D) is similar to that reported in F508del/F508del HBE cultures [11-13] (Fig. 1E). Epithelial sodium channel (ENaC) function was reduced by ETI treatment (Fig. 1F) although ENaC currents were not affected by SMM (Fig. 1F). Therefore, the change in ENaC function likely reflects the inhibitory effect of the rescued CFTR on ENaC activity [16]. Moreover, UTP-induced calcium-activated chloride channel (CaCC) activity, which can be reduced by ivacaftor contained in ETI ([17] and Fig. 1G), was drastically enhanced by SMM (Fig. 1G), reinforcing the view that CaCCs can serve as an alternative pathway for Cl- secretion that may further support airway hydration.

These findings suggest that ETI may achieve clinically relevant levels of CFTR activity in pwCF bearing the N1303K mutation in the presence of inflammation. To explore translational relevance, we examined outcomes of two pediatric pwCF heterozygous for N1303K treated with ETI. The first individual was a 10-year-old, 31.9 kg, Caucasian male (genotype N1303K/I507del) initiated on ETI in October 2021. Just prior to initiation, he had been treated with a one-year course of sulfamethoxazole/trimethoprim for Nocardiainfection that had decreased his lung function from baseline 100% to 73% percent predicted FEV\(_1\) (ppFEV\(_1\)), though with treatment his lung function improved to 94% ppFEV\(_1\) prior to starting ETI. Due to the presence of CF-associated liver disease (CFLD), a modified ETI dose of one orange tablet (elexacaftor 100/tezacaftor 50/ivacaftor 75 mg) once daily was started. On follow up visit two months after ETI initiation, his ppFEV\(_1\) had increased to 99%, and family noted subjective improvements in cough, work of breathing, and energy levels. Bronchoscopy after 1 month on ETI demonstrated minimal secretions and healthy mucosa with no growth on cultures, compared to bronchoscopy in 2016 with evidence of mild bronchitis and mild yellow lower airway secretions. Repeat sweat chloride testing performed
after six months on ETI was 95 mmol/L and 99 mmol/L, compared to baseline sweat chloride of 105 mmol/L and 103 mmol/L at two months of age.

The second patient was a 10-year-old, 38.6 kg, African American female (genotype N1303K/Q552P) started on ETI in October 2022. Shortly before starting ETI, new-onset pancreatic insufficiency was diagnosed, prompting pancreatic enzyme replacement therapy (PERT) initiation. In the two years before starting ETI she had multiple exacerbations requiring antibiotics, including two admissions for intravenous antibiotics. Her maximum lung function in that period was 80% ppFEV₁. ETI was initiated two days prior to discharge from an admission for intravenous antibiotics and inhaled tobramycin for newly acquired *Pseudomonas*, during which her lung function improved (56% ppFEV₁ on admission to 80% ppFEV₁ upon discharge). One month post-ETI, the patient reported no cough or other respiratory symptoms, with decreased sputum production. Her ppFEV₁ improved to 117%, and BMI increased from 38th%ile to 62nd%ile (weight gain of 2.3 kg). Repeat sweat chloride testing four months after initiation of ETI was 97 mmol/L and 94 mmol/L, compared to prior sweat chloride values of 83 mmol/L and 75 mmol/L obtained at three months of age.

Overall, these findings suggest that inflammation plays a critical role in response of N1303K CFTR to CFTR modulators. N1303K arrests CFTR folding at a late stage after partial assembly of its N-terminal domains; endoplasmic reticulum (ER)-associated degradation-resistant pools of N1303K are concentrated in the ER tubules that associate with autophagy initiation sites [4]. N1303K processing is affected by chaperone proteins (e.g., transmembrane Hsp40, DNAJB12, and cytosolic Hsp70) that cooperate to facilitate the triage of nascent polytopic membrane proteins for folding versus degradation. Airway inflammation leads to several epithelial responses that enhance the protein folding [18, 19], which could provide a mechanism for the enhancement of N1303K rescue observed in the present study.

The findings from this study have two important implications for CF research and clinical care. First, they suggest that theratyping of CFTR mutation responses to modulators needs to account for the impact of inflammation. Current theratyping studies have not included inflammatory stimuli and may underestimate potential clinical efficacy within
inflamed environments such as the CF airway. Furthermore, different cell culture media have been shown to induce different levels of basal inflammation in HBE, which could account for lab-to-lab variability in assessments of N1303K response to modulators [20]. Second, the findings raise concerns about the utility of sweat chloride as a clinical marker of efficacy. Sweat chloride glands are not inflamed, and responses to modulators may be more limited than in the CF airway or gut [21]. The role of inflammation may also help explain why changes in sweat chloride in individual pwCF are poorly predictive of clinical responses despite robust associations between sweat chloride responses and clinical efficacy on the population level [22]. The significant clinical responses despite minimal to no change in sweat chloride in our study suggest that lack of change in sweat chloride should not be used to determine if an individual patient might benefit from modulator treatment.

This study does have several limitations. Clinical responses are complex and difficult to attribute to ETI specifically. However, in both treated individuals subjective and objective clinical measures after ETI initiation improved beyond what aggressive traditional therapies had achieved. Furthermore, we cannot rule out the possibility that the second mutations, I507del and Q552P, respectively, contributed to the ETI response. I507del is an in-frame deletion variant class II mutation, whereas very little is known about Q552P. While further studies will be needed to determine the impact of ETI in individuals with these alleles, these should account for the impact of inflammation on efficacy.

In summary, our findings suggest that changes are needed in the testing paradigm for CF mutation responses to modulator therapy, both in vitro and in the clinic, to account for the impact of inflammation. Such changes could potentially increase the number of mutations that qualify for HEMT and provide significant benefit for pwCF with these mutations.

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Author contributions:
M.G. and C.M.P.R. oversaw in vitro experimental design and interpretations of data and wrote the in vitro portions of the manuscript. C.R.E. designed and coordinated the clinical studies and wrote the clinical portions of the manuscript. M.G. designed graphs and performed statistical analyses. C.M.R.P. generated and provided pooled SMM. S.E.B. performed tissue culture of CF HBE cells, D.M.C. coordinated and conducted treatments of cultures with SMM and modulators and assisted with writing and editing the manuscript. N.L.Q. performed Ussing chambers analyses. C.J.M., B.B., C.W.K. helped with conceptualization of the case series at the time. B.B. wrote the majority of the clinical portions of the manuscript. C.J.M., C.W.K., K.A.D. assisted with writing and editing the manuscript.

Conflict of interest:
M. Gentzsch directs the CFTR Functional Analysis Core of the CF Foundation Research Development Program BOUCHE19R0 and the CF Molecular/Functional Measurement Core of the NIH CFRTCC Program P30DK065988. M. Gentzsch and C.M.P. Ribeiro are recipients of a Boost Award from the School of Medicine at the University of North Carolina and have obtained support for registration (NACFC) or travel (ECFS Conference) to conferences. C.W. Kam reports grants from the CFF, Friends Fighting Cystic Fibrosis, and the American Society of Health-System Pharmacists Foundation. C.J. McKinzie received funding from the CFF and consulting fees from Vertex Pharmaceuticals, Inc. K.A. Despotes received funding from the CFF. C.R. Esther directs a National Resource Center Core that is funded by the CFF and obtained consulting fees from the CFF. B. Baker, D.M. Cholon, S.E. Boyles, and N.L. Quinney report no conflict of interest.

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**Figure:**

**Figure 1. SMM-induced inflammation increases the activity of N1303K CFTR and enhances its rescue by ETI.** N1303K/394delTT HBE cultures were treated with elexacaftor, tezacaftor and ivacaftor/VX-770 (ETI; 3, 10, and 5 µM, respectively) for 24 h in presence or absence of mucosal SMM to inflame the cultures. SMM was prepared as previously described [11-13]. I_sc was measured in Ussing chambers to examine CFTR function. **A)** Representative Ussing chamber traces in response to various agonists and antagonists. **B)** Representative Ussing chamber traces shown in A at a different scale to better visualize CFTR responses. **C)** Quantification of maximal CFTR responses (10 µM Forskolin + 1 µM VX-770; n=4). **D)** Quantification CFTR inhibition by CFTR(inh)-172. **E)** For comparison, CFTR inhibition by CFTR(inh)-172 in F508del/F508del HBE is shown. **F)** Quantification of ENaC responses (n=4). **G)** Quantification of UTP-activated CaCC responses. **C-G:** Mean ± SEM. * P ≤0.05.
References:


elexacaftor+tezacaftor+ivacaftor mediated in part by the dual activities of elexacaftor as both corrector and potentiator. *Eur Respir J* 2021: 57(6).


Figure 1