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

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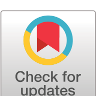
To the Editor:

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 the CFTR potentiator ivacaftor, referred to as ETI, has been approved by the US Food and Drug Administration (FDA) and European agencies [3–5] as highly effective modulator 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 the F508del *CFTR* mutation [1, 2, 6]. 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 [7]. 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) [8] or rectal organoids [9], and modest and varying CFTR responses were observed in ETI-treated N1303K nasal epithelial cells [10, 11]. 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 [12]. 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) [13].

Inflammation is commonly found in the airways of pwCF. Using supernatant of mucopurulent material (SMM), a pro-inflammatory material derived from cystic fibrosis lungs that includes bacterial products, neutrophil factors, cytokines, purines, mucins and peptides [14], we have shown that inflammation enhances the efficacy of CFTR modulator-promoted folding and activity of F508del CFTR in HBE [15–17]. These findings have been subsequently supported by a study by other investigators [18] 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 [19], 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 (figure 1). SMM exposure increased N1303K activity (figure 1a and b), based on quantification of maximal CFTR responses (figure 1c) and CFTR inhibition by CFTR inhibitor 172 (figure 1d). Notably, SMM-enhanced rescue of N1303K CFTR by ETI (figure 1a–d) is similar to that reported in F508del/F508del HBE cultures [15–17] (figure 1e). Epithelial sodium channel (ENaC) function was reduced by ETI treatment (figure 1f) although ENaC currents were not affected by SMM (figure 1f). Therefore, the change in ENaC function likely reflects the inhibitory effect of the rescued CFTR on ENaC activity [20]. Moreover, UTP-induced calcium-activated chloride channel (CaCC) activity, which can be reduced by the ivacaftor in ETI [21], was drastically enhanced by SMM (figure 1g), reinforcing the view that CaCCs can serve as an alternative pathway for Cl^- secretion that may further support airway hydration.



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Rescue of N1303K *CFTR* by highly effective modulator therapy (HEMT) is enabled by CF airway inflammation. These findings suggest that evaluation of HEMT for rare *CFTR* mutations must be performed under inflammatory conditions relevant to CF airways. https://bit.ly/3tTcoJE

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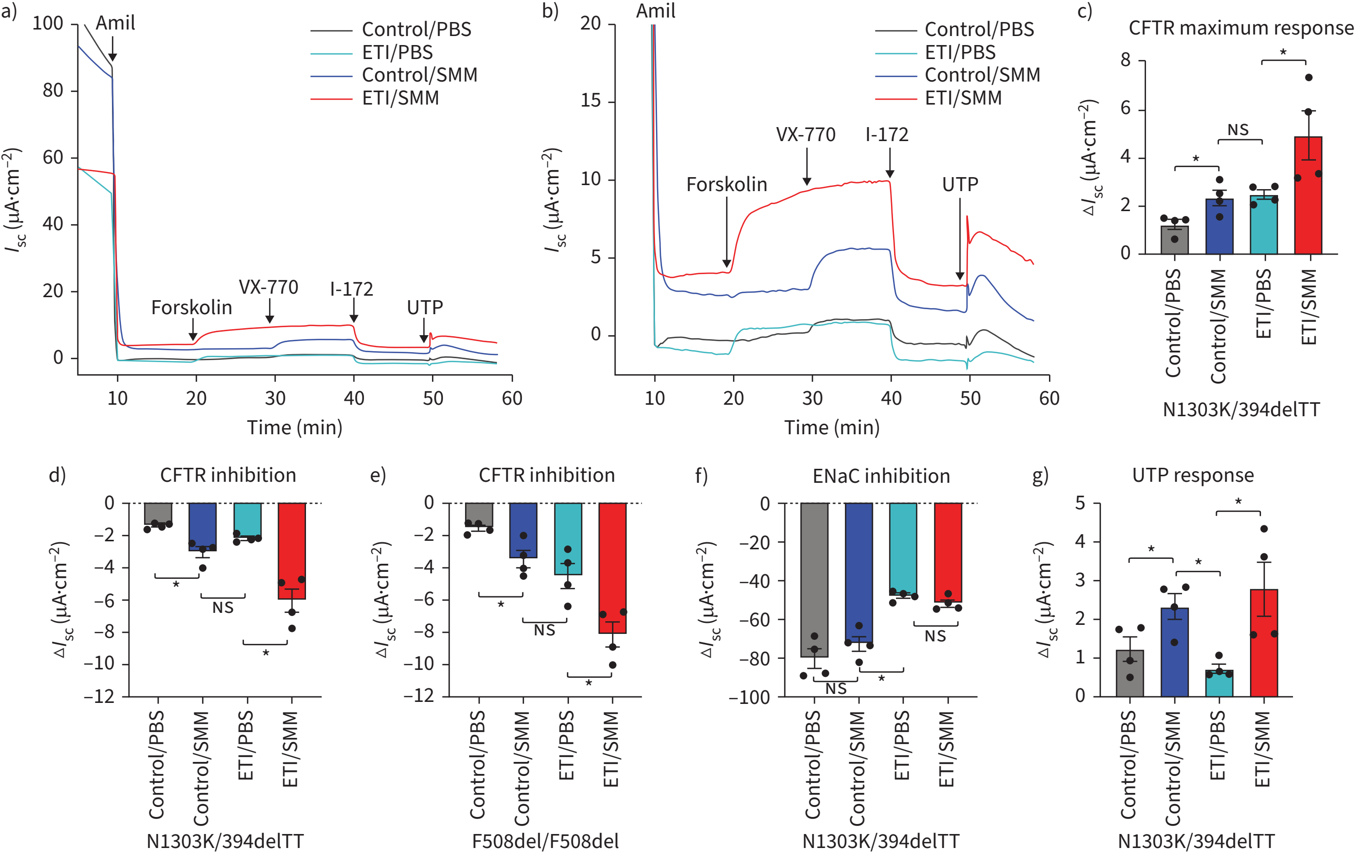

FIGURE 1 Supernatant of mucopurulent material (SMM)-induced inflammation increases the activity of N1303K cystic fibrosis transmembrane conductance regulator (CFTR) and enhances its rescue by ETI (elexacaftor/tezacaftor/ivacaftor). N1303K/394delTT human bronchial epithelia (HBE) cultures were treated with elexacaftor, tezacaftor and ivacaftor (VX-770) (3, 10 and 5 μM, respectively) for 24 h in the presence or absence of mucosal SMM to inflame the cultures. SMM was prepared as previously described [15–17]. Short-circuit current (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, the same as shown in a, at a different scale to better visualise CFTR responses. c) Quantification of maximal CFTR responses (10 μM forskolin + 1 μM VX-770; n=4). d) Quantification of CFTR inhibition by CFTR inhibitor 172 (I-172). e) For comparison, CFTR inhibition by I-172 in F508del/F508del HBE is shown. f) Quantification of epithelial sodium channel (ENaC) responses (n=4). g) Quantification of UTP-activated calcium-activated chloride channel responses. c–g) Data are presented as mean±SEM. *: p⩽0.05; NS: not significant.

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 the translational relevance of this, we examined the outcomes of two paediatric 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 1-year course of sulfamethoxazole/trimethoprim for *Nocardia wallacei* infection that had decreased his lung function from baseline 100% to 73% predicted forced expiratory volume in 1 s (FEV_1), though with treatment his lung function improved to 94% predicted FEV_1 prior to starting ETI. Due to the presence of cystic fibrosis-associated liver disease, a modified ETI dose of one orange tablet (elexacaftor 100/tezacaftor 50/ivacaftor 75 mg) once daily was started. On a follow-up visit 2 months after ETI initiation, his FEV_1 had increased to 99% predicted, and his 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. Results of repeat sweat Cl^- testing performed after 6 months on ETI were 95 and 99 mmol·L^{-1}, compared to baseline sweat Cl^- of 105 and 103 mmol·L^{-1} at 2 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 initiation. In the 2 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% predicted FEV_1. ETI was initiated 2 days prior to discharge from an admission for intravenous antibiotics and inhaled tobramycin for newly acquired *Pseudomonas* infection, during which her lung function improved (56% predicted FEV_1 on admission to 80% upon discharge). 1 month post-ETI, the patient reported no cough or other respiratory symptoms, with decreased sputum production. Her FEV_1 improved to 117% predicted, and her BMI increased from the 38th to the 62nd centile (weight gain of 2.3 kg). Repeat sweat Cl^- testing 4 months after initiation of ETI was 97 and 94 $mmol \cdot L^{-1}$, compared to prior sweat Cl^- values of 83 and 75 $mmol \cdot L^{-1}$ obtained at 3 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 amino-terminal domains; endoplasmic reticulum (ER)-associated degradation-resistant pools of N1303K are concentrated in the ER tubules that associate with autophagy initiation sites [8]. N1303K processing is affected by chaperone proteins (*e.g.* transmembrane 40-kDa heat shock protein (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 [22, 23], 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 cystic fibrosis 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 cystic fibrosis airway. Furthermore, different cell culture media have been shown to induce different levels of basal inflammation in HBE, which could account for laboratory-to-laboratory variability in assessments of N1303K response to modulators [24]. Second, the findings raise concerns about the utility of sweat Cl^- as a clinical marker of efficacy. Sweat Cl^- glands are not inflamed and responses to modulators may be more limited than in the cystic fibrosis airway or gut [25]. The role of inflammation may also help explain why changes in sweat Cl^- in individual pwCF are poorly predictive of clinical responses despite robust associations between sweat Cl^- responses and clinical efficacy on the population level [26]. The significant clinical responses despite minimal to no change in sweat Cl^- in our study suggest that lack of change in sweat Cl^- 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 cystic fibrosis 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. Gentzsch and C.M.P. Ribeiro oversaw *in vitro* experimental design and interpretations of data, and wrote the *in vitro* portions of the manuscript. C.R. Esther Jr designed and coordinated the clinical studies, and wrote the clinical portions of the manuscript. M. Gentzsch plotted graphs and performed statistical analyses. C.M.P. Ribeiro generated and provided pooled SMM. S.E. Boyles performed tissue culture of cystic fibrosis human bronchial epithelial cells, D.M. Cholon coordinated and conducted treatments of cultures with SMM and modulators, and assisted with writing and editing the manuscript. N.L. Quinney performed Ussing chamber analyses. C.J. McKinzie, B. Baker and C.W. Kam helped with conceptualisation of the case series at the time. B. Baker wrote the majority of the clinical portions of the manuscript. C.J. McKinzie, C.W. Kam and K.A. Despotes assisted with writing and editing the manuscript.

Conflict of interest: M. Gentzsch directs the CFTR Functional Analysis Core of the CF Foundation (CFF) 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 Jr 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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References

1 Middleton PG, Mall MA, Drevinek P, *et al.* Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single Phe508del allele. *N Engl J Med* 2019; 381: 1809–1819.
2 Heijerman HGM, McKone EF, Downey DG, *et al.* Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019; 394: 1940–1948.
3 US Food and Drug Administration. FDA approves new breakthrough therapy for cystic fibrosis. https://www.fda.gov/news-events/press-announcements/fda-approves-new-breakthrough-therapy-cystic-fibrosis. Date last updated: 21 October 2019.
4 European Medicines Agency. Kaftrio. https://www.ema.europa.eu/en/medicines/human/EPAR/kaftrio. Date last updated: 26 July 2023.
5 European Commission. Kaftrio. https://ec.europa.eu/health/documents/community-register/html/h1468.htm. Date last updated: 4 September 2023.
6 Zemanick ET, Taylor-Cousar JL, Davies J, *et al.* A phase 3 open-label study of elexacaftor/tezacaftor/ivacaftor in children 6 through 11 years of age with cystic fibrosis and at least one F508del allele. *Am J Respir Crit Care Med* 2021; 203: 1522–1532.
7 Cystic Fibrosis Foundation. Patient registry. https://www.cff.org/medical-professionals/patient-registry.
8 He L, Kennedy AS, Houck S, *et al.* DNAJB12 and Hsp70 triage arrested intermediates of N1303K-CFTR for endoplasmic reticulum-associated autophagy. *Mol Biol Cell* 2021; 32: 538–553.
9 Dekkers JF, Gogorza Gondra RA, Kruisselbrink E, *et al.* Optimal correction of distinct CFTR folding mutants in rectal cystic fibrosis organoids. *Eur Respir J* 2016; 48: 451–458.
10 Laselva O, Bartlett C, Gunawardena TNA, *et al.* Rescue of multiple class II CFTR mutations by elexacaftor +tezacaftor+ivacaftor mediated in part by the dual activities of elexacaftor as both corrector and potentiator. *Eur Respir J* 2021; 57: 2002774.
11 Veit G, Roldan A, Hancock MA, *et al.* Allosteric folding correction of F508del and rare CFTR mutants by elexacaftor-tezacaftor-ivacaftor (Trikafta) combination. *JCI Insight* 2020; 5: e139983.
12 Ensinck MM, De Keersmaecker L, Ramalho AS, *et al.* Novel CFTR modulator combinations maximise rescue of G85E and N1303K in rectal organoids. *ERJ Open Res* 2022; 8: 00716-2021.

13 Huang Y, Paul G, Lee J, *et al.* Elexacaftor/tezacaftor/ivacaftor improved clinical outcomes in a patient with N1303K-CFTR based on *in vitro* experimental evidence. *Am J Respir Crit Care Med* 2021; 204: 1231–1235.

14 Abdullah LH, Coakley R, Webster MJ, *et al.* Mucin production and hydration responses to mucopurulent materials in normal *versus* cystic fibrosis airway epithelia. *Am J Respir Crit Care Med* 2018; 197: 481–491.

15 Ribeiro CMP, Gentzsch M. Impact of airway inflammation on the efficacy of CFTR modulators. *Cells* 2021; 10: 3260.

16 Gentzsch M, Cholon DM, Quinney NL, *et al.* Airway epithelial inflammation *in vitro* augments the rescue of mutant CFTR by current CFTR modulator therapies. *Front Pharmacol* 2021: 12: 628722.

17 Gentzsch M, Cholon DM, Quinney NL, *et al.* The cystic fibrosis airway milieu enhances rescue of F508del in a pre-clinical model. *Eur Respir J* 2018; 52: 1801133.

18 Rehman T, Karp PH, Tan P, *et al.* Inflammatory cytokines TNF-alpha and IL-17 enhance the efficacy of cystic fibrosis transmembrane conductance regulator modulators. *J Clin Invest* 2021; 131: e150398.

19 Kahre T, Teder M, Panov M, *et al.* Severe CF manifestation with anaemia and failure to thrive in a 394delTT homozygous patient. *J Cyst Fibros* 2004; 3: 58–60.

20 Gentzsch M, Dang H, Dang Y, *et al.* The cystic fibrosis transmembrane conductance regulator impedes proteolytic stimulation of the epithelial Na^+ channel. *J Biol Chem* 2010; 285: 32227–32232.

21 Cholon DM, Quinney NL, Fulcher ML, *et al.* Potentiator ivacaftor abrogates pharmacological correction of DeltaF508 CFTR in cystic fibrosis. *Sci Transl Med* 2014: 6: 246ra96.

22 Ribeiro CMP, Hull-Ryde EA. Functional role of the ER stress transducer IRE1α in CF airway epithelial inflammation. *Curr Opin Pharmacol* 2022; 65: 102258.

23 Ribeiro CM, Lubamba BA. Role of IRE1α/XBP-1 in cystic fibrosis airway inflammation. *Int J Mol Sci* 2017; 18: 118.

24 Lee RE, Reidel B, Nelson MR, *et al.* Air-liquid interface cultures to model drug delivery through the mucociliary epithelial barrier. *Adv Drug Deliv Rev* 2023; 198: 114866.

25 Quinton PM. Cystic fibrosis: lessons from the sweat gland. *Physiology* 2007; 22: 212–225.

26 Sermet-Gaudelus I, Nguyen-Khoa T, Hatton A, *et al.* Sweat chloride testing and nasal potential difference (NPD) are primary outcome parameters in treatment with cystic fibrosis transmembrane conductance regulator (CFTR) modulators. *J Pers Med* 2021; 11: 729.