Differential BK channel potentiation by vanzacaftor enantiomers enables therapy for modulator-ineligible people with cystic fibrosis

Nathalie Baumlin[1], Sumedha Gunewardena[2], Scott H. Randell[3], Frank Horrigan[4], and Matthias Salathe[1]

[1]Internal Medicine and [2]Cell Biology and Physiology, KUMC.

[3]Cell Biology and Physiology, UNC Chapel Hill.

[4]Integrative Physiology, Baylor College of Medicine.

Correspondence: Matthias Salathe, KUMC, 3901 Rainbow Blvd., 2025 Murphy, Kansas City, KS 66160, USA. 913-588-6000, msalathe@kumc.edu.

Authorship Assignment: Intellectual contributions.

COI: Some authors filed related intellectual property.

Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have significantly improved outcomes for people with cystic fibrosis (pwCF), yet those with unresponsive mutations remain without effective treatments. One promising strategy involves enhancing ion channels alternative to CFTR (reviewed in 1), including apically expressed large conductance, calcium-activated potassium (BK) channels. BK channels, composed of the pore-forming subunit KCNMA1 (Slo1) and the regulatory subunit LRRC26 (γ1), are critical for airway surface hydration and mucociliary transport (MCT). Inhibition or knockdown of these components leads to reduced airway surface liquid, increased mucus concentration, and impaired MCT in CF models *in vitro* and *in vivo* (2, 3). BK plays a crucial role in enhancing chloride exit, a phenomenon called apical loop current. Our simulations indicated that small apical increases in potassium conductance (from 0 to 0.002 S/cm^2) enhance Cl^- efflux ~3-fold. Reanalyzing airway single cell RNA sequencing data from (4), we show KCNMA1, LRRC26, and ANO1 expression in secretory cells (Fig. 1A). Among BK β-subunits, only KCNMB2/4 are detected, with KCNMB2 in ciliated cells and KCNMB4 in large airways (Fig. 1A), supporting the hypothesis that, in peripheral airways where CF disease starts, apical loop currents between ANO1 and BK, made of KCMNA1/LRRC26, can be enhanced therapeutically.

Our study investigates the restoration of mucociliary function in CF airway epithelia by pharmacologically activating BK channels. Beyond acute BK activation shown by others (5), we found that the CFTR modulator elexacaftor, at concentrations reached in pwCF, potentiates BK activity in normal and CF bronchial epithelial cells ~2-fold (Fig. 1B). It is unclear if this is clinically meaningful as pwCF with minimal function variants treated with elexacaftor/tezacaftor/ivacaftor showed on average only a marginal, clinically not meaningful FEV1 increase (6) and as expected no sweat chloride level changes.

Others (5) described that vanzacaftor, a new CFTR modulator, acutely activates BK. We therefore explored the effects of vanzacaftor, especially its enantiomers. Both S- and R-vanzacaftor activate BK channels, but with distinct temporal profiles.

S-vanzacaftor increases basal BK activity in *Xenopus* oocytes expressing BK channels (hSlo1±LRRC26) >1000-fold over control (Fig. 1E and S2; at -80 mV with 0 Ca^{2+}) comparable to saturating Ca^{2+}, with detectable activity at concentrations as low as 50 nM (Fig. S1). Similarly, R-vanzacaftor increases BK activity acutely by 455-fold at 5 μM, much greater than 10 μM elexacaftor (3.17±0.28-fold, Fig. S1). In CF airway epithelia, S-vanzacaftor acutely activates BK (Fig. 1F/G) and modulates F508del-CFTR (Fig. 1C) yet fails to potentiate BK long-term (Fig. 1D, 24h). In contrast, R-vanzacaftor does not modulate CFTR but activates BK and, more importantly, potentiates BK activity long-term (Fig. 1H), thereby improving MCT in CF cells (Figs. 1I and S3C). These improvements were related to BK as KCNMA1 or LRRC26 knockdowns blunt currents and physiological responses (Fig. 1J-L).

The differential effects of enantiomers raise important mechanistic questions. That both activate BK in excised patches with concentration-response curves well fit by Hill coefficients of 2 but not 1 (Fig. S2E), suggest similar mechanisms of acute action involving direct cooperative channel binding with modest stereoselective efficacy. However, the lack of sustained potentiation by S-vanzacaftor reveals additional differences, potentially involving mechanisms such as desensitization or stereoselective metabolism, that will require additional experiments to resolve. Thus, our data uniquely highlight enantiomer-specific effects of vanzacaftor with long-term therapeutic implications.

In summary, R-vanzacaftor emerges as a promising candidate for pwCF with minimal function mutations, offering a CFTR-independent mechanism to restore mucociliary clearance. Further studies are warranted to elucidate the molecular basis of its sustained BK potentiation and to evaluate clinical efficacy, likely via an inhaled drug delivery route.

References

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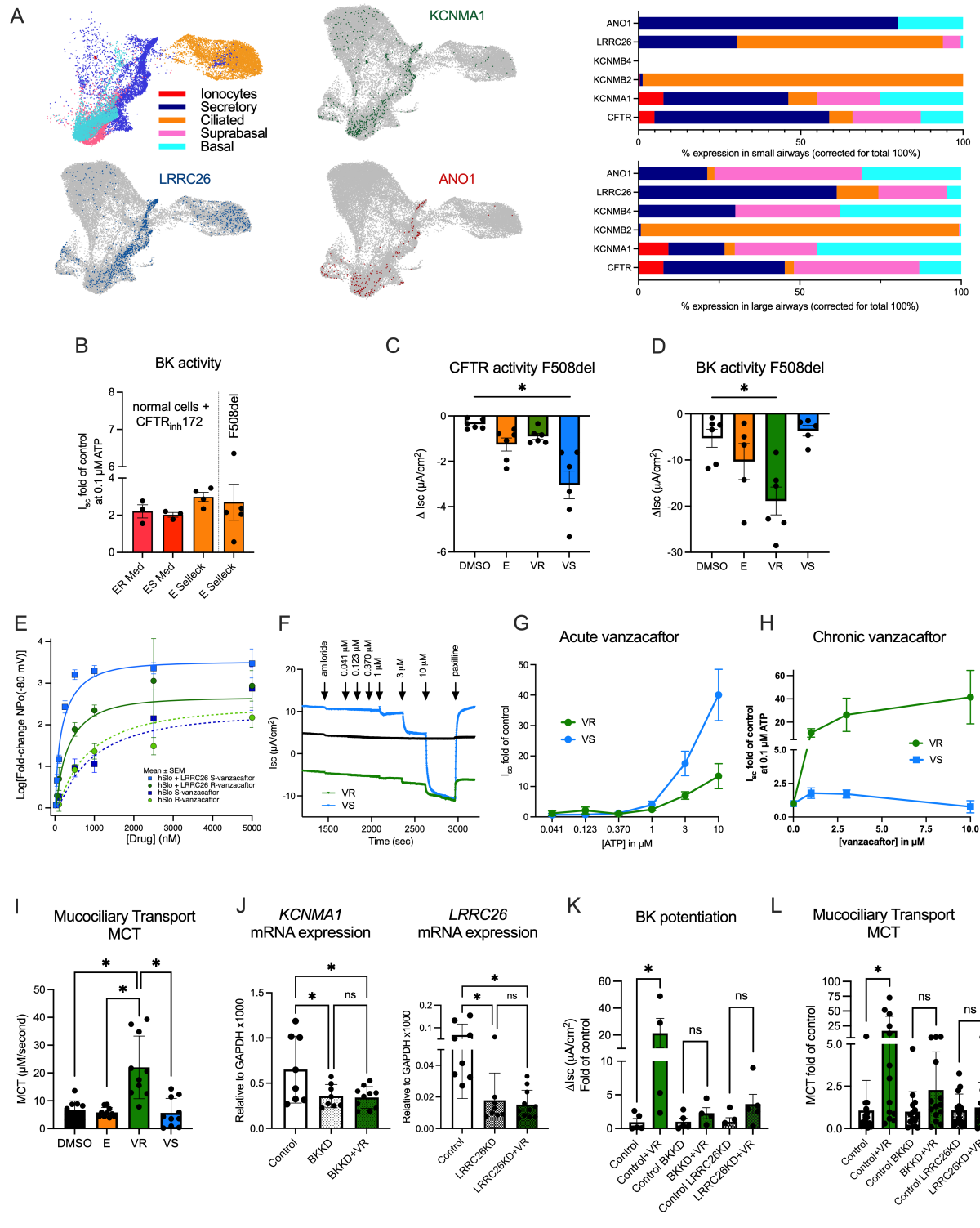
Figure 1 Legend:

(**A**) Data re-analyzed from (4). Left: UMAP projections of major cell types. Right: Ion channel expression in large/small airways. (**B**) Fully differentiated, primary normal human bronchial epithelial cells exposed basolaterally to 5 μM elexacaftor (24h). To assess BK activity, cells were permeabilized basolaterally in Ussing chambers under a basolateral to apical potassium gradient (3). To increase intracellular calcium, cells were stimulated with 0.1 μM ATP in the acute presence of 10 μM $CFTR_{inh}172$ mimicking minimal function CFTR variants (CFTRMF). Short circuit currents represent basolateral to apical potassium flux. F508delCFBE cells were used without $CFTR_{inh}172$. Differently sourced elexacaftor potentiated BK ~2-fold. (**C/D**) F508delCFBE cells exposed basolaterally to 5 μM elexacaftor and 5 μM R- or S-vanzacaftor for 24h. (**C**) S-vanzacaftor modulates CFTR (***$p<0.05$ Friedman; short circuit current inhibition by $CFTR_{inh}172$ after forskolin stimulation). (**D**) R-vanzacaftor potentiates BK (*$p<0.05$ Kruskal-Wallis). (**E**) Oocytes; Log or fold-increase in BK (hSlo1±LRRC26) activity (NP_O at -80 mV, 0 Ca) over vehicle (mean±SEM, n=13-25 patches per condition), fit with Hill equations (see Fig. S2). (**F/G**) Acute exposures of CFTRMF cells to vanzacaftor enantiomers after basolateral permeabilization with basolateral to apical potassium gradient (Ussing traces in **F** and summary data in **G**). S-vanzacaftor is the most efficacious BK activator (n=4). (**H**) CFTRMF cells: 24h basolateral exposures before testing BK potentiation with ATP (see above) show that R-vanzacaftor is the most efficacious BK potentiator (n=4). (**I**) Only R-vanzacaftor (3 μM basolaterally for 24h) improves mucociliary transport (MCT) in CFTRMF cells (****$p<0.0001$ ANOVA and Tukey after passing normality test, n=10). (**J-L**) CFTRMF cells with KCNMA1 or LRRC26 knockdown (BKKD, LRRC26KD). (**J**) *KCNMA1* (left) and *LRRC26* (right) mRNA expression (n=8-11). One-way ANOVA / Tukey (left) or Kruskal Wallis (right). (**K**) BK potentiation by 3 μM R-vanzacaftor (24h basolaterally) is eliminated by KCNMA1 and LRRC26 KD. (**L**) R-vanzacaftor's effect on MCT (treatment/DMSO) in CFTRMF (3 μM for 24h; n=15). Baselines: 2.9±1.3 μm/s. *<0.05 by Mann-Whitney.

Figure 1


A
Ionocytes
Secretory
Ciliated
Suprabasal
Basal
KCNMA1
LRRC26
ANO1
ANO1
LRRC26
KCNMB4
KCNMB2
KCNMA1
CFTR
% expression in small airways (corrected for total 100%)
% expression in large airways (corrected for total 100%)
B
BK activity
normal cells + CFTRinh172
F508del
Isc fold of control at 0.1 µM ATP
ER Med
ES Med
E Selleck
E Selleck
C
CFTR activity F508del
Δ Isc (µA/cm²)
DMSO
E
VR
VS
D
BK activity F508del
ΔIsc (µA/cm²)
E
Log[Fold-change NPo(-80 mV)]
[Drug] (nM)
Mean ± SEM
hSlo + LRRC26 S-vanzacaftor
hSlo + LRRC26 R-vanzacaftor
hSlo S-vanzacaftor
hSlo R-vanzacaftor
F
amiloride
0.041 µM
0.123 µM
0.370 µM
1 µM
3 µM
10 µM
paxilline
Isc (µA/cm²)
VR
VS
Time (sec)
G
Acute vanzacaftor
Isc fold of control
VR
VS
[ATP] in µM
H
Chronic vanzacaftor
Isc fold of control at 0.1 µM ATP
VR
VS
[vanzacaftor] in µM
I
Mucociliary Transport MCT
MCT (µM/second)
DMSO
E
VR
VS
J
KCNMA1 mRNA expression
Relative to GAPDH x1000
Control
BKKD
BKKD+VR
LRRC26 mRNA expression
Relative to GAPDH x1000
Control
LRRC26KD
LRRC26KD+VR
K
BK potentiation
ΔIsc (µA/cm²) Fold of control
Control
Control+VR
Control BKKD
BKKD+VR
Control LRRC26KD
LRRC26KD+VR
L
Mucociliary Transport MCT
MCT fold of control
Control
Control+VR
Control BKKD
BKKD+VR
Control LRRC26KD
LRRC26KD+VR