TAVT-135, a novel chloride ion transporter for the pan-genotypic treatment of cystic fibrosis: electrophysiological and mucus-hydration properties

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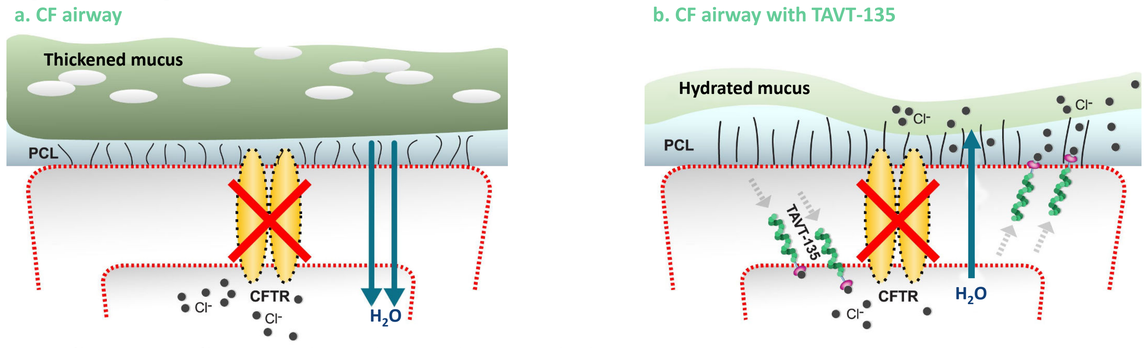
Background and objectives

- The treatment of cystic fibrosis (CF) has been transformed by the introduction of modulators of the CF transmembrane conductance regulator (CFTR)[1,2]
- Approximately 10% of patients have ineligible genotypes,[3] while others may experience inadequate response or intolerance to CFTR modulators[4]
- TAVT-135, a novel small molecule-peptide conjugate composed of a chloride ion-binding moiety and a cell-penetrating peptide (CPP; **Figure 1**), is being investigated as a potential treatment for CF, regardless of CFTR mutational status
- We performed a series of *in vitro* studies to characterize the electrophysiological and mucus-hydration properties of TAVT-135

Figure 1. Working model of TAVT-135 mechanism of action



CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; PCL, periciliary layer
Cl^- ions depicted as black circles and bacterial colonization depicted as gray ovals. Blue arrows indicate the overall direction of water movement

Methods

Intracellular to extracellular chloride ion transport

- The effects of TAVT-135 and its separate functional components on intra- to extracellular chloride ion transport were evaluated in *Xenopus laevis* oocytes using a two-electrode voltage-clamp technique in the presence of TAVT-135 (10 µM) or negative controls – the CPP alone (10 µM) and the chloride-binding moiety alone (10 µM)

Electrophysiological correlates of chloride ions

- Anion efflux was evaluated using a modified Ussing chamber system with human bronchial epithelial (HBE) cells harboring mutations for non-functional CFTR (non-functional genotype with W1282X/R1162X mutation)
- Following amiloride-induced inhibition of the epithelial sodium channel, the impact of TAVT-135 exposure (0.01–50 µM) on short-circuit current (I_{SC}) and transepithelial electrical resistance (TEER) was determined

Mucus hydration

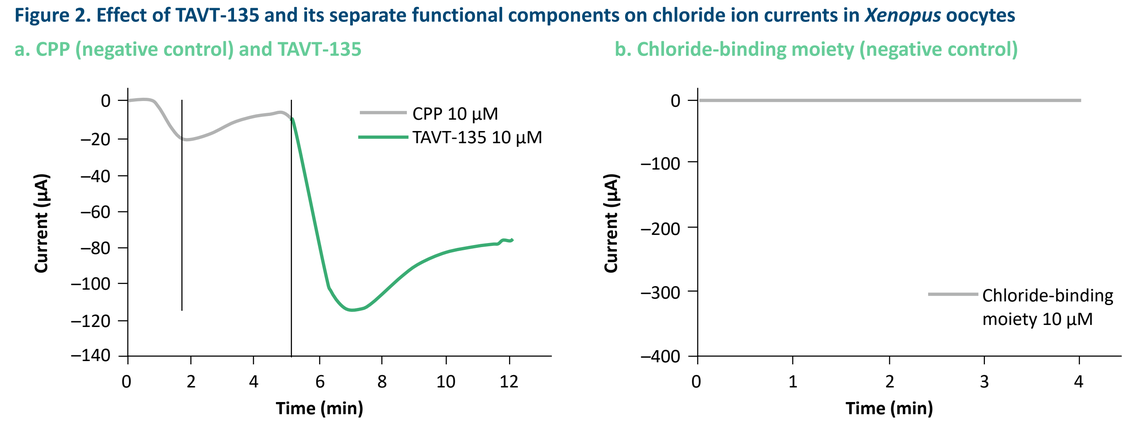
- Mucus hydration in HBE cells with CFTR mutations (homozygous for ΔF508) was assessed by measuring the height of the airway surface liquid (ASL) and periciliary layer (PCL)
- Following chronic exposure to TAVT-135 (1, 10, and 100 µM) for 48 hours, ASL and PCL were visualized with apical application of FITC-dextran
- Heights (µm) were determined using Z-stack images from confocal microscopy

Results

Intracellular to extracellular chloride ion transport

- In *X. laevis* oocytes, TAVT-135 induced rapid chloride ion efflux, demonstrating chloride ion transport from the intracellular to the extracellular space (**Figure 2a**)
- In comparison, CPP alone had minimal activity on chloride current **(Figure 2a**), and the unconjugated chloride-binding component did not have any detectable effect on chloride current (**Figure 2b**)

Figure 2. Effect of TAVT-135 and its separate functional components on chloride ion currents in *Xenopus* oocytes

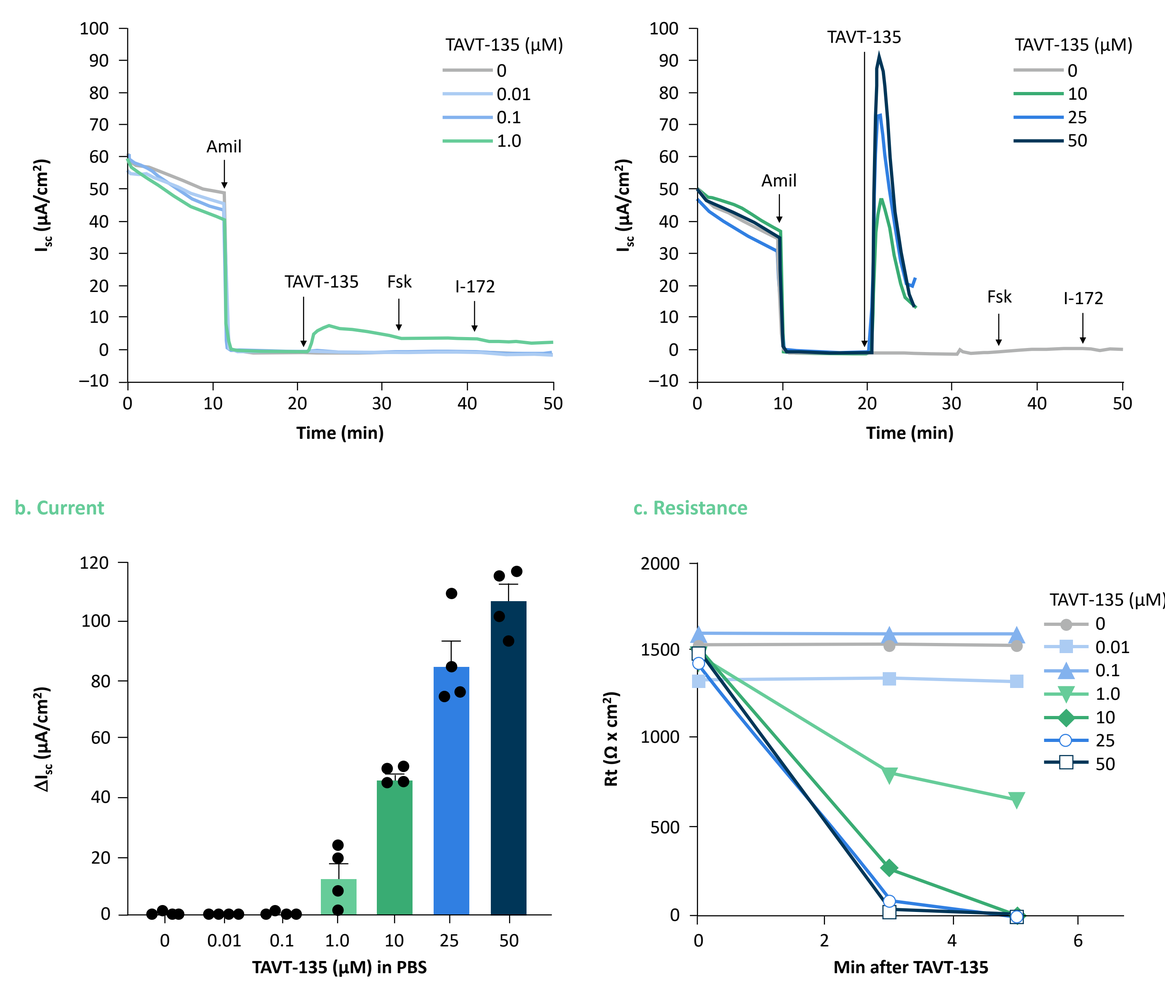


CPP, cell-penetrating peptide

Electrophysiological correlates of chloride ions

- In HBE cells, there was a significant, dose-dependent increase in I_{SC} following acute application of TAVT-135 ≥1 µM, demonstrating anion efflux (**Figures 3a, b**)
- Within 5 min of acute exposure, TEER was maintained at TAVT-135 concentrations ≤1 µM and decreased at concentrations ≥10 µM (**Figure 3c**)

Figure 3. Effect of acute application of TAVT-135 on current and resistance in primary airway cultures

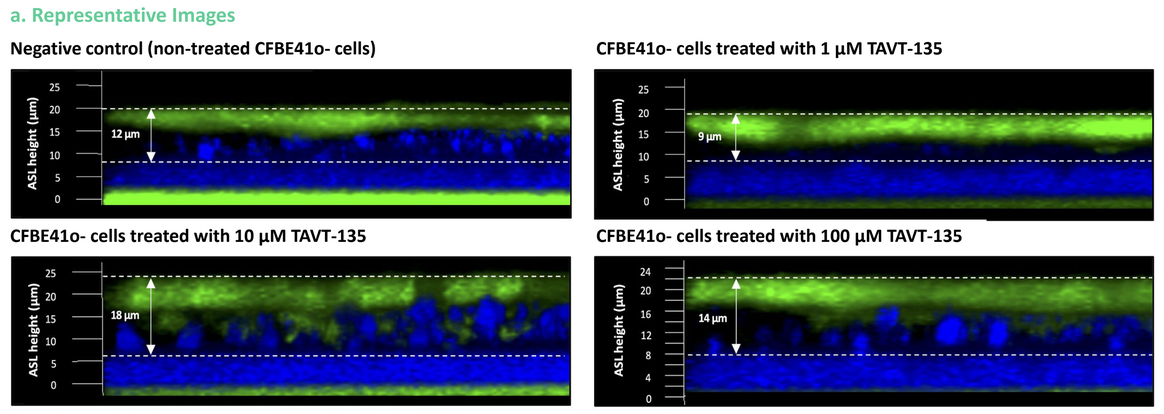


Amil, amiloride; fsk, forskolin; I_{sc}, short-circuit current; PBS, phosphate-buffered saline; Rt, transepithelial resistance
Black dots are individual measurements; bars are mean ± standard error of the mean (SEM)

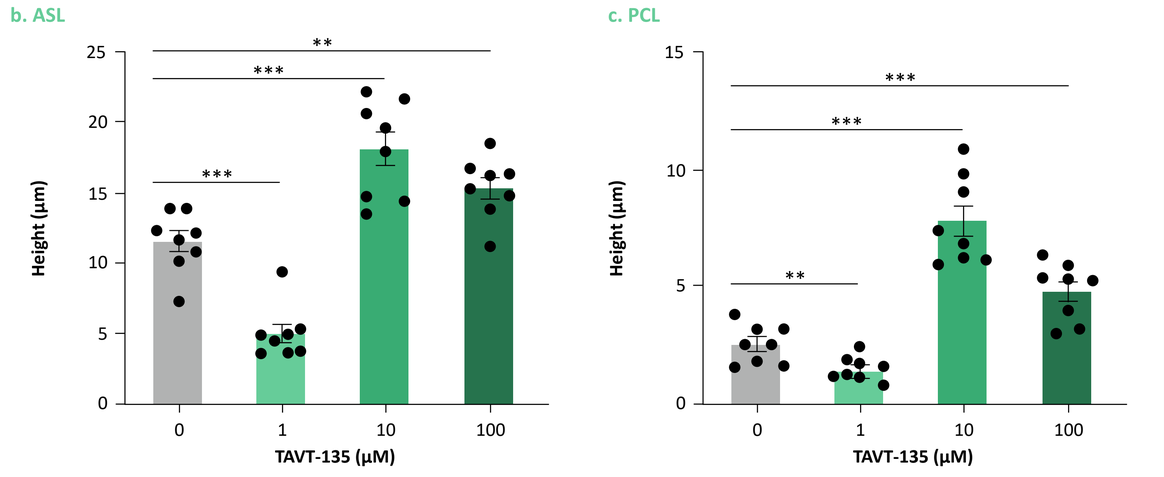
Mucus hydration

- Following 48 hours' incubation of CFTR-mutated HBE cells with TAVT-135 (10 and 100 µM), statistically significant increases in ASL and PCL height were observed in comparison with the' untreated control cells (**Figure 4**)

Figure 4. Effect of TAVT-135 on ASL and PCL height in CFTR-mutated HBE cells



Green: Fluorescein isothiocyanate-dextran (4 kDa) Blue: Hoechst 33342
Objective: 20x



ASL, airway surface liquid; CFTR, cystic fibrosis transmembrane conductance regulator; HBE, human bronchial epithelial; PCL, periciliary layer
**p<0.01; ***p<0.001
Black dots are individual samples; bars are mean ± standard error of the mean (SEM)

Conclusions

- In this series of *in vitro* experiments, TAVT-135 rapidly induced intracellular chloride transport across plasma membranes without negatively impacting the epithelial barrier
- TAVT-135 also increased ASL and PCL height, which may suggest a mucociliary clearance effect *in vivo*
- These data support the potential for TAVT-135 to address significant unmet needs in patients with CF, including those who are ineligible for or do not respond to CFTR modulators
- Additional studies into this novel artificial chloride ion transporter are ongoing

References

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Disclosures
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