In vitro evaluation of TAVT-135, an artificial pan-genotypic chloride ion transporter

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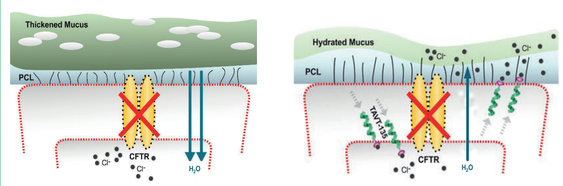
Background

- Cystic fibrosis (CF) transmembrane conductance regulator (CFTR) modulators have revolutionized treatment in patients with F508del or gating mutations[1,2]
- A significant unmet need, however, remains for patients with ineligible genotypes (approximately 10% of the CF population[3]), inadequate response or intolerance[4]
- Alternative agents, including those capable of mediating chloride (Cl^-) transport, are being investigated[5]
- TAVT-135, a novel Cl^- transporter (**Figure 1**), could potentially benefit all patients with CF, independent of CFTR mutation

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



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

Objective

- To characterize the electrophysiological correlates of Cl^- transport and the mucus-penetrating properties of TAVT-135 through a series of *in vitro* studies

Methods

Intra- to extracellular Cl^- transport

- Human embryonic kidney 293 (HEK293) cells were loaded with Cl^--sensitive fluorescent dye MQAE [*N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide[6]] and exposed to TAVT-135 or a negative control
- Fluorescence was measured in the culture medium with fluorescence microscopy

Cell viability

- The impact of TAVT-135 on HEK293 cell viability was assessed using a commercially available apoptosis/necrosis detection kit (blue, green, red; Abcam; cat no.: ab176749)

Mucus penetration

- Mucus harvested from human bronchial epithelial cell cultures was prepared to concentrations mimicking healthy airways and mild and severe CF airway disease, containing 2%, 4%, and 8% solids (w/w), respectively
- Test solutions (2 µL) of fluorescently labeled TAVT-135 in phosphate-buffered saline (PBS) or 80/20 glycerol/water control were added to mucus samples (30 µL) in capillary tubes
- Well intensity was measured every 15 minutes for 24 hours using a Tecan plate reader
- The intensity pattern for each capillary tube was fit using Gaussian distribution. Mucus-penetration times for the diffusion of TAVT-135 through mucus layers (100 µm thickness) were calculated using a simplified version of the Stokes Einstein relationship

Electrophysiological correlates of Cl^- transport in CF airway cells

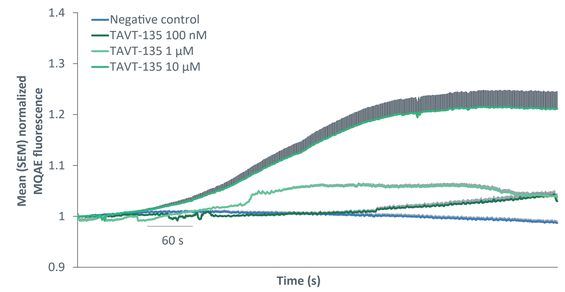
- Ion transport in cultured primary airway cells of a non-functional CF genotype (with W1282X/R1162X mutation) was measured in a modified Ussing chamber system
- TAVT-135 (diluted to 1, 10, and 100 µM with PBS) or PBS control was delivered (acutely and over 24 hours) to the apical side of the cultures
- Transepithelial resistance was measured, and the impact of TAVT-135 exposure on short-circuit current (I_{SC}) was determined using amiloride-induced sodium channel suppression
- Electrophysiological responses were studied in the presence of Cl^- transport agonists (forskolin, uridine triphosphate [UTP]) and an antagonist (CFTR inhibitor-172)
- Treatment differences for TAVT-135 versus controls were evaluated using a two-tailed Student's t-test

Results

Intra- to extracellular Cl^- transport

- In HEK293 cells, TAVT-135 induced dose-dependent Cl^- transport from the cytosol to the extracellular space (**Figure 2**), resulting in decreased intracellular Cl^- levels

Figure 2. MQAE fluorescence in cultured HEK293 cells exposed to TAVT-135

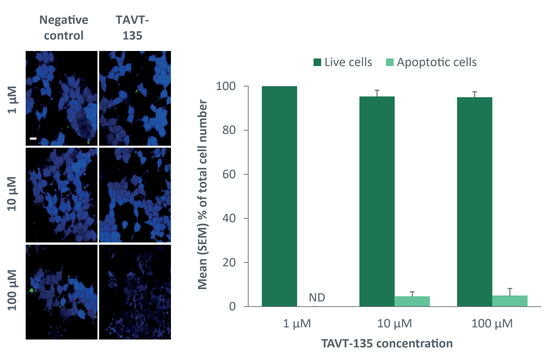


HEK, human embryonic kidney; MQAE, *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide; SEM, standard error of the mean

Cell viability

- At 1 µM, TAVT-135 had no measurable effect on the viability of HEK293 cells (**Figure 3**)
- A limited rate of apoptotic cell death was observed at 10 and 100 µM (**Figure 3**), but no necrotic cell death was detected at any tested concentration of TAVT-135

Figure 3. Effect of TAVT-135 on HEK293 cell viability



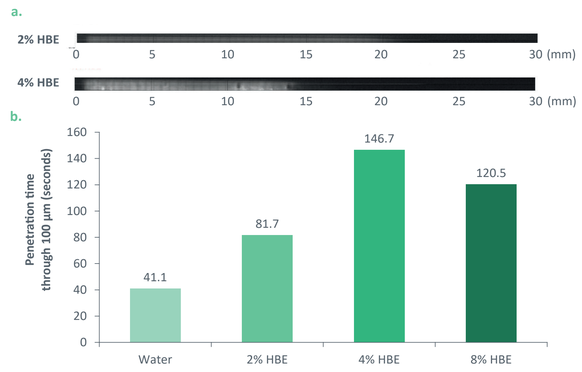
HEK, human embryonic kidney; ND, not detectable; SEM, standard error of the mean

Results (continued)

Mucus penetration

- TAVT-135 readily penetrated all mucus layers representing varying severities of CF to a greater extent in less concentrated mucus, as indicated by the wider smear in the intensity pattern in 2% versus 4% mucus (**Figure 4a**) and the shorter penetration time (**Figure 4b**)

Figure 4. Intensity pattern of TAVT-135 diffusion (a) and mucus penetration times (b)



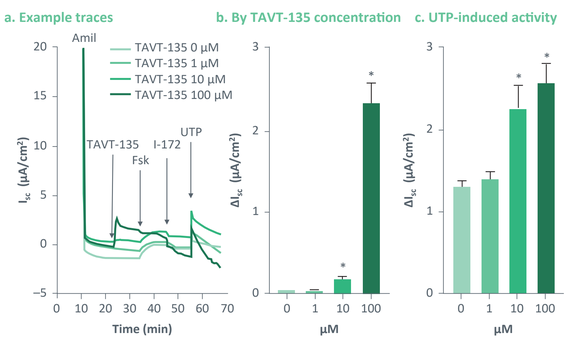
HBE, human bronchial epithelial cell mucus

Electrophysiological correlates of Cl^- transport in W1282X/R1162X CFTR primary airway cultures

Acute exposure

- TAVT-135 (10 and 100 µM) induced significant increases in I_{SC} compared with control (**Figure 5**)
  - The effect on I_{SC} was partially sustained over 10 minutes and was independent of CFTR
- UTP-induced activity of Ca^{2+}-activated Cl^- channels increased 1.7- and 2-fold after exposure to TAVT-135 10 µM (p=0.02) and 100 µM (p=0.003), respectively
- Neither forskolin nor CFTR inhibitor-172 had any significant effect on ion transport due to the absence of CFTR protein in the cultures
- TAVT-135 exposure had no effect on responses to forskolin or CFTR inhibitor-172

Figure 5. Preliminary electrophysiological effects of acute administration of TAVT-135 in W1282X/R1162X CFTR primary airway cultures

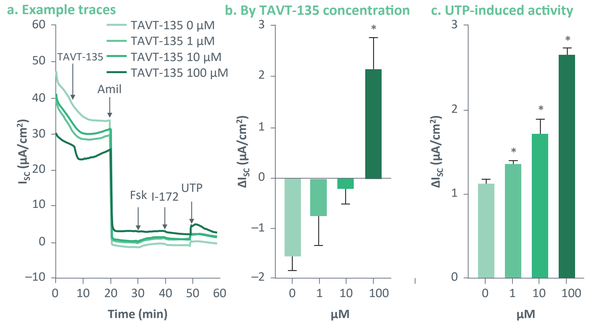


*p<0.05 versus control
Amil, amiloride; CFTR, cystic fibrosis transmembrane conductance regulator; Fsk, forskolin; I-172, CFTR inhibitor-172; I_{SC}, short-circuit current; UTP, uridine triphosphate

Chronic (24-hour) exposure

- The mean potential difference of cultures exposed to TAVT-135 (100 µM) was significantly decreased compared with control (**Figure 6**)
- UTP-induced activity increased 1.2-, 1.5- and 2.3-fold after exposure to TAVT-135 1 µM (p=0.02), 10 µM (p=0.024), and 100 µM (p<0.0001), respectively
- TAVT-135 (100 µM) appeared to reduce the response to forskolin

Figure 6. Electrophysiological effects of chronic administration of TAVT-135 to W1282X/R1162X CFTR primary airway cultures



*p<0.05 versus control
Amil, amiloride; CFTR, cystic fibrosis transmembrane conductance regulator; Fsk, forskolin; I-172, CFTR inhibitor-172; I_{SC}, short-circuit current; UTP, uridine triphosphate

Conclusions

- Initial investigations show that TAVT-135 has a favorable electrophysiological response profile for therapeutic purposes
  - Acutely activating short circuit current while potentially inducing purinergic activation of Ca^{2+}-activated chloride channels in response to UTP and causing basal ion-channel activation in the absence of agonist
- TAVT-135 readily penetrated mucus layers, including those mimicking severe CF
- TAVT-135 had no *in vitro* toxicity, even at high concentrations
- TAVT-135 could potentially alleviate symptoms associated with highly viscous mucus by increasing electrolyte levels of the airway surface liquid layer and facilitating water transport out of epithelial cells
- Additional studies into this novel artificial chloride ion transporter are ongoing

References

1. Despotes KA, Donaldson SH. Curr Opin Pharmacol 2022;65:102239.
2. Regard L et al. Cells 2022;11:1769.
3. Fajac I, Sermet I. Cells 2021;10:2793.
4. Guimbellot J et al. Pediatr Pulmonol 2017;52(Suppl 48):S4–S14.
5. Quesada R, Dutzler R. J Cyst Fibros 2020;19(Suppl 1):S37–S41.
6. West MR, Molloy CR. Anal Biochem 1996;241:51–58.

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Disclosures

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