hCFTRΔR expression and correction of human CF airway epithelia increase with increasing SP-101 MOI and doxorubicin concentration

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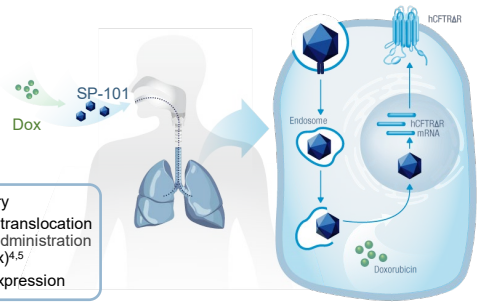
POSTER 672
(Also see Poster 621)

SP-101 – A novel, inhaled AAV-based gene therapy to treat CF

DESIGN FEATURES

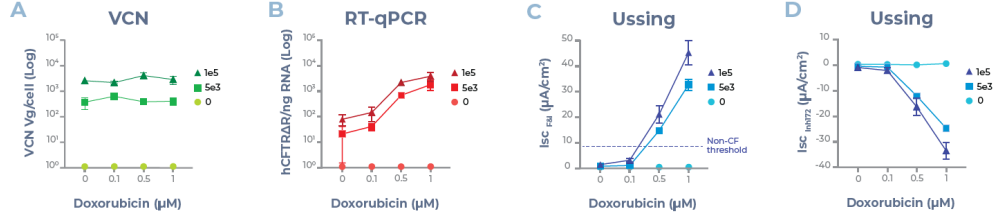
- AAV capsid selected for tropism to the apical surface of human airway epithelia (HAE)[1]
- hCFTRΔR minigene with regulatory elements[2,3]



MECHANISM OF ACTION

- Efficient apical entry
- Enhanced SP-101 translocation to the nucleus by administration of doxorubicin (Dox)[4,5]
- Increased CFTR expression

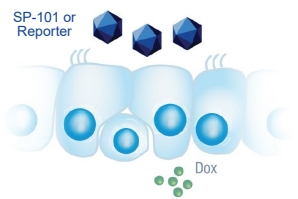
Doxorubicin concentration directly correlates with hCFTRΔR function and mRNA transcripts but not with vector copy number (VCN)



Primary CF HAE (W1282X/R1162X) transduced apically with SP-101 at MOIs of 0, 5e3 and 1e5 Vg/cell, with/without increasing concentrations of Dox at the basal surface. One week post transduction A) VCN, B) *hCFTRΔR* mRNA, C) peak forskolin/IBMX stimulated hCFTRΔR activity (short circuit Cl^- current; $Isc_{F\&I}$), and D) CFTR-specific Inhibitor 172 decrease in Cl^- current were measured. Increasing SP-101 MOI resulted in a dose-dependent increase in VCN, *hCFTRΔR* mRNA, and hCFTRΔR function. Dox concentration correlated with *hCFTRΔR* mRNA and function, but not VCN.

Experimental Methods

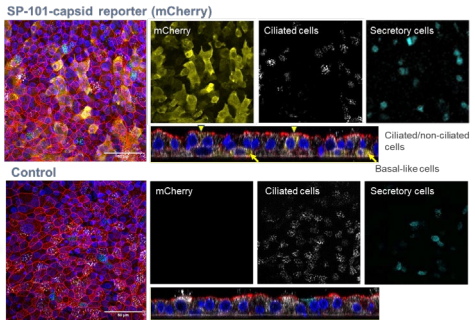
Polarized primary CF HAE



SP-101 or reporter/Dox 16 h incubation, analysis at D7

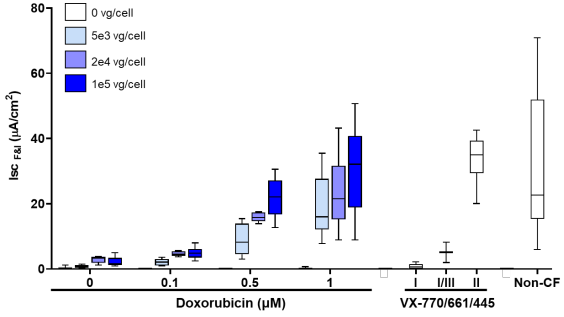
- Cell tropism by 5-color immunostaining/ confocal microscopy with reporter vector
- SP-101 vector copy number per cell by ddPCR
- *hCFTRΔR* mRNA copies per ng RNA by RT-qPCR
- *hCFTRΔR* function by Ussing chamber assay

SP-101 is tropic to human CF airway epithelia (HAE)



SP-101-capsid reporter encoding mCherry transduces many epithelial cell types in CF HAE (F508del/F508del). SP-101-capsid-reporter (mCherry, yellow) showed >30 % positive cells that colocalized with markers for ciliated (α-tubulin, white) or secretory cells (MUC5AC, teal) and to basally-oriented cells.

SP-101 MOI and doxorubicin concentration directly correlates with hCFTRΔR function



Functional correction of epithelia from 5 different CF donors (2 Class I, 1 Class I/III, 2 Class II). CF correction reaching non-CF levels starts with SP-101 MOI as low as 5e3 Vg/cell with 0.5 μM Dox. As expected, no correction with VX-770/661/445 is observed in epithelia with Class I, partial correction if heterozygous for Class I/III, and full correction with Class II mutations. Box (25th – 75th percentile) and whisker (10th – 95th percentile) plots, with the horizontal line representing the median of the values are shown. n = 4-16 epithelia per condition except n=2 for Class I/III Vertex treated epithelia.

SP-101 holds great promise for people living with CF

- Doxorubicin is required for efficient CF correction
- SP-101 is tropic to many human airway epithelial cell types
- VCN is SP-101 MOI but not Dox dose responsive indicating that Dox enhances post-entry steps
- CFTRΔR mRNA expression and CFTR correction are SP-101 MOI and Dox dose responsive
- SP-101-mediated correction is CF mutation agnostic

ACKNOWLEDGEMENTS: Spirovation: Scott Randell, Samuel Gallant, Tony Church; Spirovant: Melane James and Awal Jimah

REFERENCES [1]Excoffon et al., PNAS 2009; [2]Ostedgaard et al., PNAS 2002; [3]Yan et al., Hum Gene Ther. 2015; [4]Yan et al. J Virol. 2004; [5]Zhang et al., Mol Ther. 2004