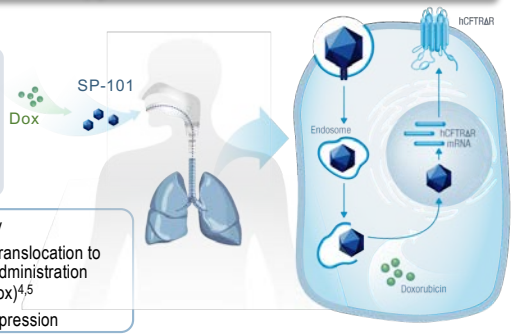
POSTER 584

Delivery of SP-101 restores CFTR function in human CF airway epithelial cultures and drives hCFTRΔR transgene expression in the airways of ferrets

SP-101 – A novel, inhaled 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]

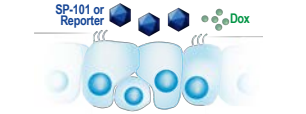


MECHANISM OF ACTION

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

SP-101 is tropic to and corrects human CF airway epithelia

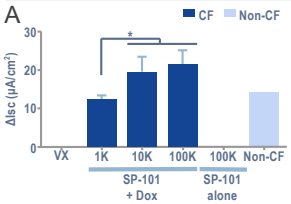
Polarized primary human CF airway epithelia



CFTR Function by Ussing or 5-color immunostain/confocal microscopy

AAV/Dox 16 h incubation, analysis at D7

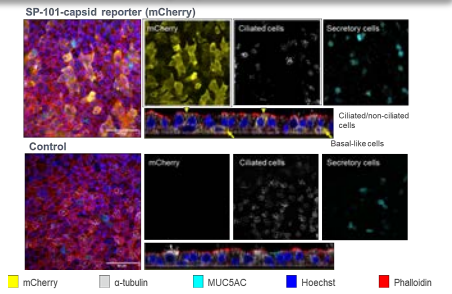
A



*p < 0.05

VX - CFTR triple modulators VX-770/661/445

B

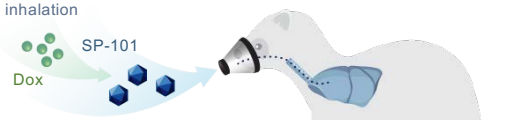


A) Apical SP-101 demonstrates dose-dependent functional correction of primary human CF airway epithelia (HAE). As expected for a donor with class I mutations (W1282X/R1162X), treatment with a combination of VX-770/661/445 did not stimulate currents. MOI 1K, 10K, and 100K of SP-101 + doxorubicin (Dox) significantly increased currents in a dose-dependent manner to levels similar to non-CF HAE. SP-101 transduction without dox was insufficient to produce current.

B) SP-101-capsid reporter encoding mCherry transduces many epithelial cell types in CF HAE (F508del/F508del). SP-101-reporter (yellow stain) showed >30 % positive cells that colocalized with markers for ciliated (α-tubulin, white) or secretory cells (MUC5AC, teal) or did not colocalize with any cell type markers (non-ciliated or basally-oriented cells).

Ferrets as a model to evaluate inhaled SP-101

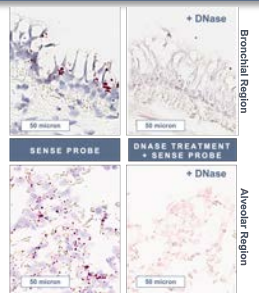
- SP-101 capsid is tropic to ferret airway cells[6]
- CF ferret model recapitulates human CF lung pathology[7]
- Ability to administer via inhalation



Methods: Ferrets were exposed to SP-101 or diluent followed by doxorubicin or diluent on a plenum system connected to a mesh nebulizer. Animals were sacrificed 2 or 12 weeks post-exposure and tissues harvested for *in situ* hybridization (ISH) or copies of hCFTRΔR mRNA. ISH: Sections from formalin-fixed paraffin-embedded lung were evaluated by RNAScope using z probes designed to the sense strand unique regions of the vector genome. hCFTRΔR mRNA copies: RNA was isolated from 25-50 mg samples taken from 9 different regions of the airway (3 from trachea, 4 from bronchial, 2 from alveolar/lobe regions) followed by qPCR +/- reverse transcriptase with primers and a probe for a unique region of the hCFTRΔR mRNA. No signal was observed in the absence of reverse transcriptase indicating the complete removal of vector genomes (data not shown). Data are shown as box and whisker plots around the median value.

Non-CF ferrets

A



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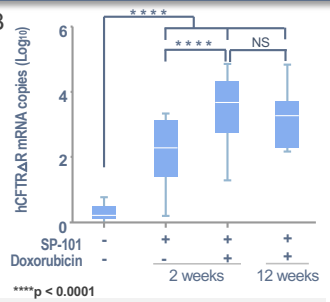


****p < 0.0001

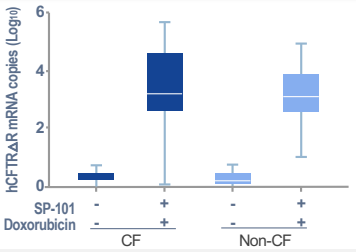
A) SP-101 vector genomes are abundant in many regions of ferret lungs. SP-101 vector genomes (red dots) were detected in multiple cells whereas pretreatment with DNase did not show staining indicating the specificity of staining.

B) Doxorubicin increases hCFTRΔR mRNA expression >10 fold and is durable in ferret lungs. In contrast to control samples, samples from animals exposed to SP-101 alone demonstrated signal in the majority of samples. Signal from animals exposed to the same amount of SP-101 that was subsequently followed by dox were ~17-fold higher demonstrating that the addition of dox results in a significant (p<0.0001) increase in hCFTRΔR mRNA expression that did not significantly decrease (NS) 12 weeks post-administration indicating the importance of dox as well as durability.

SP-101 holds great promise for people living with CF

- SP-101 functionally corrects CF HAE
- Doxorubicin is required for efficient CF correction
- SP-101 is tropic to many human epithelial cell types
- hCFTRΔR expression and CF correction are dose responsive and durable
- hCFTRΔR mRNA expression is similar in CF and wildtype ferrets, suggesting that the CF airway is not an additional barrier to SP-101

CF and non-CF ferrets



A) hCFTRΔR mRNA expression is similar in the lungs of CF and wildtype ferrets. In contrast to control animals administered diluent only, hCFTRΔR mRNA was detectable to a similar extent in both CF (G551D) and non-CF animals suggesting that the CF lung is not an additional barrier to SP-101.

ACKNOWLEDGEMENTS

Lovelace Biomedical: Philip Kuehl, Adam Werts, Bryan Gullick; **Cystic Fibrosis Foundation:** Martin Mense, Yi Cheng, Kevin Coote, Hermann Bihler; **Spirovation:** Scott Randall, Samuel Gallant, Tony Church; **University of Iowa:** John Englehardt, Yinghua Tang, Ziying Yan; **Spirovant:** Joan Lau, Eric Yuen, Roland Kolbec, Mark Smith, Donna Henry, Matthew Glatfelter, Shen Lin, Madhu Mahankali, Poornima Kotha; **Statistical Support:** Wei Zhu

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; [6]Tang et al., Mol Ther Methods Clin Dev. 2020; [7]Sun et al., Sci Transl Med. 2019