Intratracheal administration of AAV2.5T-SP183-fCFTRΔR in combination with doxorubicin corrects the mucociliary clearance defect in cystic fibrosis model ferrets

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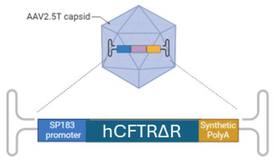
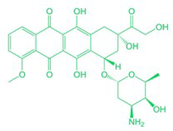
SAAVe Clinical Trial: inhaled SP-101 followed by inhaled Doxorubicin for the treatment of cystic fibrosis (CF)

SP-101



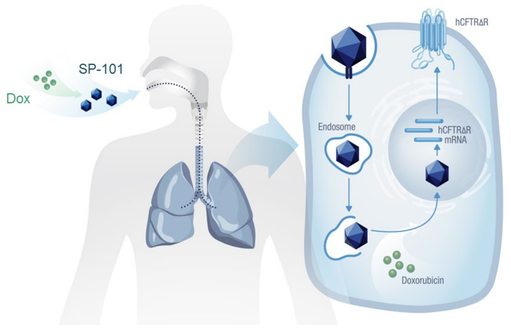
- Novel capsid **(AAV2.5T)**[1] selected for efficient apical **human** airway epithelial cell transduction
- A **minigene (CFTRΔR)**[2] with **wild-type activity** + a **strong promoter (SP183)**[3]

Doxorubicin (Dox)



- Small molecule Augmenter (doxorubicin hydrochloride)
- **Enhances AAV translocation** to the nucleus and **CFTRΔR expression and activity**[4,5,6,7]

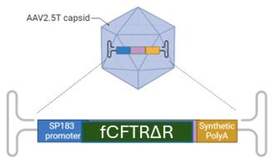
SAAVe clinical trial
Single inhaled dose of SP-101 followed by single inhaled low dose of Dox



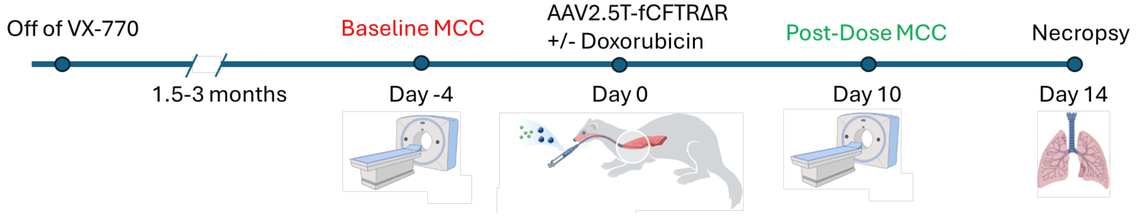
Background, experimental design and methods

- SP-101 capsid is tropic to ferret airway cells[6]
- CF ferret model recapitulates human CF lung pathology[8]
- Administration via MADgic Atomizer™ to the distal trachea

AAV2.5T-SP183-fCFTRΔR

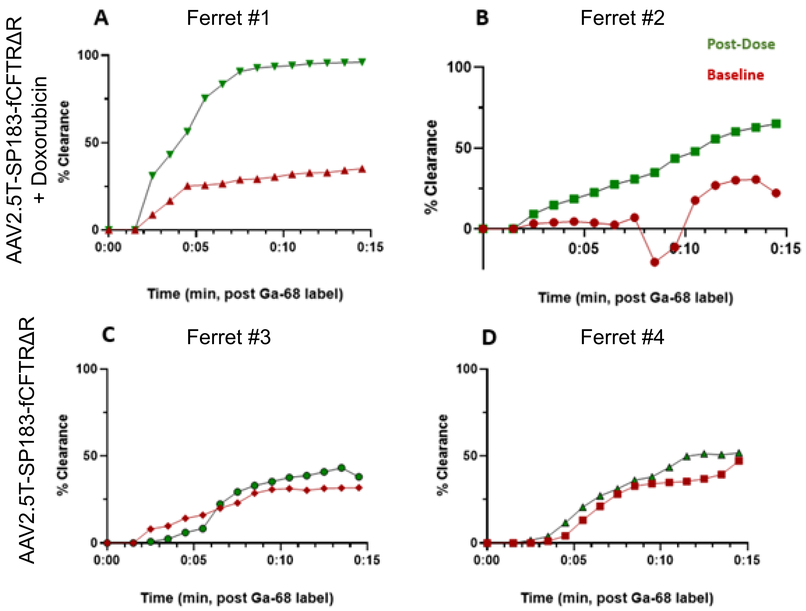


Hypothesis: The mucociliary clearance (MCC) defect in CF ferrets can be corrected by AAV2.5T-SP183-fCFTRΔR with or without Doxorubicin.



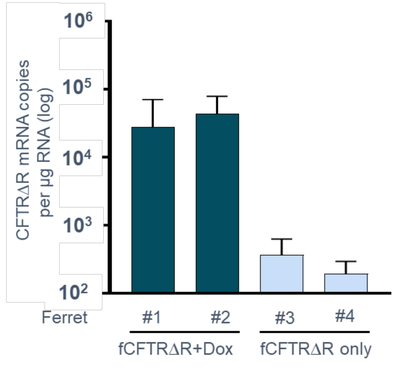
- CF (G551D) ferrets were raised on Ivacaftor (Iva) for at least 10 weeks. Then Iva was withdrawn for at least 6 weeks to establish impaired mucociliary clearance (MCC) phenotype in the distal trachea.
- MCC was determined via instillation of ^{68}Ga-macro aggregated albumin (^{68}Ga-MAA) to the distal trachea, with subsequent measurement of its rate of clearance (baseline) by positron emission tomography and computed tomography (PET/CT)[9]
- 4 – 7 days after baseline MCC measurement, a single dose of AAV2.5T-SP183-fCFTRΔR (1E13 vg/kg) or AAV2.5T-SP183-fCFTRΔR + doxorubicin (200μM) was instilled to the distal trachea via MADgic atomizer.
- MCC was then determined 10-days post-dose, with animals necropsied 2 weeks post-dose for lung tissue collection to quantitate vector genomes (qPCR) and *fCFTRΔR* mRNA (RT-qPCR)

Intratracheal administration of AAV2.5T-SP183-fCFTRΔR in combination with Doxorubicin (Dox) corrects the mucociliary clearance defect in cystic fibrosis model ferrets

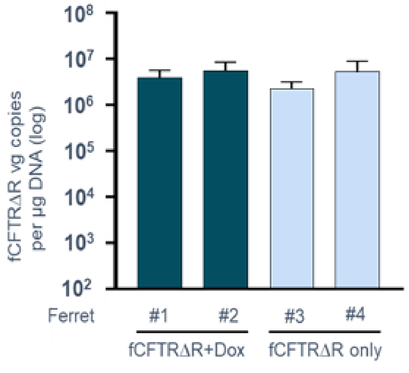


Panels represent baseline (red) and post-dose (green) MCC clearance rates for instilled ^{68}Ga-MAA in individual ferrets.
A) and B) **AAV2.5T-SP183-fCFTRΔR + dox.**
C) and D) AAV2.5T-SP183-fCFTRΔR only.

Increased MCC correlates with *fCFTRΔR* mRNA level



Doxorubicin does not affect lung vector genome levels



Conclusions

- Accumulated thick mucus in the ferret CF airway is not a barrier to AAV2.5T+Dox.
- *fCFTRΔR* mRNA levels have predictive value for fCFTRΔR activity.
- Doxorubicin co-administration is required to achieve *fCFTRΔR* expression for functional activity.
- Functional improvement of MCC in CF ferrets supports the potential for clinical efficacy of SP-101+Dox in pwCF.

REFERENCES: 1. Excoffon KJ, et al. *Proc Natl Acad Sci U S A*. 2009;106(10):3865-3870. **2.** Tang Y, et al. *Mol Ther Methods Clin Dev*. 2020;19:186-200. **3.** Ostedgaard LS, et al. *Proc Natl Acad Sci U S A*. 2002;99(5):3093-3098. **4.** Yan Z, et al. *Hum Gene Ther*. 2015;26(6):334-346. **5.**Yan Z, et al. *J Virol*. 2004;78(6):2863-2874. **6.** Zhang LN, et al. *Mol Ther*. 2004;10(6):990-1002. **7.** Excoffon KJ, et al. *Hum Gene Ther* 2024 in press. **8.** Sun X, et al. *Sci Transl Med*. 2019;11(485):eaau7531. **9.** Yuan F, et al *Nature* 2023; 621:857-867.