Inhalation of SP-101 followed by doxorubicin results in wide-spread SP-101 distribution and hCFTRΔR transgene expression in the airways of CF and non-CF ferrets

Katherine Excoffon, Mark Smith, Lillian Falese, Robert Schulingkamp, Shen Lin, Poornima Narayan, Madhu Mahankali, Matt Glatfelter, Eric Yuen and Roland Kolbeck

Spirovant Sciences, Inc., Philadelphia, PA

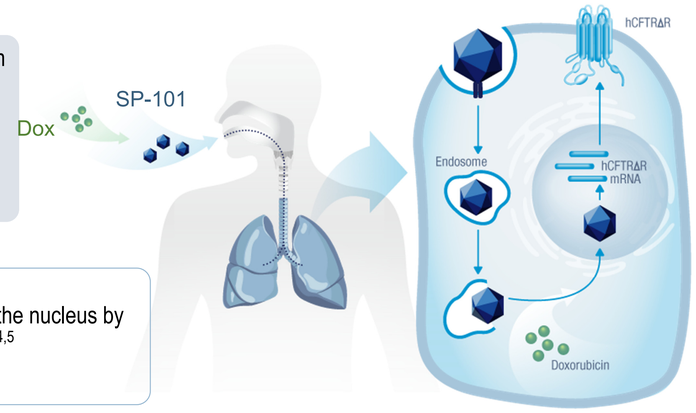
SP-101 – A novel, inhaled AAV-based gene therapy to treat CF

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

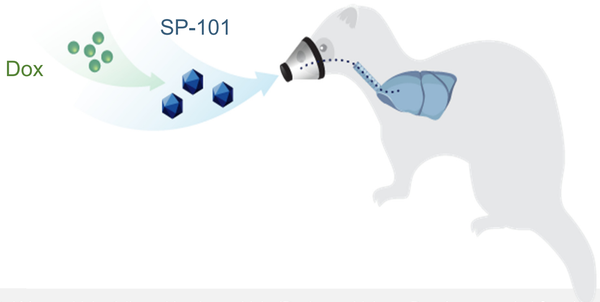


Ferret as a model to evaluate inhaled SP-101

SP-101 capsid is tropic to ferret airway cells[6]

CF ferret model recapitulates human CF lung pathology[7]

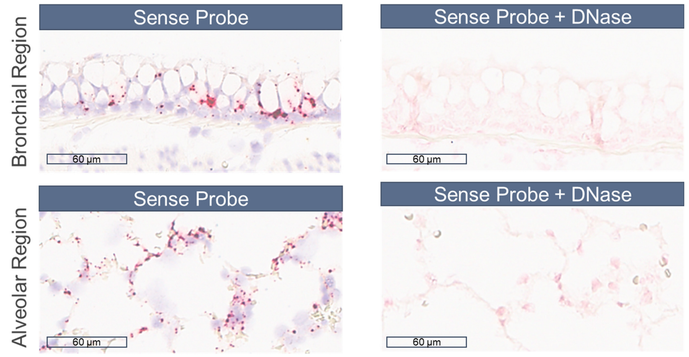
Ability to administer via inhalation



Methods: Non-CF and CF ferrets were exposed to nebulized SP-101 or vehicle, followed by doxorubicin (Dox) or vehicle on Day 1, necropsied 2 or 12 weeks post-exposure, and respiratory tissues harvested for *in situ* hybridization (ISH) or for determination of SP-101 vector genomes (vg) *or hCFTRΔR* mRNA copies. **ISH:** Sections from formalin-fixed, paraffin-embedded lung were evaluated by RNAScope,(+/- DNase treatment) using zz-probes designed to the anti-sense strand unique regions of the SP-101 vg.

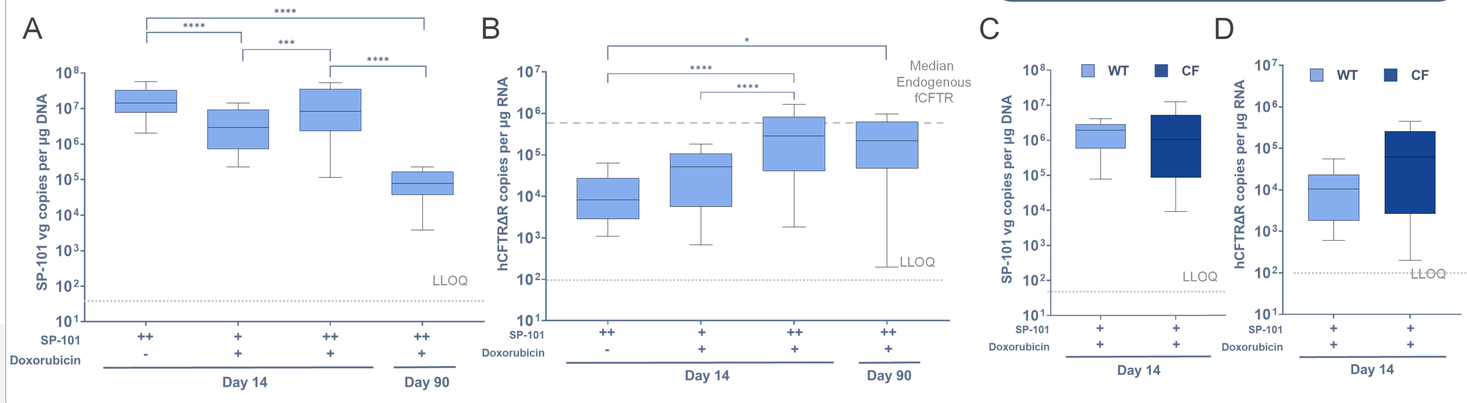
SP-101 vector genomes (vg) and *hCFTRΔR* mRNA copies: DNA or RNA was isolated from ~25 mg samples taken from 8-9 different regions of the airway (tracheal, bronchial, lobe). RNA was treated with Turbo DNase procedure to ensure removal of vg. qPCR +/- reverse transcriptase was performed with primers and a probe for unique regions of SP-101 and the *hCFTRΔR* mRNA. Control signals were below the lower limit of quantitation (LLOQ) and for mRNA, no signal was observed in the absence of reverse transcriptase indicating the complete removal of vg (data not shown). Data are shown as box and whisker plots of lung and bronchus around the median value (SP-101 or *hCFTRΔR* copy count normalized to 1 µg total DNA or RNA).

SP-101 is tropic to the ferret airway



SP-101 vector genomes are abundant in many regions of non-CF 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 for Vg.

hCFTRΔR mRNA expression is enhanced by doxorubicin, dose responsive, durable, and similar in both CF and wild-type ferrets



In wild type ferrets (A) SP-101 vg levels increase with MOI, are not affected by doxorubicin and decline from Day 14 (n=6/sex) to Day 90 (n=3/sex, end of study). (B) *hCFTRΔR* mRNA expression is increased >10 fold by administration of doxorubicin and is durable. SP-101 vg and *hCFTRΔR* mRNA were detected in the majority of samples from animals exposed to SP-101 with or without doxorubicin. While vg dropped by Day 90 (end of study), *hCFTRΔR* mRNA expression remained high indicating the retention of functional genomes. Control samples (vehicle) were below LLOQ, (not shown). **(C) SP-101 vg and (D) *hCFTRΔR* mRNA expression is similar in the lungs of wild-type (WT, n=3) and CF ferrets (n=3).** SP-101 vg and hCFTRΔR mRNA were detectable to a similar extent in both CF and wild-type ferrets suggesting that the CF airway is not an additional barrier to SP-101. Control animals (vehicle) were below LLOQ (not shown).

SP-101 holds great promise for people living with CF

- SP-101 is distributed throughout the airways and tropic to various airway cell types
- Doxorubicin does not alter SP-101 biodistribution in the airways
- *hCFTRΔR* mRNA expression is MOI and doxorubicin dose responsive, durable, and reaches endogenous fCFTR levels
- SP-101 vg and *hCFTRΔR* mRNA expression is similar in CF and wild-type ferrets, suggesting that the CF airway is not an additional barrier to SP-101

spirovant

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