Cystic Fibrosis lung disease is caused by the loss of function of CFTR. This loss leads to hyperactivation of the epithelial sodium channel (ENaC), enhancing Na+ uptake by airway epithelial cells and dehydration of the lung airway surface. In normal lungs, short palate lung and nasal epithelial clone S (SPLUNC1) is secreted to the airway surface liquid and binds to ENaC inducing its internalization. As the protein is rapidly degraded by neutrophil elastase, SPLUNC1 regulation of ENaC is lost in the CF lung. Previous work has identified an 18 amino acid residue (S18) of the SPLUNC1 N-terminus that is responsible for the regulation of ENaC. We have optimized SPX-101 to perform a similar regulatory role on ENaC in the protease-rich environment of the CF lung. Here we present the preclinical safety and effectiveness of SPX-101 in animal models.

**Results**

SPX-101 fully restores mucus transport to normal levels in the sheep model of CF, while amiloride demonstrates only a transient increase in mucus transport.

**Fig 1:** Baseline tracheal mucus velocity (TMV) was obtained (n=0) and immediately thereafter CFTRinh-172 was nebulized to inhibit CFTR. (A) Four hours later SPX-101, control peptide, or 0.9% isotonic saline was administered via nebulization and TMV measured hourly for 8 hs. (B) Sheep were nebulized with amiloride (0.06 mg/kg) formulated in isotonic (0.9%) or hypertonic saline (4.2%). Data represent mean ± SEM, n=3; *P<0.05

SPX-101 increases survival to more than 90% and also reduces neutrophil and eosinophil infiltration into the lungs.

**Fig 2:** (A) Kaplan-Meier survival curve of βENaC-Tg mice treated once-daily with the indicated concentrations of SPX-101, control peptide, or saline starting two days after birth. (B) Leukocyte composition in the BALF of mice treated in (A).

Injection of a bolus of SPX-101 has no respiratory or cardiac effects.

**Fig 3:** Dogs were monitored by telemetry 2 hours prior to intravenous injection and monitored continuously for 24 hours after dose administration. Note: Similar results were obtained after oro-nasal inhalation for up to 240 minutes to conscious, telemetered dogs in a single escalating dose design. (data not shown)

SPX-101 has no effect on serum or urinary sodium or potassium levels in a 28 day nebulized toxicity study in dogs.

**Fig 4:** (A) Blood and (B) Urine were collected at the termination of the study and analyzed for sodium and potassium levels. Note: Similar results were obtained in a 28 day study in rats (data not shown).

SPX-101 half life once it reaches systemic circulation is 12.5 min.

**Fig 5:** SPX-101 was determined by mass spectrometry in plasma of dogs at the indicated times, after intra-peritoneal injection of SPX-101

**Conclusions**

1. **Peptide Binds to Epithelial Sodium Channel**
   - Restores mucus mucus transport in a sheep model.
   - Increases the βENaC mouse survival to more than 90%.
   - Reduces leukocyte infiltration in the βENaC mouse.

2. **Robust Removal of Channels from Airway Surface**
   - Nebulization or I.P. injection of SPX-101 has no respiratory or cardiac effects in dogs and rats, and does not induce hyperkalemia.
   - Half life of 12.5 min in systemic circulation.

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