Methods: The straightforward protocol and standardized calibration were used to measure the viscoelasticity of mucus and sputum in a fast-paced clinical setting. Dynamic oscillation with a shear-strain sweep provides linear viscoelastic moduli ($G'$, $G''$, $\delta$, and $\gamma$) and gel point characteristics ($G^*$, $\eta_c$) for clinical samples within 5 minutes. Device performance was validated using various concentrations of a mucous simulant, 8 MDA polyethylene oxide (PEO) against a traditional bulk rheometer (DHR-3, TA Instruments). A clinical isolate harvested from an intubated patient with status asthmaticus was assessed in triplicate measurements, as well as the effects of a potent mucus reducing agent, tris (2-carboxylethyl) phosphine hydrochloride (TCEP).

Results: PEO solutions were viscous-dominated ($G' > G''$) and values were comparable between Rheomuco and the traditional DHR-3 rheometer. Tripletic measurements performed on 1.5% PEO solution and asthmatic mucus reducing agent, tris (2-carboxylethyl) phosphine hydrochloride (TCEP) resulted in a decrease in elastic modulus from 8.62 Pa to 1.67 Pa and a change toward a more liquid-like behavior overall (e.g., higher $\delta$).

Conclusions: These results demonstrate that Rheomuco measures the viscoelasticity of mucus samples rapidly and reliably and could therefore be used to explore the effects of approved mucolytic drugs (e.g., rhDNase, N-acetyl cysteine) in clinics to adapt treatment on a case-by-case basis or in laboratory preclinical studies to test the efficacy of novel mucoactive compounds.

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Toxicology of ELD607, a novel immunomodulator of inflammatory responses in cystic fibrosis lungs
S. Almad1, M. Sassano2, J. Wrennali2, R. Tarran2, 1Eldec Pharmaceuticals, Durham, NC; 2Department of Cell Biology and Physiology, School of Medicine, University of North Carolina, Chapel Hill, NC

Background: Management of airway inflammation is a vital aspect of cystic fibrosis (CF) treatment, but beyond ibuprofen, there are no approved anti-inflammatory drugs to treat people with CF. Orai1 is a plasma membrane Ca2+ channel that regulates inflammation by controlling gene expression and cytokine secretion. We have generated ELD607, a fully optimized Orai1 inhibitory peptide that is CF transmembrane conductance regulator (CFTR)-mutation agnostic and proteolytically stable and has a half maximal inhibitory concentration (IC50) of 9 nM. ELD607 inhibits Orai1 in the lungs of neutrophilic mouse models to reduce pulmonary inflammatory responses. Although we have shown efficacy of ELD607, toxicological effects must be determined before it can be advanced as a therapeutic. We assess the safety and toxicology of ELD607.

Methods: To determine potential cytotoxicity, HEK293T and human primary bronchial epithelial cells were incubated with 100 µM ELD607 (approximately 10 000 times the IC50) for 24 hours, and cell viability was measured using calcine-AM. The human ether-a-go-related gene (hERG) K+ channel plays a vital role in cardiac repolarization, and inhibition of hERG is fatal. hERG activity in response to 100 µM ELD607 was measured using a patch clamp assay. 100 µM ELD607 was also screened against the Eurofins Safety 44 Panel. We next switched to an in vivo model to evaluate the safety of ELD607. C57Bl/6j mice were dosed with 10 mg/kg ELD607 (20 times the predicted therapeutic dose) intratracheally for 7 days. To determine the location of inhaled ELD607, mice were dosed with 1.0 mg/kg of ELD607-labeled Tamra intratracheally, and the lung was excised 1 hour later. Whole lungs were fixed and imaged using light sheet microscopy. To analyze the pharmacokinetics of ELD607, mice were exposed to 0.5 mg/kg of ELD607 intratracheally. Bronchoalveolar lavage fluid (BALF) and blood were collected at timed intervals after inhalation, and samples were measured for ELD607 using high-performance liquid chromatography (HPLC). We are currently dosing nonhuman primates with ELD607 because nonhuman primate airways better mimic human airways.

Results: There was no change in cell viability when exposed to ELD607 and no change in the transspetithelial electrical resistance, indicating that ELD607 does not change epithelial integrity and permeability. ELD607 did not have any effect on hERG activity and did not bind to any of the targets. After a 7-day dosing, mouse weight did not change, and there were no histopathological changes in the lungs, heart, liver, or kidneys. Light sheet microscopy revealed that ELD607-Tamra was found in the airway lumen (from the main bronchi to the alveoli) but was not detected outside of the airways. HPLC analysis detected low ELD607 levels in the BALF with 20% of the initial dose found at 10 minutes, declining to 10% 2 hours after dosing. Extremely low ELD607 levels were found in the blood (0.2% of initial dose) throughout the timed-interval collections. Results from the nonhuman primate studies will be discussed at the time of presentation.

Conclusions: ELD607 has no significant toxic effects in vitro or in vivo in small animal models and may serve as a safe, novel therapeutic for anti-inflammatory effects in people with CF.

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