Evaluation of a Human 3D Airway Tissue Culture Model for the Study of Respiratory Syncytial Virus Infection and the Development of Antiviral Drugs

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BACKGROUND

- Despite respiratory syncytial virus (RSV) being a leading cause of acute respiratory tract infections in children, the immunocompromised, and the elderly, there are limited therapeutic options.
- Animal models do not completely reproduce human RSV infection pathogenesis. As such, there is a need for preclinical models to study RSV and evaluate novel antiviral agents.
- 2D cell culture models are widely used in drug discovery, but antiviral activity may be influenced by factors not relevant to an *in vivo* environment, such as surface stiffness, forced polarity, and uncontrolled cell spreading.
- 3D models provide a unique advantage for respiratory virus studies by recapitulating the different cell types in the airway at an air-liquid interface.
- EpiAirway standard AIR-100 culture tissue by MatTek Corporation (Ashland, MA) is composed of **p**rimary **h**uman **a**irway tracheal and bronchial **e**pithelial **c**ells grown at an **a**ir-**l**iquid **i**nterface (pHAEC-ALI). This includes basal cells, mucus-producing goblet cells, tight junctions, and functioning cilia, which are the target cells of RSV.
- EDP-323, a novel non-nucleoside RSV L-inhibitor, and EDP-938, a non-fusion RSV replication inhibitor, were evaluated against RSV-A and -B in the EpiAirway 3D model to determine antiviral activity.

METHODS

- All tissues used in this study are from a 23-year-old Caucasian male donor.
- Tissues were kept in a 12-well transwell plate, with 5 mL basal medium (MatTek) being replaced every 2-3 days. The apical surface was washed every 7 days with transepithelial/transendothelial electrical resistance (TEER) buffer (MatTek).
- RSV growth dynamics were evaluated utilizing plaque-forming units (PFUs) ranging from $3.9e^3$ to $2.5e^5$. Apical infection was performed for 1 hour at 37 ºC with 350 µL.
- Compounds were added to the basal medium at the time of infection. Antiviral activity was also assessed in the human larynx carcinoma HEp-2 cell line (established via HeLa cell contamination). For both assays, half-maximal effective concentrations (EC_{50}s) were assessed by RT-qPCR at 5 days post-infection.
- Viral growth from the tissue and apical washes over time were quantified by RT-qPCR and plaque assay, respectively.
- For immunofluorescence experiments, tissues were fixed with 10% formalin and immunostained with monoclonal antibodies (mAbs) against the RSV N and F proteins. A mAb against FoxJ1 was used to detect ciliated cells. All immunofluorescence was performed by Visikol Inc. (Hampton, NJ).

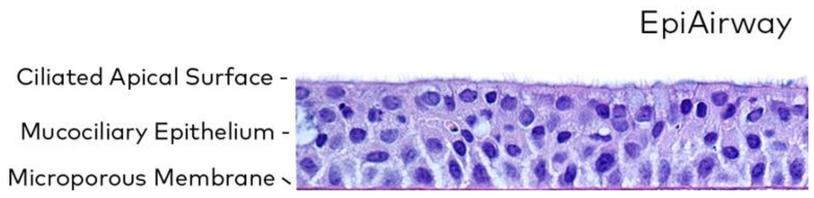

Figure 1. Diagram of a 3D EpiAirway Tissue

RESULTS

RSV RNA viral load and secreted live virus load increases exponentially over 10 days in EpiAirway tissue

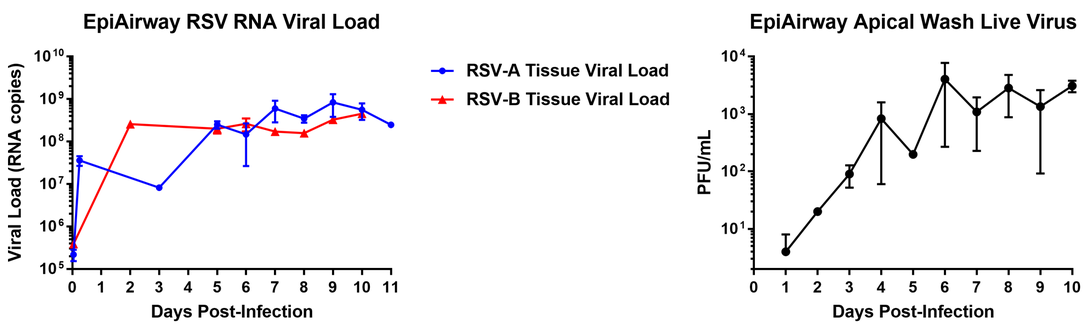

Figure 2. EpiAirway tissues were apically infected with RSV-A Long and RSV-B VR-955 with $3.1e^4$ PFU. Tissue viral RNA load was measured by RT-qPCR. Live virus in apical washes was measured by plaque assay.

Viral inoculum has minimal impact on viral load at 5 days post-infection

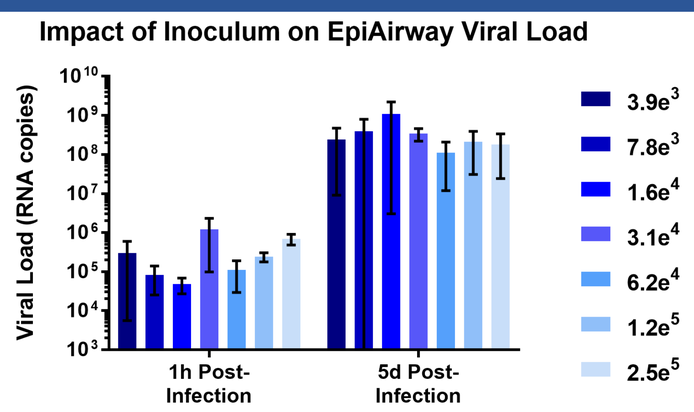

Figure 3. Tissues were apically infected with the indicated PFU. RT-qPCR was used to measure the amount of viral RNA copies in the tissue at 1-hour post-infection and at 5 days post-infection.

RESULTS

EDP-938 and EDP-323 inhibit viral RNA in the EpiAirway 3D system and 2D culture

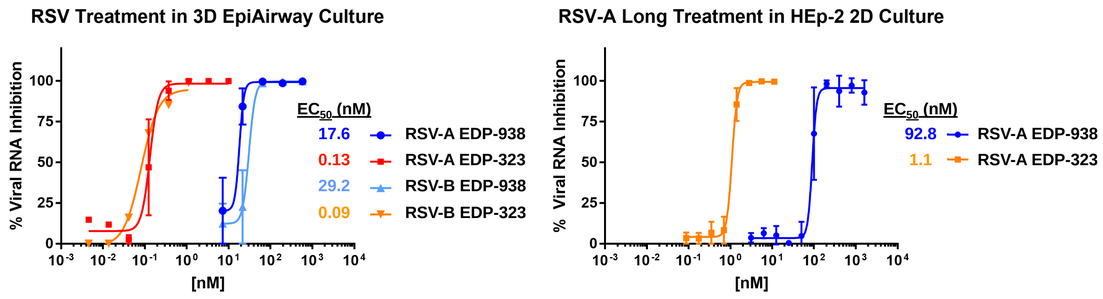

Figure 4. EpiAirway tissues were apically infected with RSV-A Long and RSV-B VR-955 with $3.1e^4$ PFU. HEp-2 cells were infected at a multiplicity of infection (MOI) of 0.1. EpiAirway tissues and HEp-2 cells were treated with EDP-938 and EDP-323 and after 5 days, EC_{50}s were determined by RT-qPCR.

Cell markers visualize ciliated cells in EpiAirway tissue

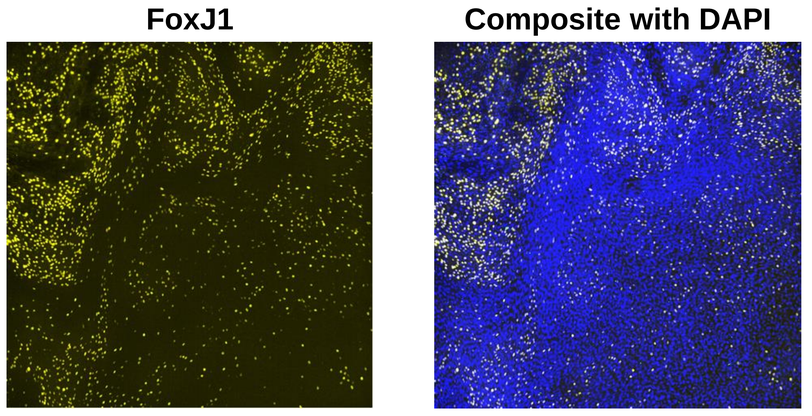

Figure 5. EpiAirway tissues were infected with RSV-A Long with $7.8e^3$ PFU. Tissues were fixed and stained for FoxJ1, shown in yellow, to indicate ciliated cells.

RSV markers are reduced in EDP-323-treated EpiAirway tissue

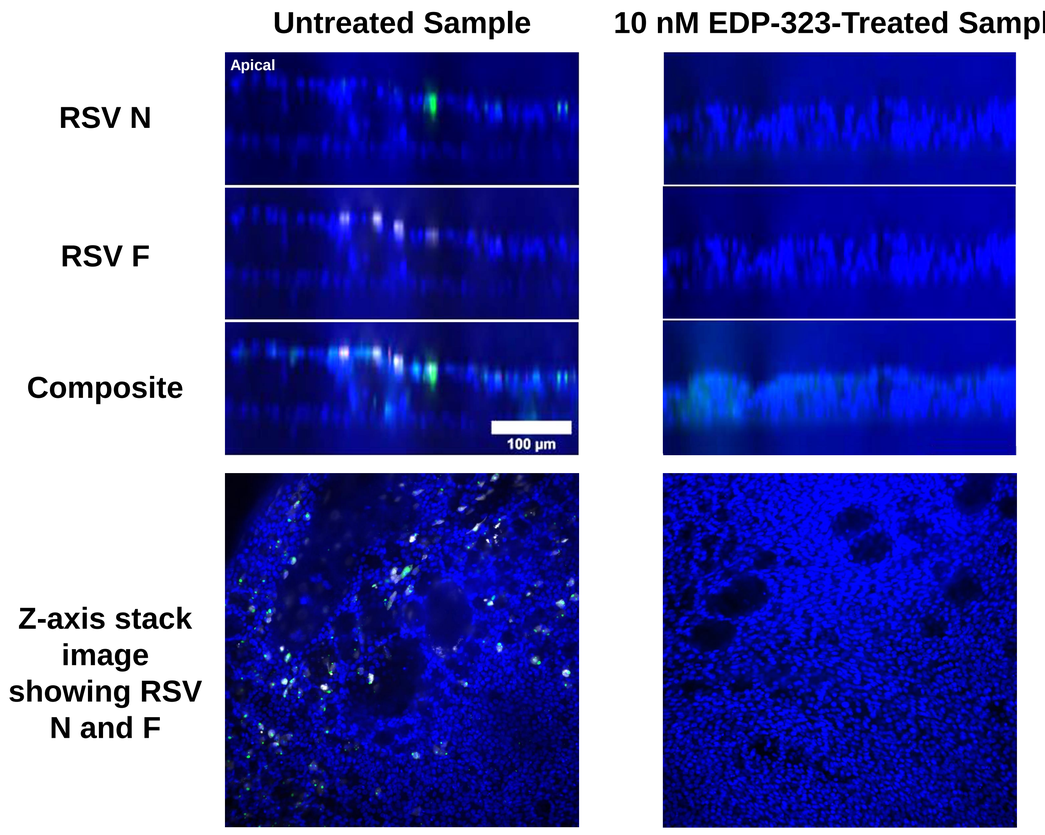

Figure 6. EpiAirway tissues were infected with RSV-A Long with $3.1e^4$ PFU and treated with 10 nM EDP-323 or vehicle control. Five days post-infection, tissues were fixed and sent to Visikol Inc. to stain for RSV N (green) and RSV F (white).

CONCLUSIONS AND ACKNOWLEDGEMENTS

- The EpiAirway model can be successfully used to study the dynamics of RSV infection and evaluate the antiviral activity of novel therapeutics.
- The EpiAirway model generates reproducible viral replication kinetics and consistent compound-induced viral inhibition while surpassing the preclinical testing limits of traditional 2D culture models.
- The EpiAirway model can be utilized for visualization of *in vivo* cell markers as well as reduction of RSV markers with antiviral treatment.
- EDP-323 is expected to begin Ph1 clinical trials in Q4 2022

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DISCLOSURE: All authors are either current or former employees of Enanta Pharmaceuticals, Inc. and received salary and stock compensation.

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