EDP-938, A Respiratory Syncytial Virus (RSV) antiviral, demonstrates a high barrier to resistance in a human challenge study

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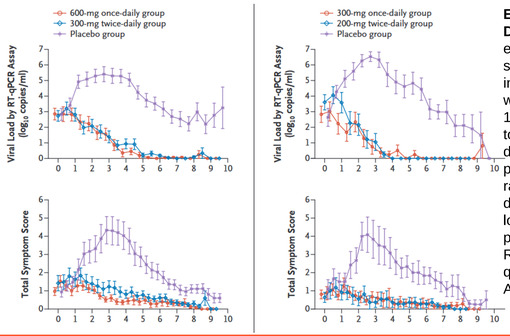
BACKGROUND

- RSV causes repeated infections throughout life, causing substantial morbidity and mortality, yet there is an unmet need for treatments
- RSV antivirals in clinical development that target the fusion protein can induce high-level resistance quickly *in vitro* and in clinical studies, with resistant mutations attaining high viral loads and retaining viral fitness[1,2,3,4,5]
- In a human challenge trial, EDP-938 significantly reduced viral load and disease severity in all tested doses[6]
- To understand the propensity for EDP-938 resistance to develop in human infection, a selection of samples underwent high-throughput sequencing



EDP-938 Reduces Viral Load and Disease Severity: Mean ± standard error RSV loads and total symptom scores from patients included in the intent-to-treat population. The study was conducted in two parts. In part 1, 115 participants were randomized 1:1:1 to 600 mg once daily, 500 mg loading dose with 300 mg twice daily, or placebo. In part 2, 63 participants were randomized 1:1:1 to a 600 mg loading dose with 300 mg twice daily, 400 mg loading dose with 200 mg twice daily or placebo. mg = milligrams; mL = milliliter; RT-qPCR = reverse-transcriptase – quantitative polymerase chain reaction. Adapted from (6).

METHODS

Selection of Participants for Next Generation Sequencing (NGS)

Visual Inspection of Viral Load Patterns Across Participants → *Selection of 37 EDP-938 treated and 11 placebo participants representative of the range of patterns observed* → *159 nasal wash samples selected from multiple pre- and post- dosing time points*

Specific nasal wash samples were selected based on the minimum viral load needed for sequencing

NGS Workflow and Variant Detection

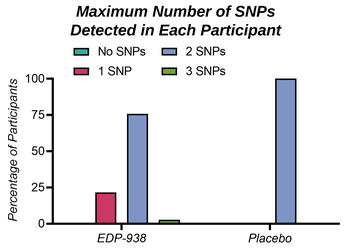
Amplification of N and surrounding regions of RSV A Memphis 37b genome → *Illumina NGS, quality assessment and filtering of reads* → *Alignment to reference RSV-A Memphis 37b genome with BBmap* → *Variant Calling with FreeBayes with a minimum of 1% frequency and at least 10 reads*

The full nucleoprotein (N) gene and surrounding region corresponding to nucleotides 1081-2227 of the RSV-A Memphis 37b genome were amplified. Initial quality assessment of NGS reads was based on data passing the Illumina Chastity filtering. A second quality assessment was performed using FASTQC quality control tool version 0.11.8. Alignment to the reference genome (RSV-A Memphis 37b, KM360090), was performed using Bbmap (version 36.77). Variants were called with FreeBayes (version 1.1.0)[8] and filtered based on having a frequency of at least 10 reads with a frequency of at least 1%.

RESULTS

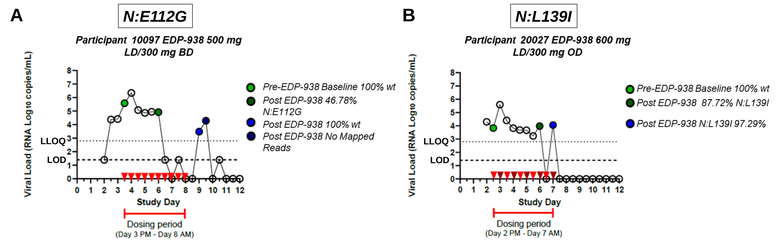
Low frequency of substitutions observed in participants

159 samples from 48 participants were chosen for sequencing. 153/159 samples had usable reads.



- 48 participants had synonymous mutations (N:K5K or P:A5A)
- 2 EDP-938-treated participants also had nonsynonymous mutations (N:E112G, N:L139I)
- Nonsynonymous mutations were not detected in any preclinical *in vitro* resistance generation studies[8]

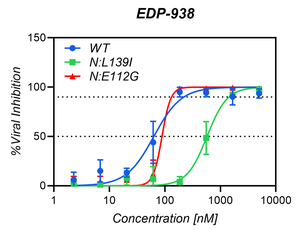
2/37 sequenced EDP-938-treated participants had treatment-emergent mutations



Viral load patterns of the two participants with nonsynonymous mutations. Nasal wash samples that underwent NGS are in green or blue circles. Non-sequenced samples are in open circles. Red triangles indicate doses of EDP-938. Dark red triangles (B) indicate dosages of placebo given to match BD dosing. LLOQ (dashed line) indicates the lower limit of quantification (2.8 log_{10} copies/mL). LOD (bold dashed line) indicates the limit of detection (1.4 log_{10} copies/mL). Amino acid numbers are in reference to the RSV nucleoprotein (N).

N:L139I is associated with low-level resistance to EDP-938

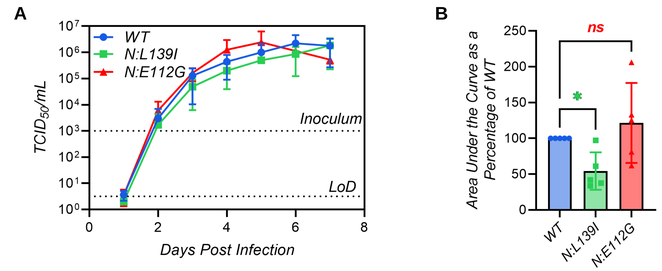
	WT		N:L139I		N:E112G	
	EC_{50} [nM]	EC_{90} [nM]	EC_{50} [nM]	EC_{90} [nM]	EC_{50} [nM]	EC_{90} [nM]
EDP-938	**61** ± 12	**91** ± 5	**590** ± 68	**1,075** ± 388	**100** ± 33	**124** ± 34
EDP-323	**0.21** ± 0.04	**0.37** ± 0.08	**0.14** ± 0.04	**0.31** ± 0.03	**0.10** ± 0.04	**0.14** ± 0.04
ArkBio 0529	**7.4** ± 0.8	**25** ± 18	**8.7** ± 6.0	**52** ± 41	**2.1** ± 0.8	**15** ± 8
RV-521	**0.42** ± 0.3	**2.7** ± 2.8	**0.37** ± 0.1	**1.2** ± 0.1	**0.62** ± 0.4	**4.0** ± 5.0



N:E112G and N:L139I recombinant RSV-A2 viruses were created using reverse genetics[9]. Data are mean ± standard deviation from at least 3 independent determinations. Amino acid numbers are in reference to the RSV nucleoprotein.

RESULTS

Clinical resistance to EDP-938 is associated with slight fitness defects



(A) HEp-2 cells were infected with the indicated recombinant virus at an MOI of 0.1 and live virus in the cells and supernatant was determined at each day post infection by $TCID_{50}$. Data are mean ± standard deviation from 5 biological experiments. (B) For each biological replicate, the area under the curve was determined. * $p < 0.05$ ANOVA followed by Dunnett's multiple comparisons test. Values below the LoD (limit of detection) (3.16E+0) are listed as 2.00E+0. Amino acid numbers are in reference to the RSV nucleoprotein.

CONCLUSIONS

- EDP-938 has a high barrier to clinical resistance, a single resistant mutation was detected in 1 of 37 sequenced EDP-938-treated participants
- The observed resistance mutation was associated with defects in viral fitness
- Observed treatment-emergent mutations do not impede viral clearance
- EDP-938 is currently in Phase 2 clinical trials for the treatment of RSV in pediatric patients under 3 years (NCT04816721) and high-risk adult patients (NCT05568706)

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