In Vivo Efficacy of EDP-323, A Novel L-Protein Inhibitor, for the Treatment of Respiratory Syncytial Virus

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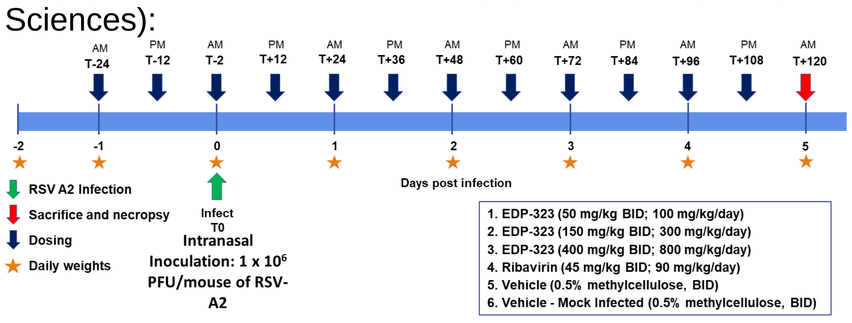
DISCLOSURE: All authors are employees of Enanta Pharmaceuticals and receive salary and stock compensation

BACKGROUND

- Almost every child is infected with respiratory syncytial virus (RSV) before age 2. In most, RSV presents as a common cold; however, for premature infants, the elderly, and the immunocompromised, RSV can result in substantial morbidity and mortality.
- Despite the availability of a prophylactic monoclonal antibody (Palivizumab) and aerosolized ribavirin, there is a high unmet medical need for RSV therapeutics.
- Here we describe the *in vivo* efficacy of EDP-323, a novel non-nucleoside, small molecule RSV L-protein inhibitor.

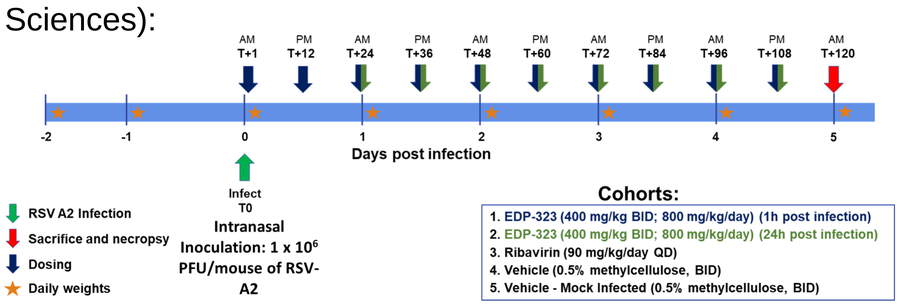
METHODS

- The antiviral activity of EDP-323 against clinical and laboratory isolates of RSV was evaluated in HEp-2 cells, primary HBECs and BALB/c lung cells, and 3-dimensional primary human airway epithelial cells grown in an air-liquid interface system using cytopathic effect and RT-qPCR as readouts.
- Generation of *in vitro* EDP-323 resistance was performed by serially passaging RSV-A Long in HEp-2 cells at increasing concentrations of EDP-323. Mutations were identified by Sanger Sequencing of the L protein region and comparison to the reference sequence (GenBank Accession #: AY911262.1).
- Prophylactic BALB/c Mouse Study (Performed by Aragen Life Sciences):



Study endpoints included terminal lung weight, terminal viral load in lung homogenates, terminal serum cytokine analysis, and next generation sequencing (NGS) analysis on terminal RNA for variant detection.

- Therapeutic BALB/c Mouse Study (Performed by Aragen Life Sciences):



Study endpoints included terminal lung weight and terminal viral load in lung homogenates.

RESULTS

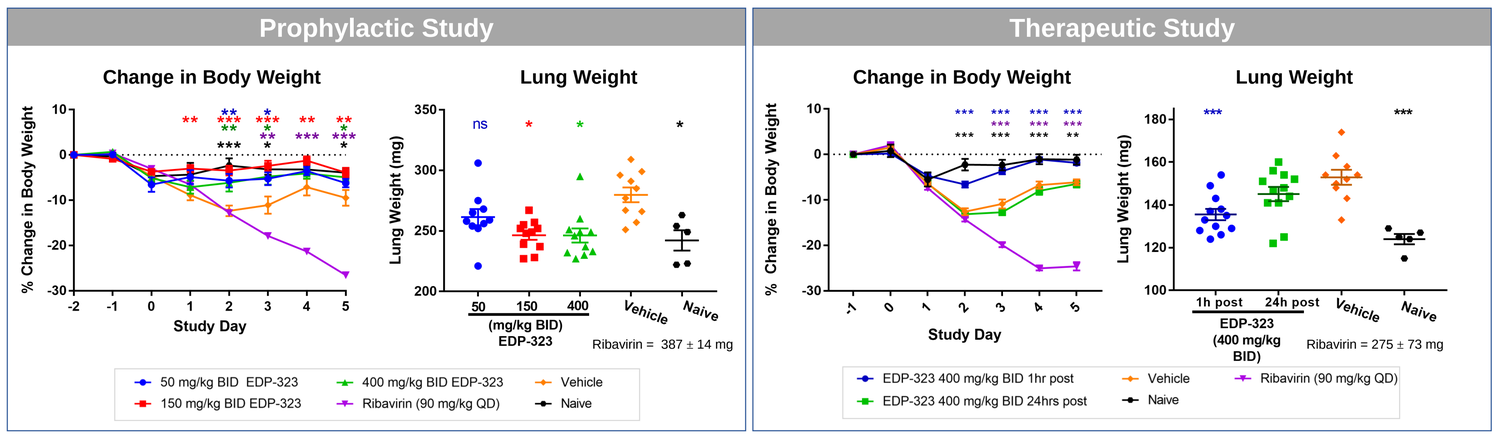
EDP-323 displays potent antiviral activity against multiple laboratory and clinical isolates of RSV-A and RSV-B

Virus	Cell Type	Assay	EC_{50} (nM)
RSV-A Long	HEp-2	CPE	0.44
	HEp-2	RT-qPCR	0.84
	HBEC	RT-qPCR	0.09
	3D pHAEC	RT-qPCR	0.16
	Primary BALB/c mouse lung	RT-qPCR	1.2
RSV- A2	HEp-2	CPE	0.15
	Primary BALB/c mouse lung	RT-qPCR	1.0
RSV-B VR-955	HEp-2	CPE	0.40
	HEp-2	RT-qPCR	0.55
	3D pHAEC	RT-qPCR	0.09
Clinical Isolate Average	HEp-2	CPE	0.20

Clinical Isolate average comes from 9 RSV-A isolates and 6 RSV-B isolates. 3D pHAEC = 3-dimensional primary human airway epithelial cells grown in an air-liquid interface system.

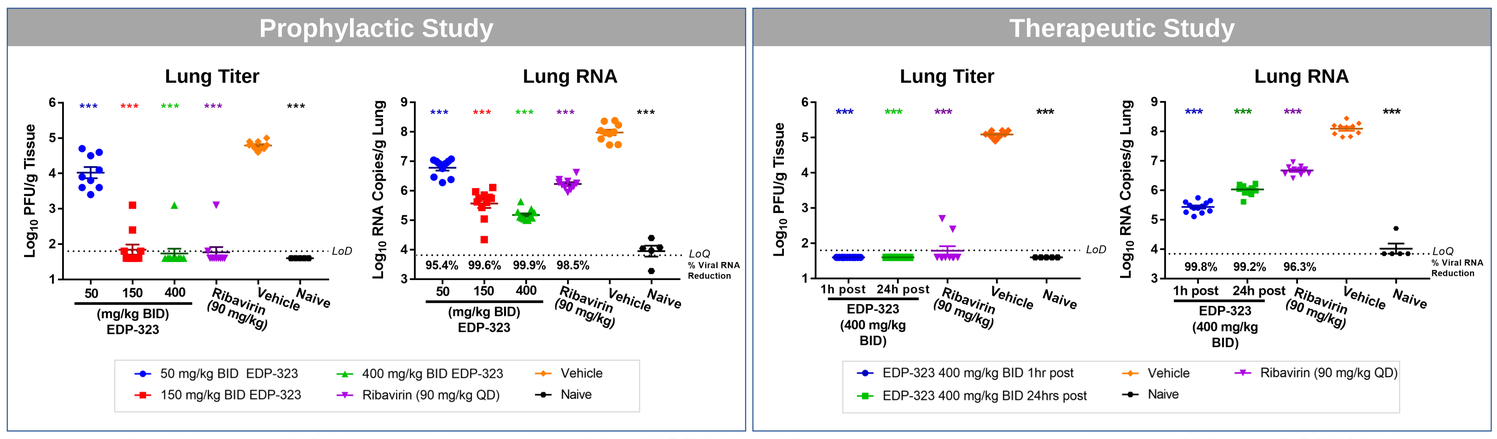
RESULTS

EDP-323 protects mice from virus-induced changes in body and lung weight in RSV-A2 infected mice



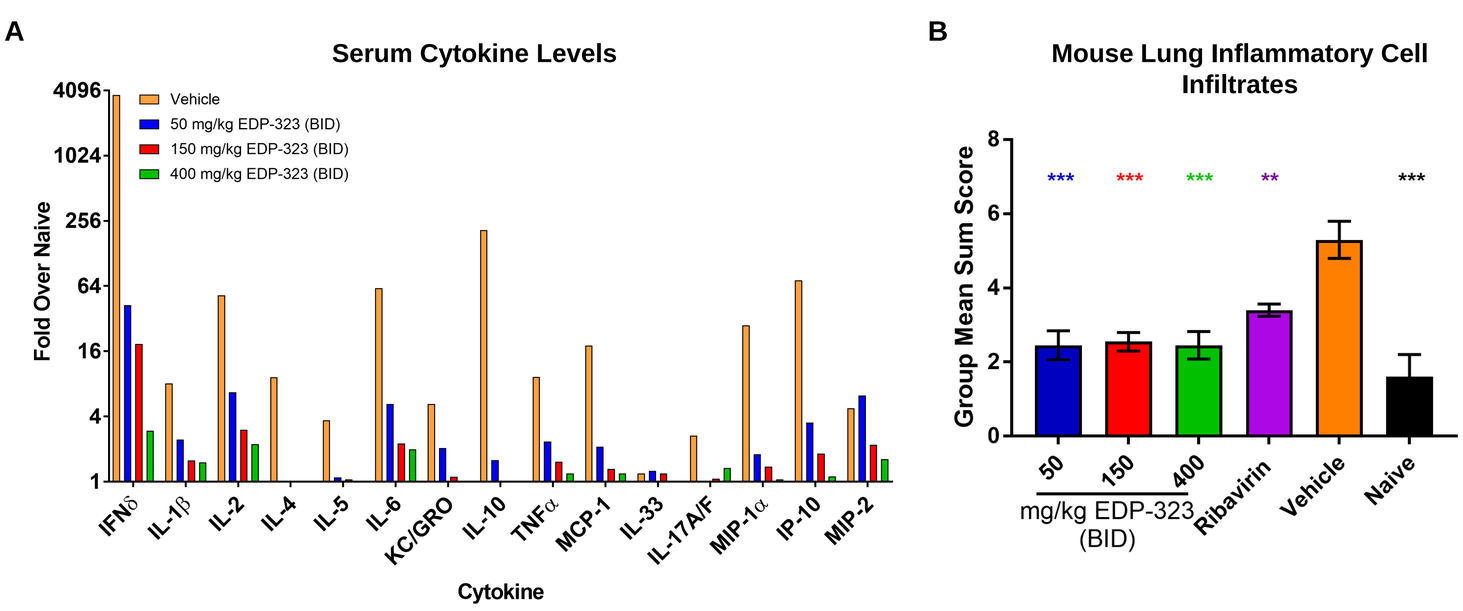
Data are mean ± standard error of the mean for each group (Naïve, n = 5; RSV-A2 infected, n = 10-12). ns = not significant, * = p <0.05, ** = p <0.01, *** = p <0.001 ANOVA followed by Dunnett's multiple comparisons test compared to Vehicle-treated animals.

EDP-323 reduces viral load in RSV-A2 infected mice



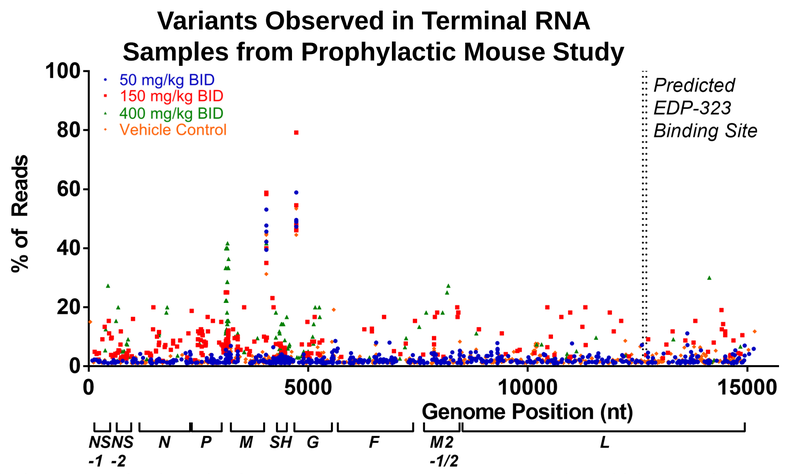
Plaque assay Limit of detection (LoD) (dotted line) for lung titers is 1.8 log10 PFU/g tissue. Data are mean ± standard error of the mean (Naïve, n = 5; RSV A2 infected, n = 10-12). *** = p <0.001 ANOVA followed by Dunnett's multiple comparisons test compared to Vehicle-treated animals.

EDP-323 reduces serum cytokine levels and lung injury in RSV-A2 infected mice



(A) Cytokine levels were determined using a Meso Scale Diagnostics multiplex assay and are expressed as fold over naïve animals. **(B)** Hematoxylin and eosin-stained lung sections were evaluated and graded for severity on a score of 0-5 (0 = not present/normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe). Data are mean ± standard error of the mean (Naïve, n = 5; RSV A2-infected, n = 10-12). ** = p <0.01, *** = p <0.001 ANOVA followed by Dunnett's multiple comparisons test compared to Vehicle-treated animals.

In vitro-derived resistance mutations were not identified in EDP-323 treated mice



- L protein mutations observed in ***in vitro*** plaque purified clones following 4 serial passages in the presence of EDP-323 (amino acid numbers in L)
  - L1372V, C1388G: 18/18
  - I392L: 3/18
  - K1532E: 3/18
- I392L, L1372V, C1388G, K1532E were not identified in any mice
- No mutations in EDP-323 treated mice within 6Å of the center of the predicted EDP-323 binding site

Terminal RNA samples from the three EDP-323 treatment groups and the vehicle control group in the prophylactic study were randomly selected for sequencing. All sequences (including inoculum) were mapped to the RSV-A2 reference genome (GenBank Accession # KT992094.1). Low frequency variant calling was performed using a threshold of 1%.

CONCLUSIONS

- EDP-323 potently inhibited RSV replication *in vitro* with low picomolar EC_{50}s versus multiple isolates of RSV
- EDP-323 blocked RSV replication and pathology in a mouse infection model
- Mutations consistent with *in vitro* EDP-323 resistance were not identified in any EDP-323 treated mice
- EDP-323 is being developed as an oral, once daily antiviral and is moving into a Phase I trial later this year

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