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Great Chemistry Cures

EDP-323, a Small Molecule L-Protein Inhibitor in Development Against Respiratory Syncytial Virus

M. H. J. Rhodin, N. V. McAllister, N. Bisht, R. E. Levene, J. S. Gibbons, A. Balakrishnan, J. Yu, A. A. Szymaniak, K. P. McGrath, T. J. Mann, I. J. Kim, B. Ludeke, R. Fearns, Y. S. Or, and B. Goodwin

Discovery on Target: New Antivirals Conference, Boston, MA. October 19th, 2022

Disclosures: **Authors M. H. J. Rhodin, N. V. McAllister, N. Bisht, R. E. Levene, J. S. Gibbons, A. Balakrishnan, J. Yu, A. A. Szymaniak, K. P. McGrath, T. J. Mann, I. J. Kim, Y. S. Or, and B. Goodwin are employees of Enanta Pharmaceuticals**

Work performed in the Fearns lab was funded under a sponsored research agreement between Boston University and Enanta Pharmaceuticals, Inc.

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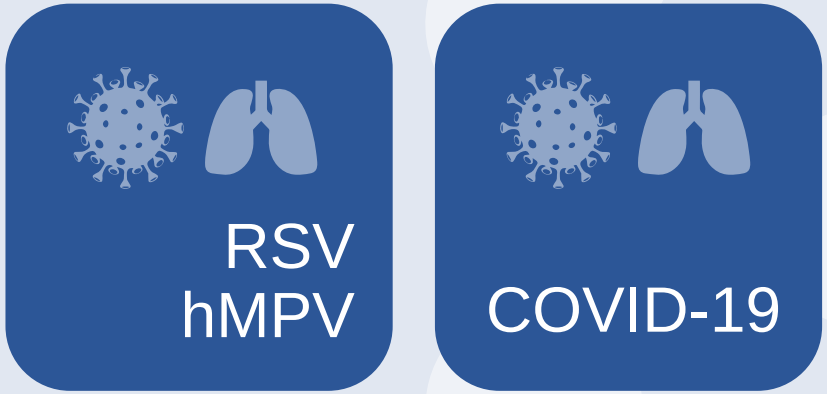
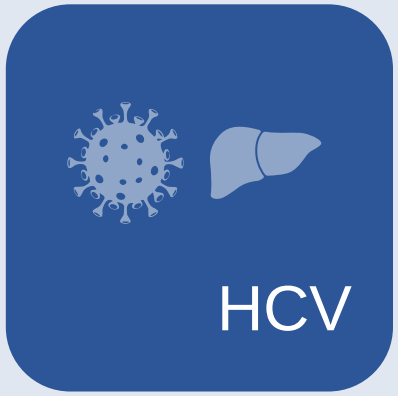
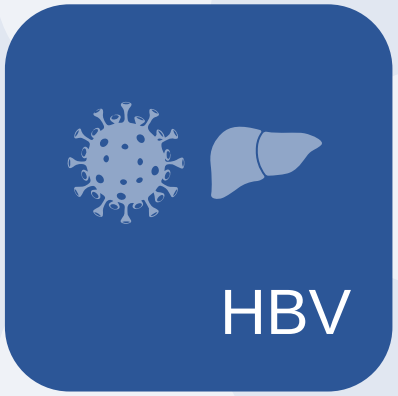
EDP-938
RSV N Inhibitor

EDP-323
RSV L Inhibitor

EDP-235
3CLpro Inhibitor







Glecaprevir-containing pangenotypic 2-DAA combo

MAVYRET
glecaprevir/pibrentasvir
100 mg/40 mg tablets

EDP-514
Core Inhibitor

Our Therapeutic Focus

Leveraging our core strength in Hepatitis C to become a leader in oral treatments for **viral** infections and **liver** diseases

Several new therapeutic areas with goal of building multiple approaches in each

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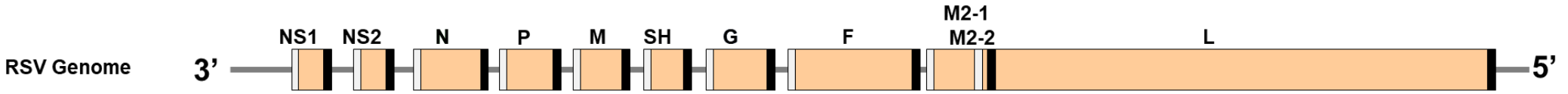
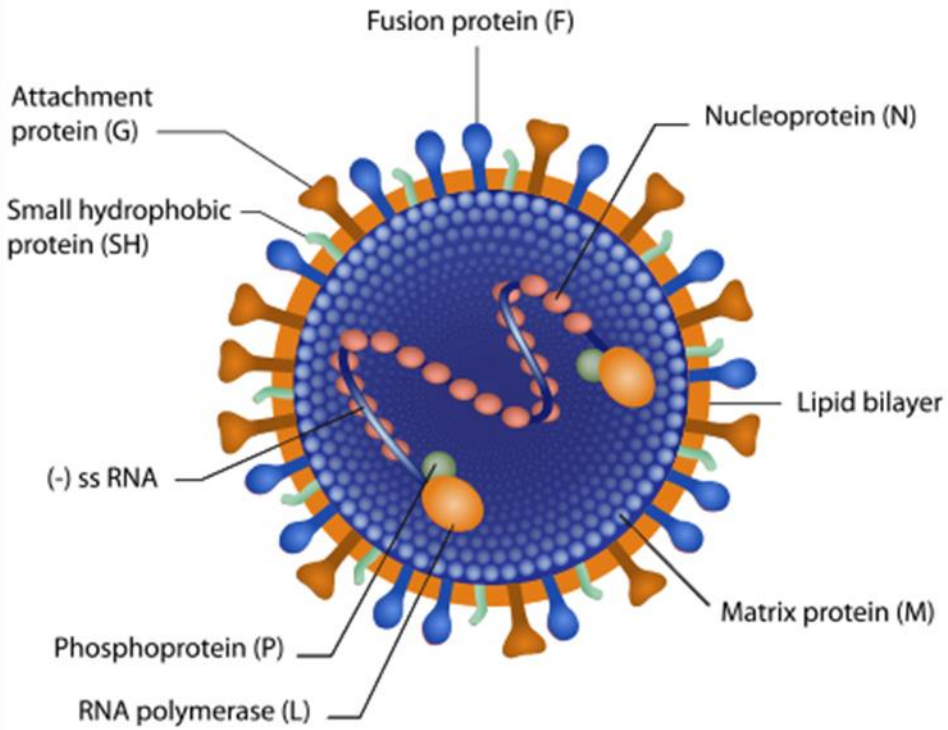
Respiratory Syncytial Virus (RSV)

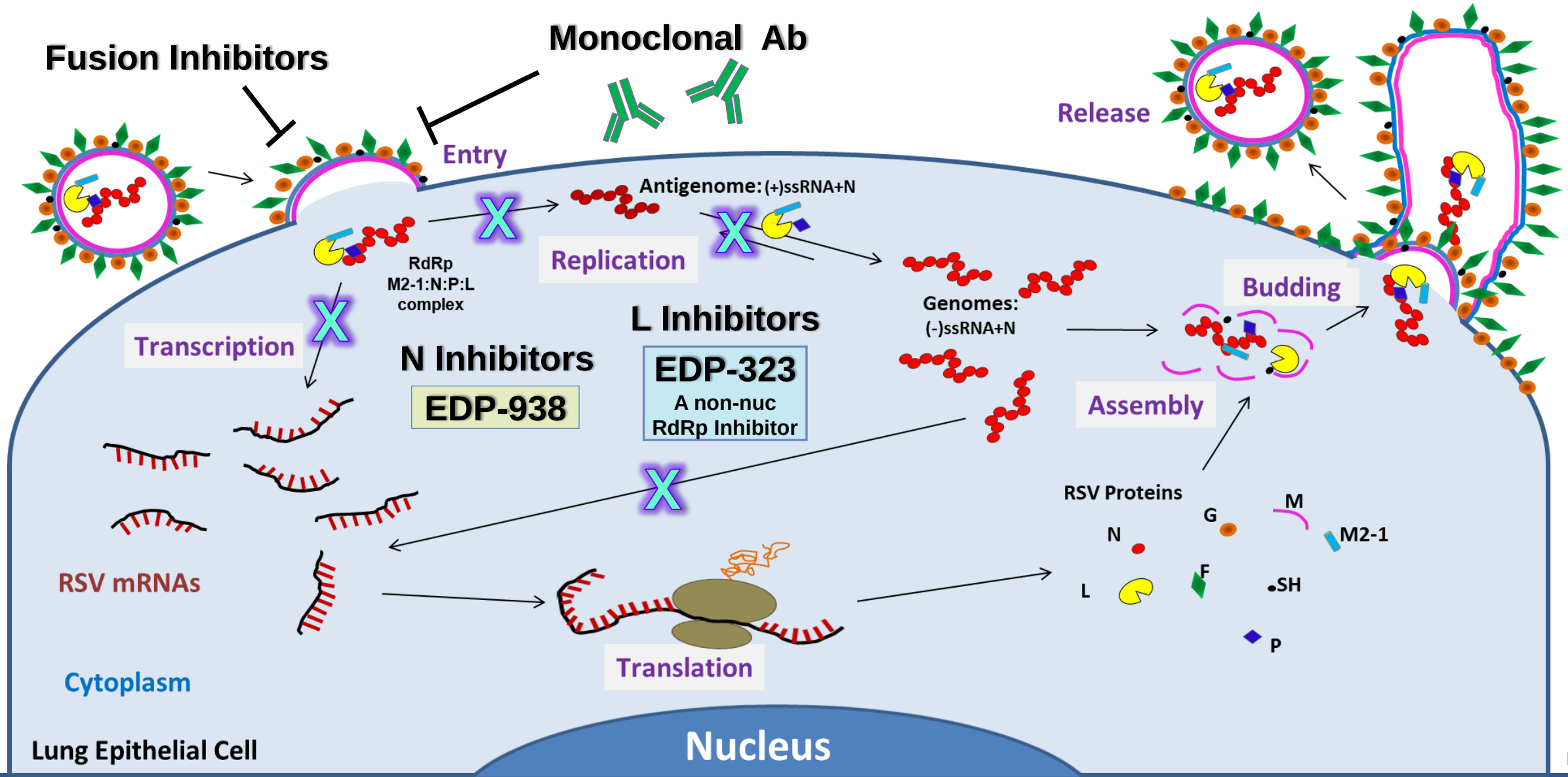
RSV is a (-)ssRNA non-segmented virus with a genome size of roughly 15 kb. It has 10 genes which code for 11 proteins.



- Causes ~25 million acute respiratory tract infections annually in children worldwide, with common re-infections in adults
- Leading cause of hospitalization of children under 5
  - Responsible for >75k deaths per year
- By 1 year of age ~60-70% have had RSV, by 3 years old ~95-99% have had RSV
- Other critically affected groups include immunosuppressed and elderly populations
- Current care:
  - mAb Palivizumab (Synagis) for premature infants in RSV season
  - Supportive care
  - **No approved vaccines**
  - **No approved direct acting therapeutics**



RSV Life Cycle and Antiviral Targets



EDP-323 Displays Potent Antiviral Activity *In Vitro*

EDP-323 Antiviral Activity: $EC_{50/90}$ [nM]

Cell Type	Virus	Readout	EC_{50}	EC_{90}
HEp-2	RSV-A Long	CPE	0.44	0.52
		RT-qPCR	0.84	1.10
	RSV-B VR-955	CPE	0.40	0.57
		RT-qPCR	0.55	0.64
	RSV-A M37	CPE	0.32	0.36
		RT-qPCR	0.34	0.69
	RSV-A2	CPE	0.15	0.19
HBEC	RSV-A Long	RT-qPCR	0.09	0.15
3D pHAEC ALI Culture	RSV-A Long	RT-qPCR	0.16	0.27
	RSV-B VR-955	RT-qPCR	0.09	0.33

EDP-323 Cytotoxicity: CC_{50}

Cells	5 days
HEp-2	18 µM

EDP-323 Activity vs Clinical Isolates $EC_{50/90}$ [nM]

Virus	Isolate	EC_{50}	EC_{90}
RSV-A HEp-2 Cells CPE Readout	80189	0.33	0.45
	Tracy	0.36	0.61
	79365	0.28	0.45
	37425	0.19	0.28
	121301018	0.19	0.21
	79309	0.36	0.68
	79223	0.21	4.20
	121301343	0.18	0.56
	61245	0.20	0.22
RSV-B HEp-2 Cells CPE Readout	57097	0.15	n/a
	60188	0.11	0.31
	79222	0.30	0.75
	65848	0.11	0.30
	65859	0.10	0.31
	121301314	0.04	0.05
	Mean	**0.21**	**0.67**

- Picomolar EC_{90}s in primary human cells, with a CC_{50}/EC_{50} selectivity index >30,000

Assay Condition: MOI 0.1, endpoint 5/6 days post infection (dpi) for RSV-A/B

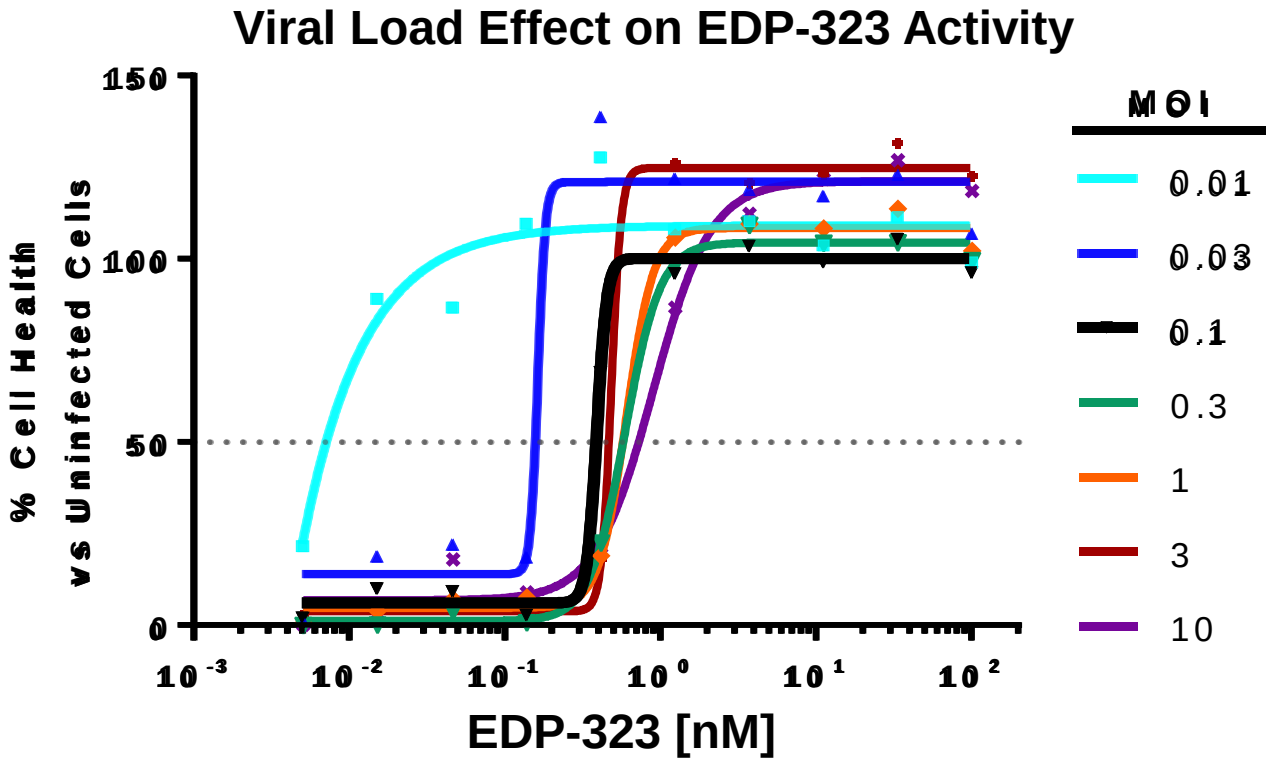
HBEC: primary human bronchial epithelial cells; **3D pHAEC-ALI**: 3-dimmenisional primary human airway epithelial cells grown at an air-liquid interface

EDP-323's Potency is Resilient to Increases in Viral Load

EDP-323 Activity vs Viral Load

Viral Load

MOI	RSV-A Long	
	EC_{50} [nM]	EC_{90} [nM]
10	0.70	1.30
3	0.47	0.53
1	0.56	0.79
0.3	0.56	0.92
0.1	0.38	0.46
0.03	0.16	0.18
0.01	0.01	0.02

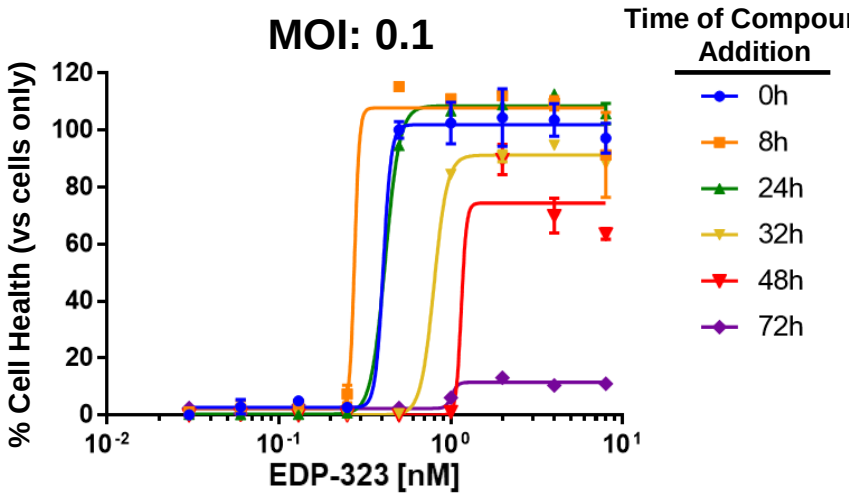


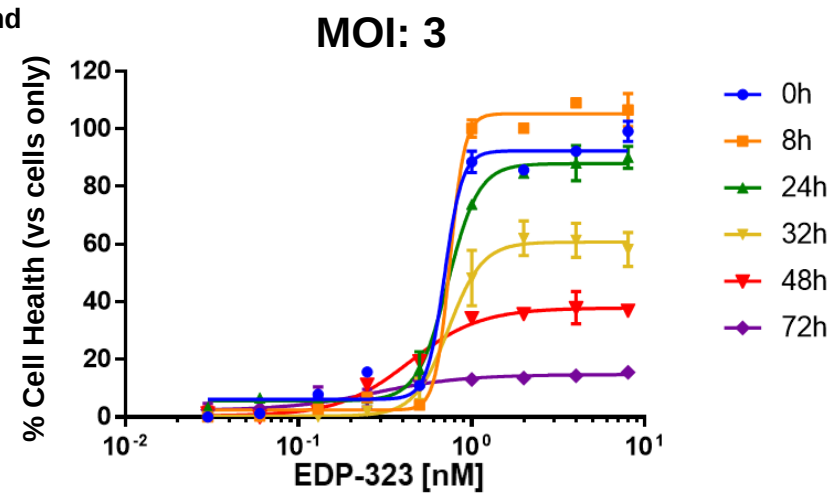
- Improved potency when MOI drops below 0.1
- Only minor potency reduction when viral load increases over MOI 0.1

Assay Condition: HEp-2 cells, RSV-A Long, endpoint 5dpi, CPE readout

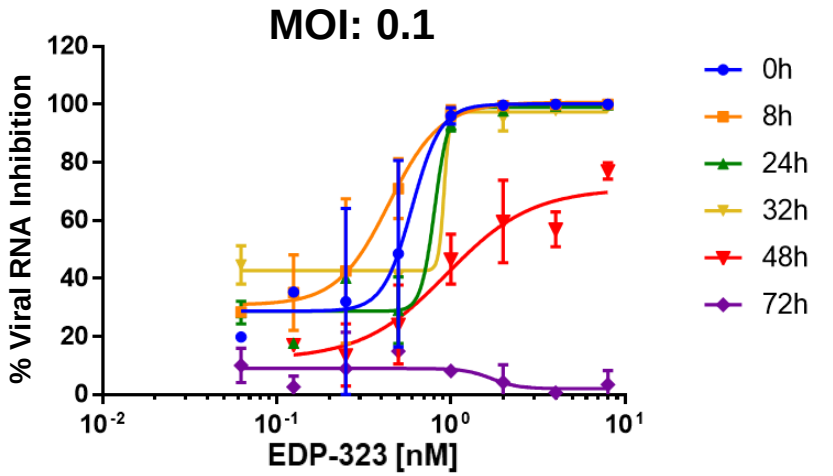
EDP-323 Maintains *In Vitro* Efficacy Post-Infection in 2D Cell Culture

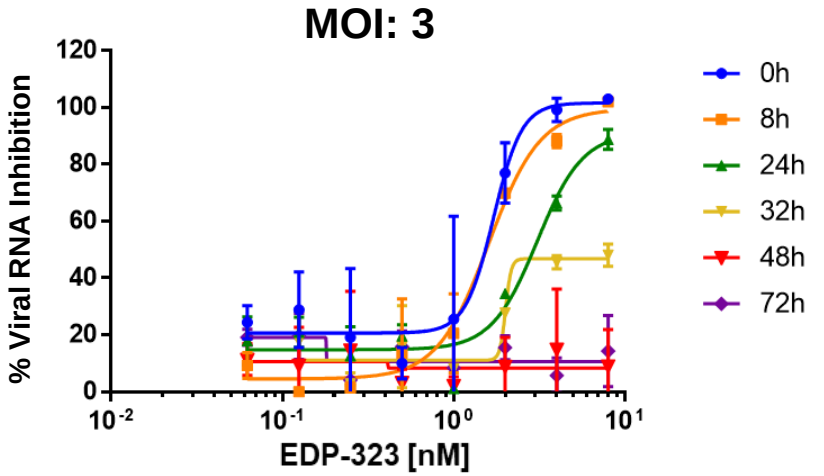
CPE





RT-qPCR





Time of Addition: CPE

ToA (hpi)	EC_{50} [nM]	
	MOI: 0.1	MOI: 3
0 h	0.36	0.7
8 h	0.28	0.76
24 h	0.42	0.76
32 h	0.84	0.97
48 h	1.2	>8.0
72 h	>8.0	>8.0

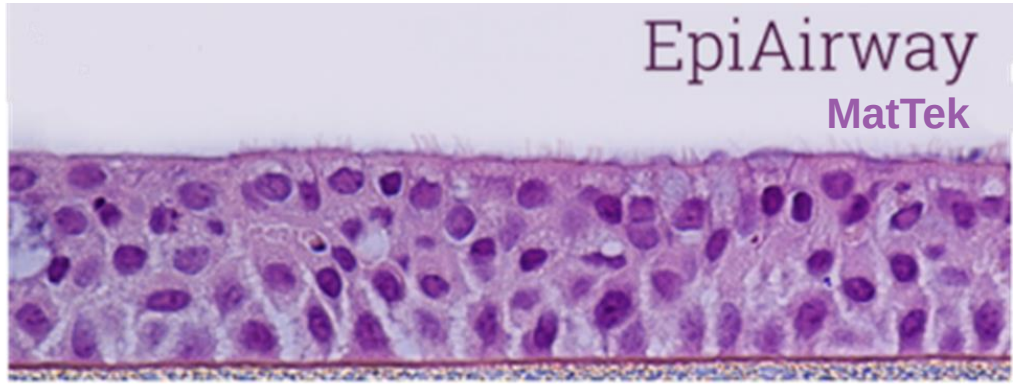
Time of Addition: RT-qPCR

ToA (hpi)	EC_{50} [nM]	
	MOI: 0.1	MOI: 3
0 h	0.44	1.5
8 h	0.35	1.5
24 h	0.74	2.9
32 h	0.72	>8.0
48 h	1.6	>8.0
72 h	>8.0	>8.0

Assay Conditions: HEp-2 cells, RSV-A Long infection, endpoint 5dpi; CPE (ATPlite) or RT-qPCR (N gene)

EDP-323 Maintains *In Vitro* Efficacy Post-Infection in 3D Cell Culture

3D pHAEC-ALI Culture
primary human airway epithelium cells
grown at an air-liquid interface



Ciliated apical surface

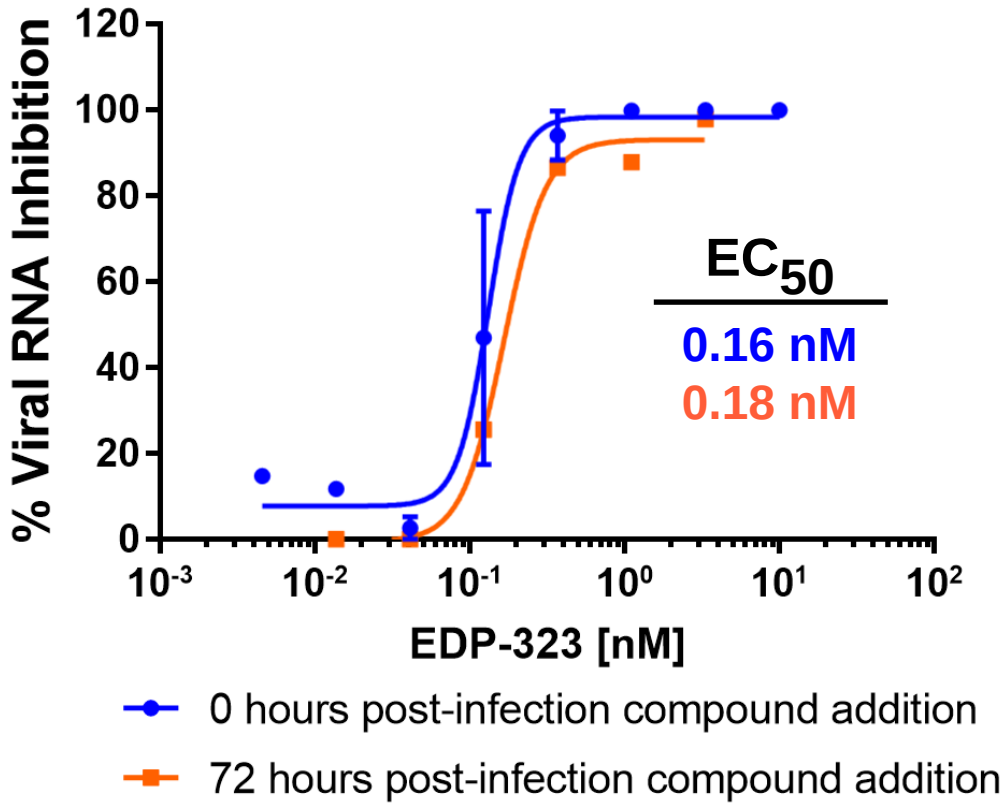
Mucociliary epithelium

Microporous membrane

Basal media supply

- **Infect at apical surface**
- **EDP-323 added to basal media**

RSV-A Long in pHAEC-ALI Culture



- EDP-323 maintains activity 72 hours post-infection in the pHAEC-ALI culture

Imaging of 3D pHAEC-ALI Culture Highlights EDP-323's ability to Reduce Viral Infection

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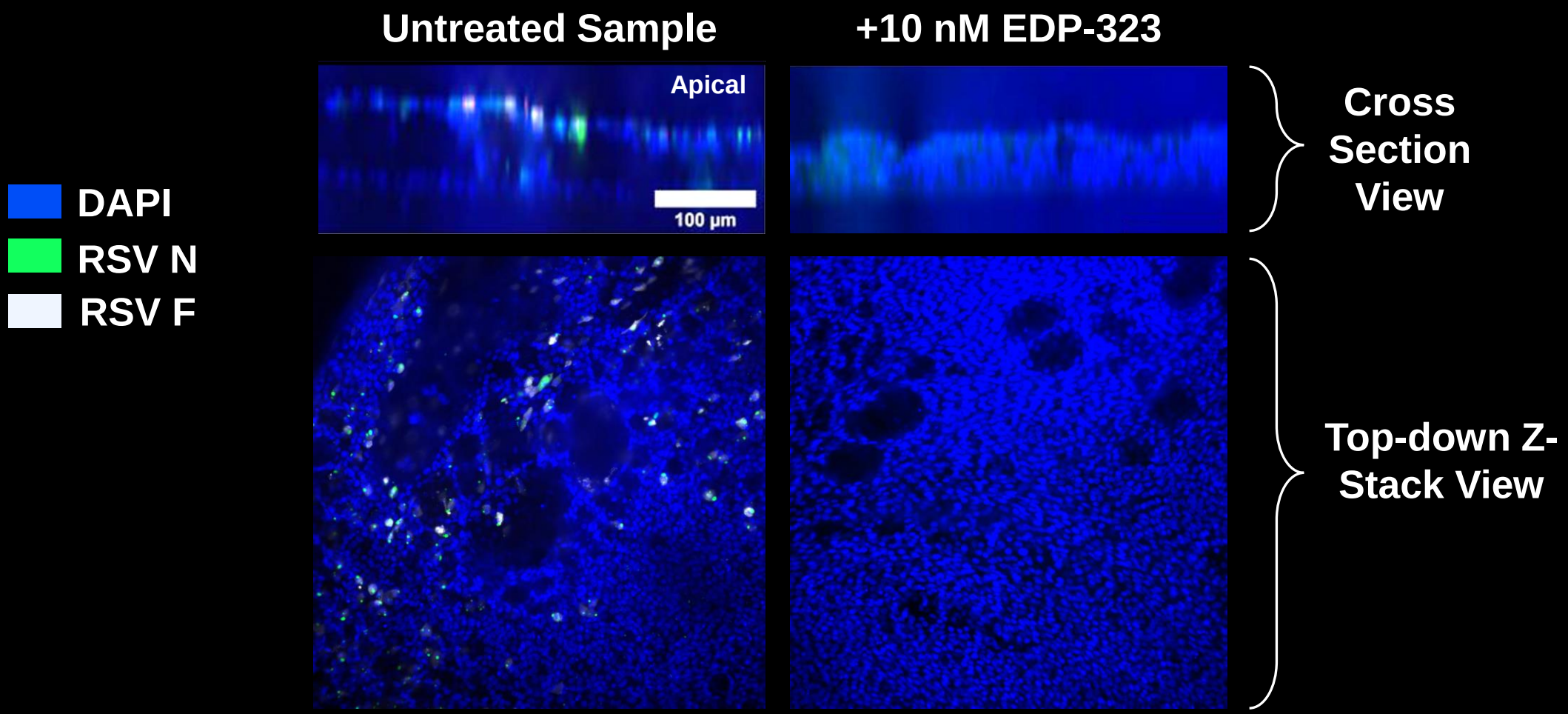
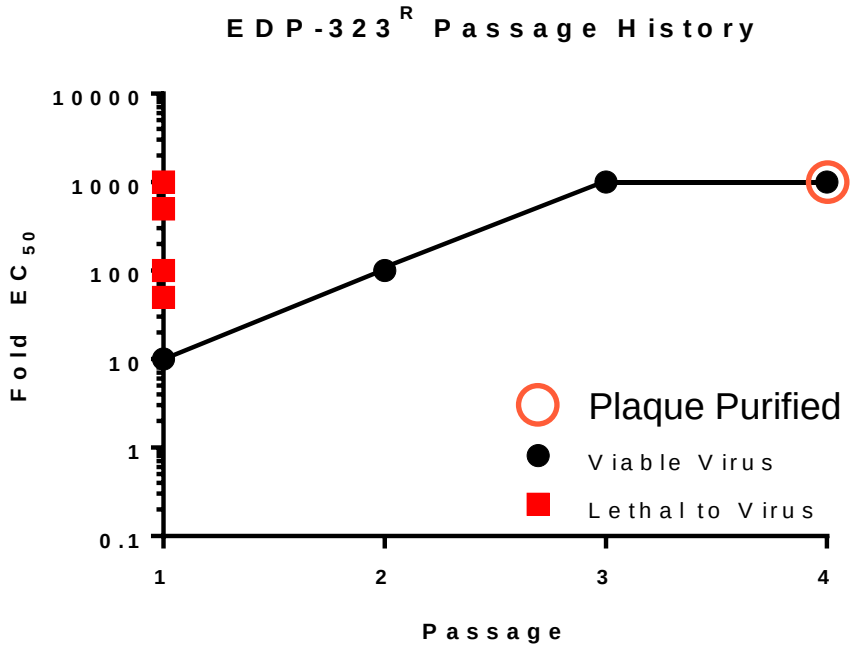


Assay Conditions: EpiAirway 3D pHAEC-ALI infected with RSV-A Long at 3.1 x 10^4 PFU and treated with EDP-323 or vehicle control. 5 days post-infection, tissues fixed and stained.

EDP-323 Resistance Mapping Indicates Binding to Capping Domain of RdRp



Plaque Purified and Sequenced

18/18 plaques: **L:** L1372V
18/18 plaques: **L:** C1388G
3/18 plaques: **L:** I392L
3/18 plaques: **L:** K1532E

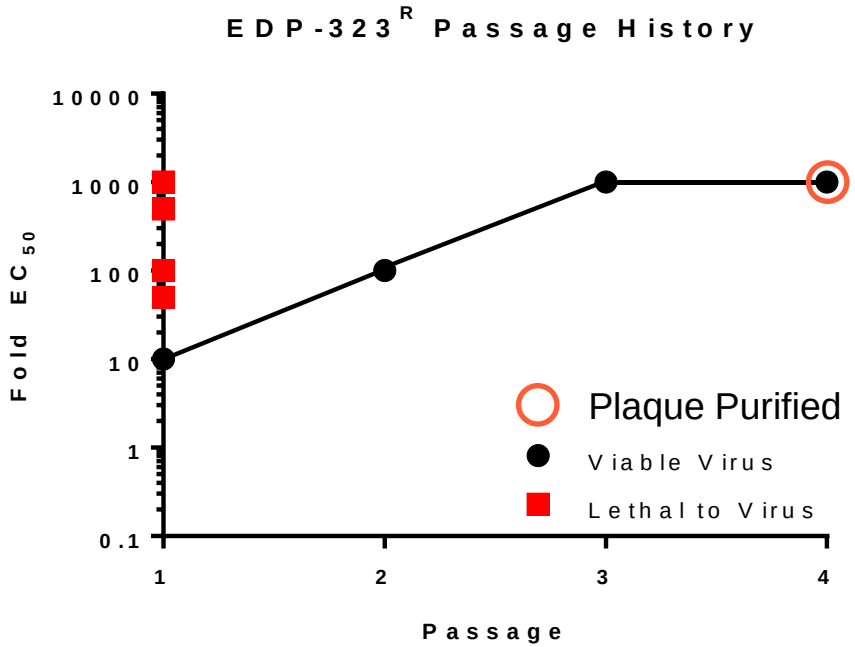
EDP-323 Cross Resistance Profiling

Compound	Target	WT	L1372V, C1388G	I392L, L1372V, C1388G	L1372V, C1388G, K1532E
		EC_{50} nM	Fold-shift	Fold-shift	Fold-shift
EDP-323	**L (non-nuc.)**	**0.3**	**10,453**	**7,963**	**7,834**
EDP-938	Nucleoprotein	21	4	3	4
AZ-27	L (non-nuc.)	15	2	1	2
PC-786	L (non-nuc.)	1.9	3	2	3
BI-D	L (non-nuc.)	56	2	3	2
ALS-8176	L (nuc.)	1,209	6	4	7
Remdesivir	L (nuc.)	54	2	1	1
GS-5806	Fusion	1.2	1	1	1
RV-521	Fusion	2.8	1	1	2

Note: WT and mutant strains are in an RSV-A Long background, CPE readout

Wild-type RSV-A Long was unable to survive initial treatment ≥50X EDP-323's EC_{50}

EDP-323 Resistance Mapping Indicates Binding to Capping Domain of RdRp



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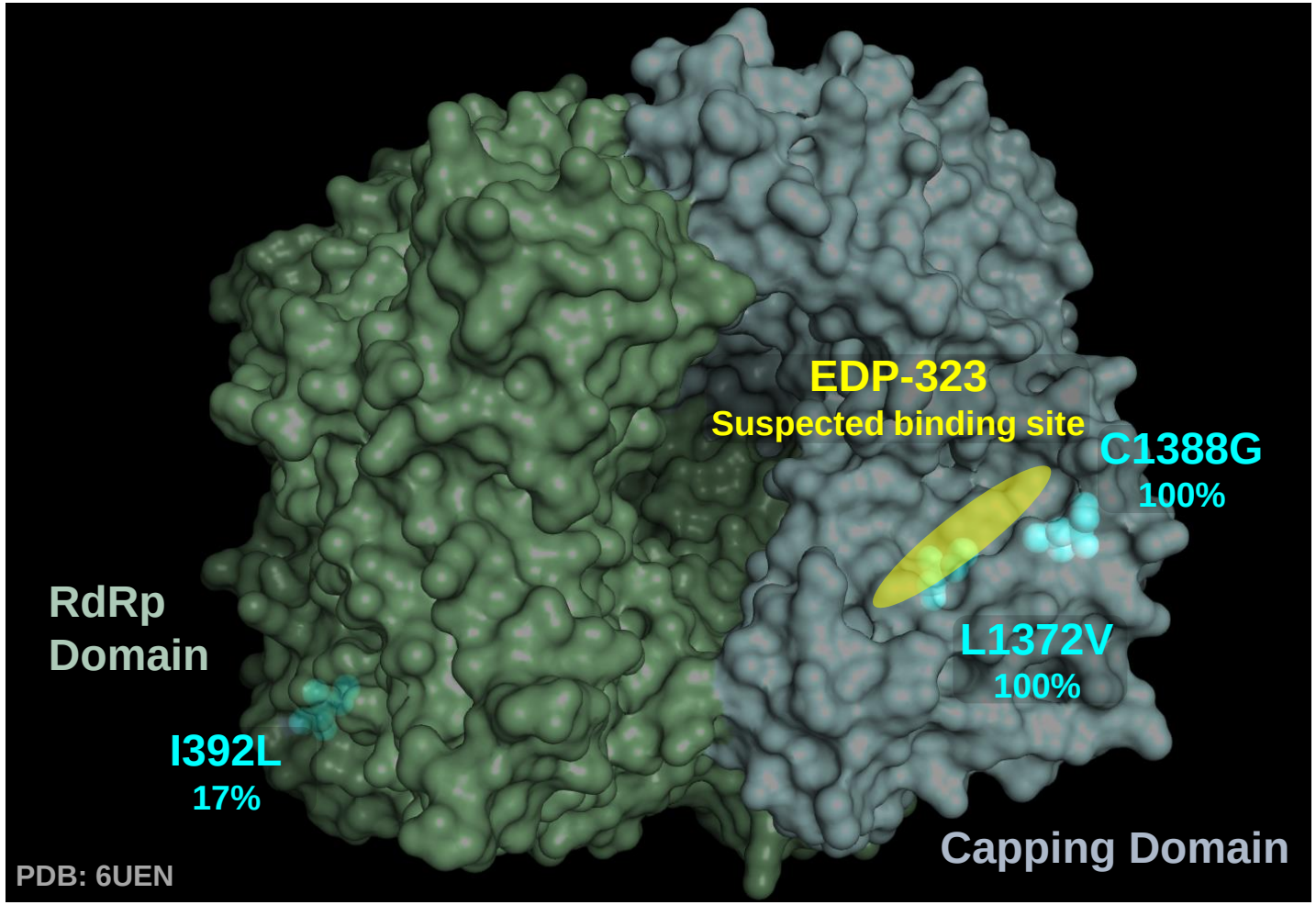
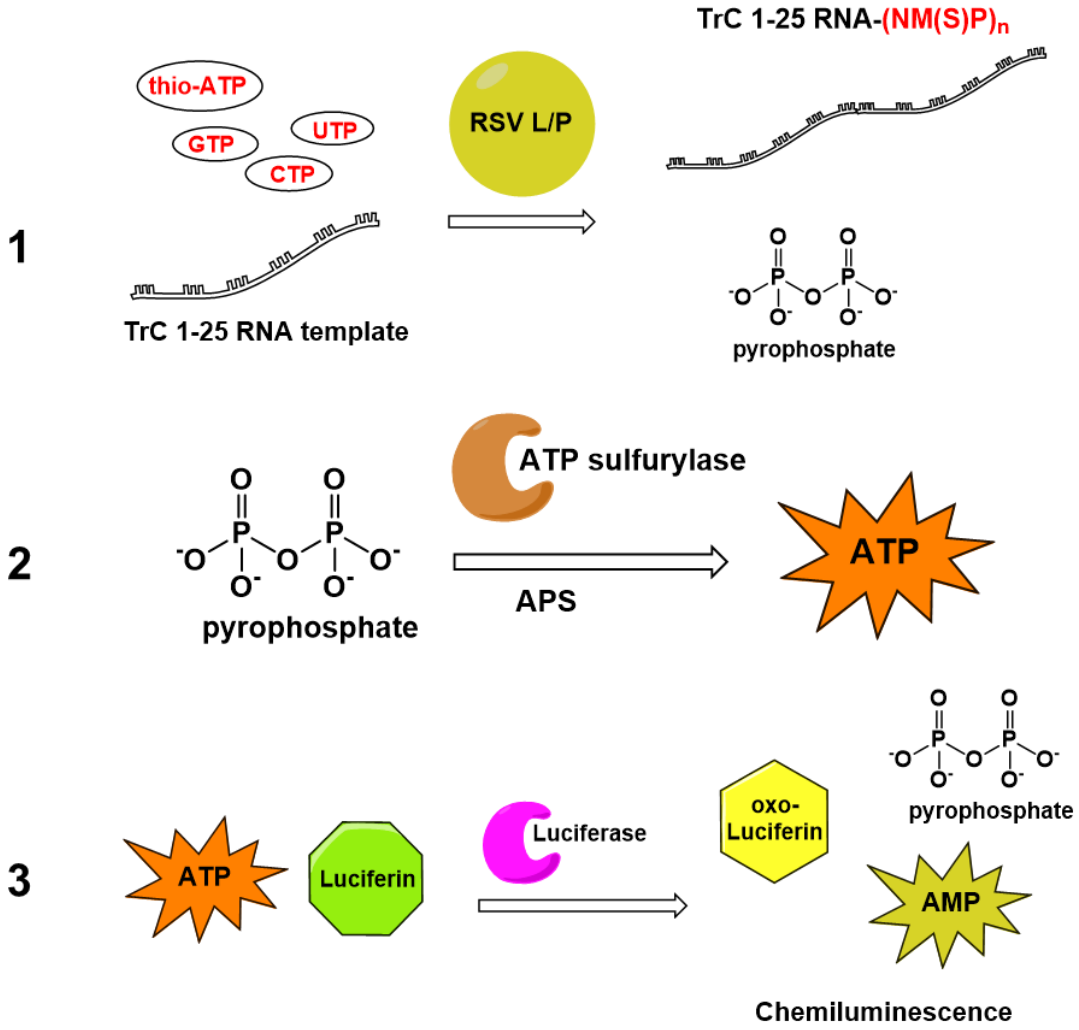


Cryo-EM: Cao, D. et al. DOI: 10.1038/s41467-019-14246-3

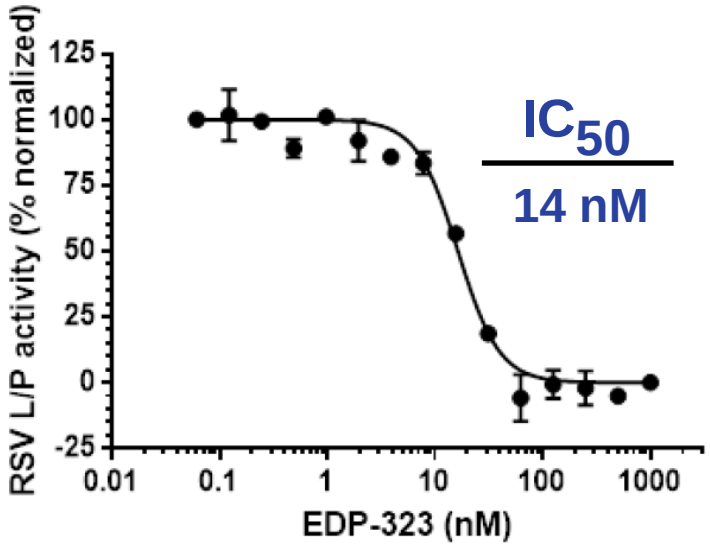
EDP-323 Directly Inhibits L Protein Function

Biochemical Assay



Inhibition of RSV L/P RdRp Activity by EDP-323

Pyrophosphate detection by enzyme-coupled luminescence assay



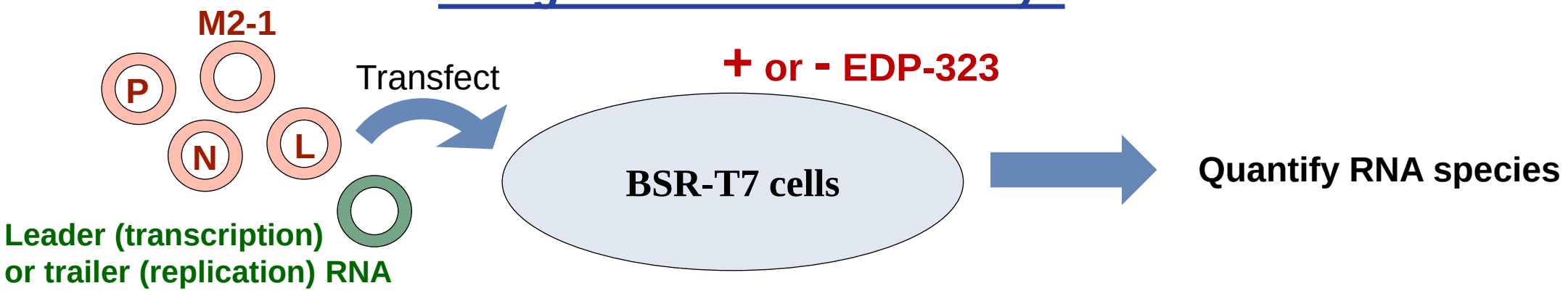
RSV Proteins:
L, P (18 µg/mL)

+ tr-25 RNA (2 µM)
+ 1-thio-ATP (100 µM)
+ C/G/UTP (0.03 µM)

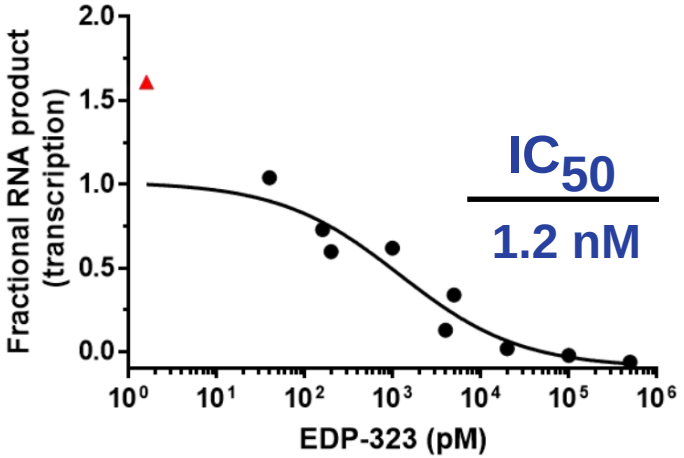
- EDP-323 directly interferes with L protein functionality

EDP-323 Directly Inhibits L Protein Function

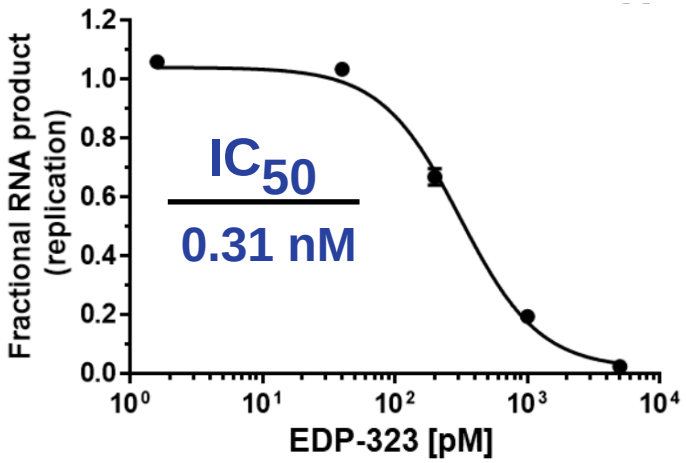
Minigenome Cellular Assays



RNA Transcription Inhibition

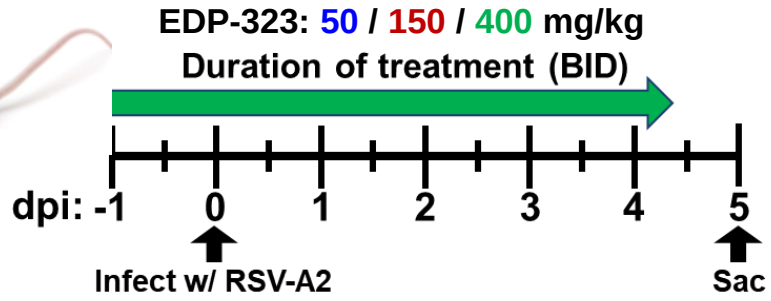


RNA Replication Inhibition



EDP-323 Protects RSV-Infected Mice in a Dose-Dependent Manner

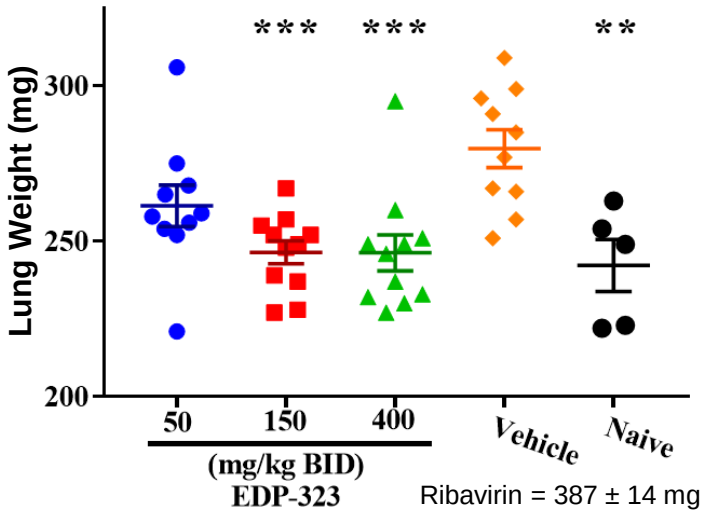
N = 12 /EDP-323 cohort



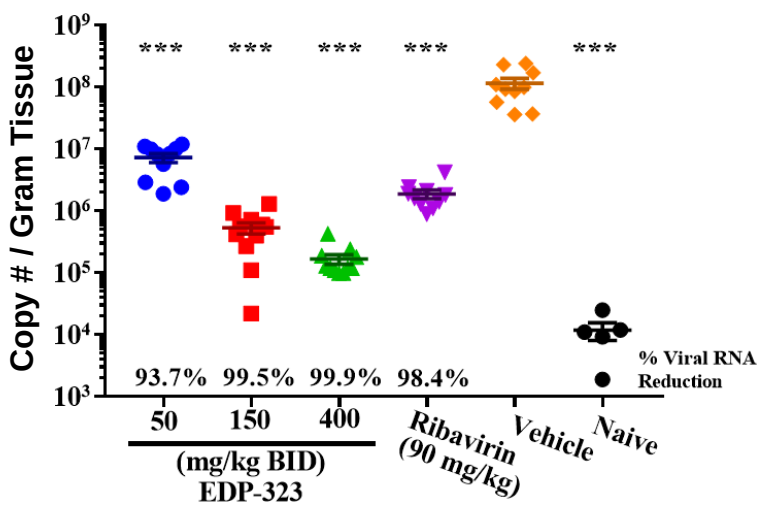
EDP-323 Antiviral Activity: $EC_{50/90}$ [nM]

Cells	Virus	Readout	EC_{50}	EC_{90}
BALB/c Lung	RSV-A2	RT-qPCR	1.00	4.10
	RSV-A Long	RT-qPCR	1.20	5.30
3D pHAEC ALI Culture	RSV-A Long	RT-qPCR	0.16	0.27
	RSV-B VR-955	RT-qPCR	0.09	0.33

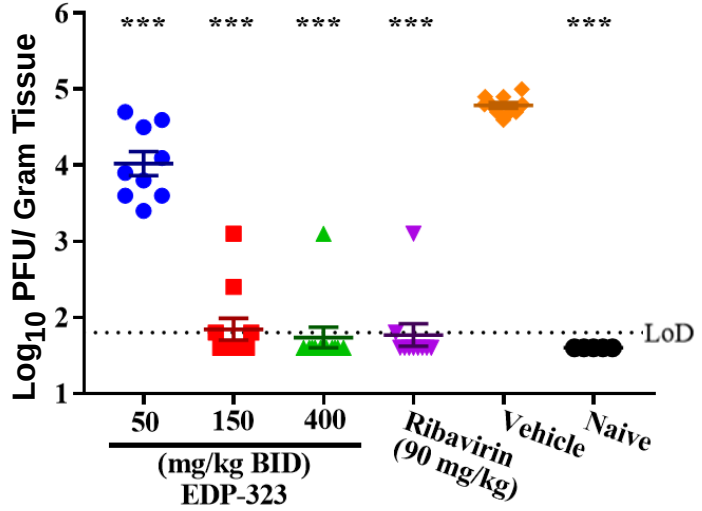
Lung Weights



Lung Titers: Viral RNA (qPCR)

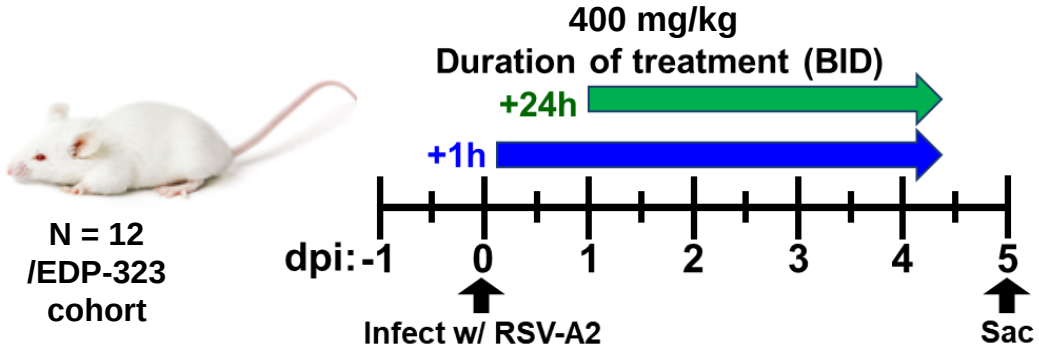


Lung Titers: Live Virus (Plaque Assay)



** p<0.01, ***p < 0.001 versus vehicle. LoD is 1.8 log10 PFU/gram tissue. Values below LoD set arbitrary at 1.6 log10 PFU/g tissue. Error bars are SEM. Ribavirin dosed SC BID from -1 - 4 dpi at 90 mg/kg/day.

EDP-323 Therapeutically Protects RSV-Infected Mice



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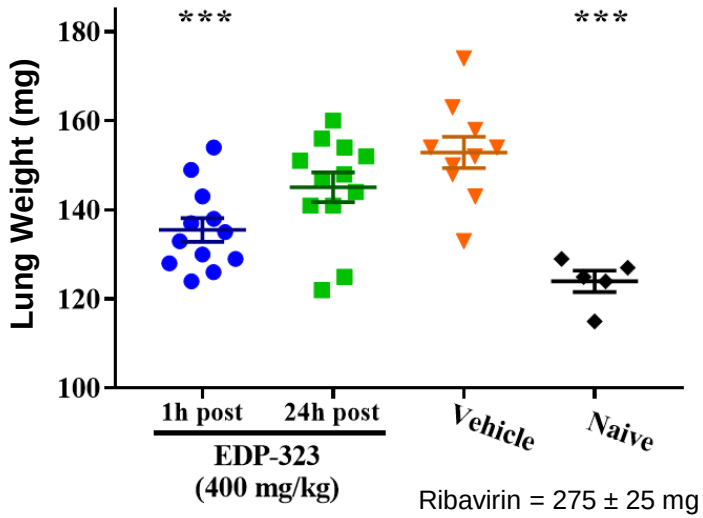

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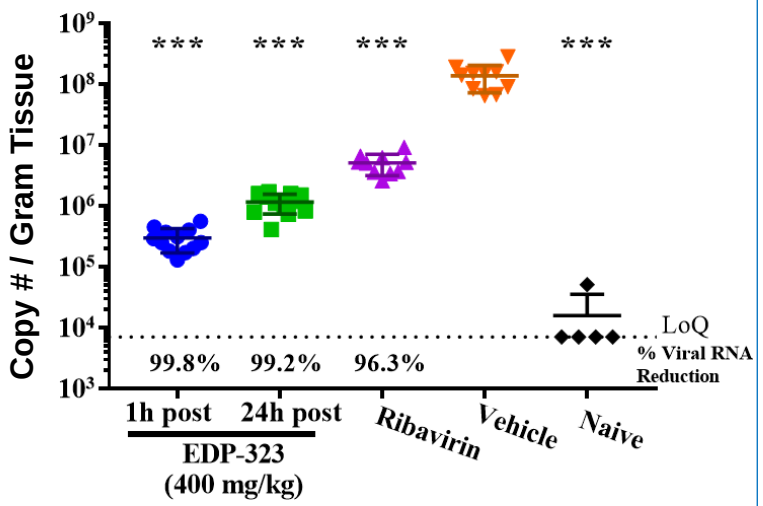

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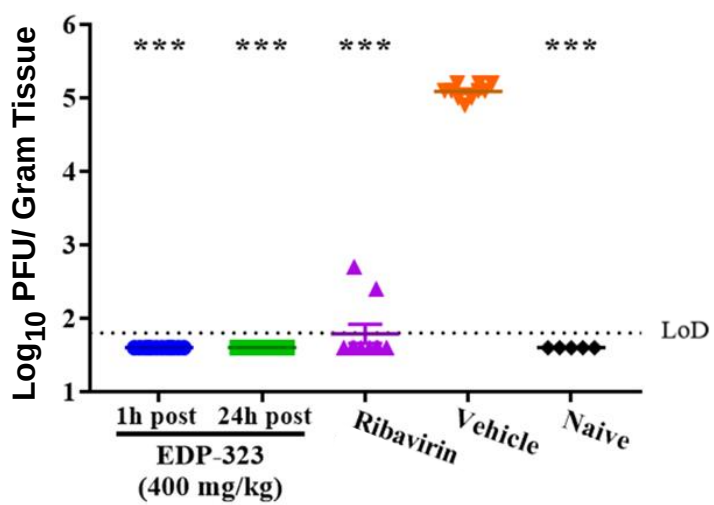
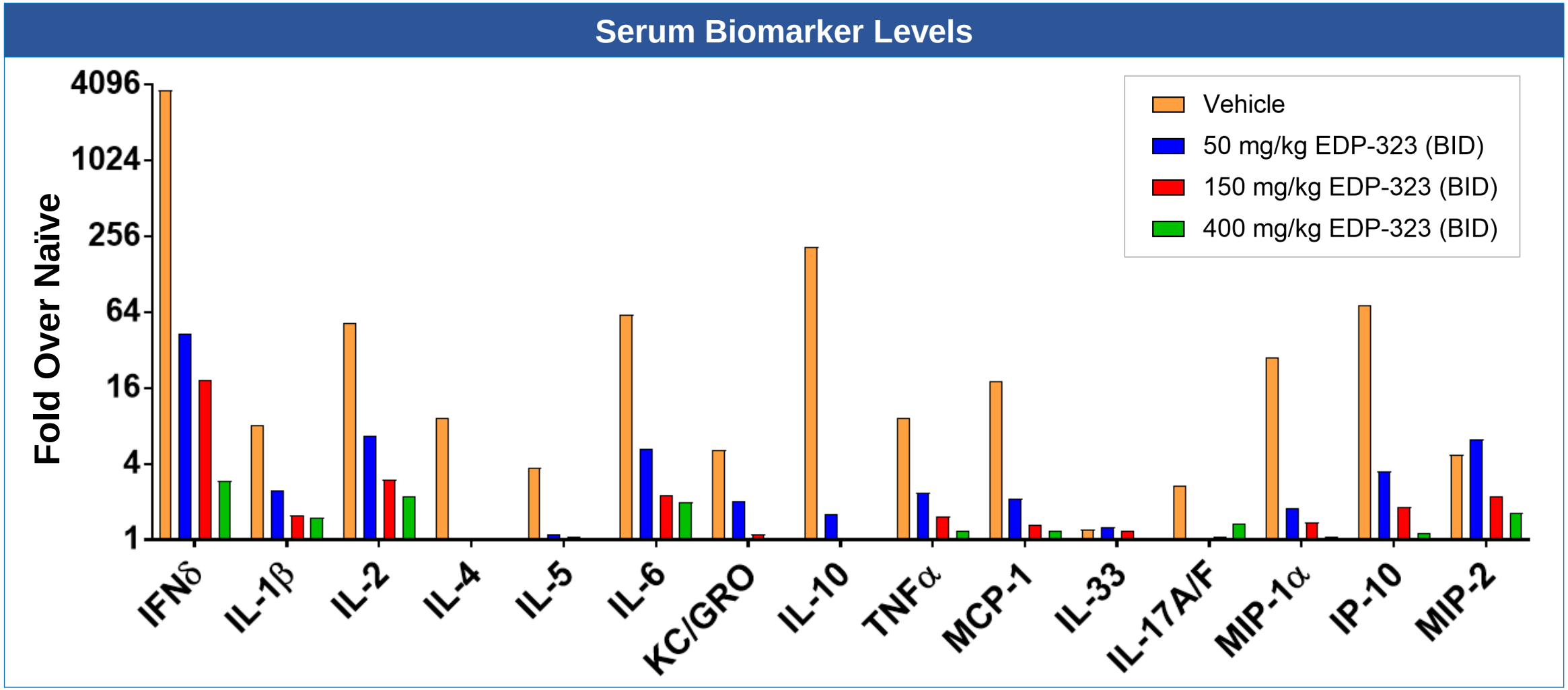


***p < 0.001 versus vehicle. LoD is 1.8 log10 PFU/gram tissue. Values below LoD set arbitrary at 1.6 log10 PFU/g tissue. LoQ is 7 x 10^3 copies/gram tissue. Error bars are SEM. Ribavirin dosed SC QD from +1 hpi - 4 dpi at 90 mg/kg/day.

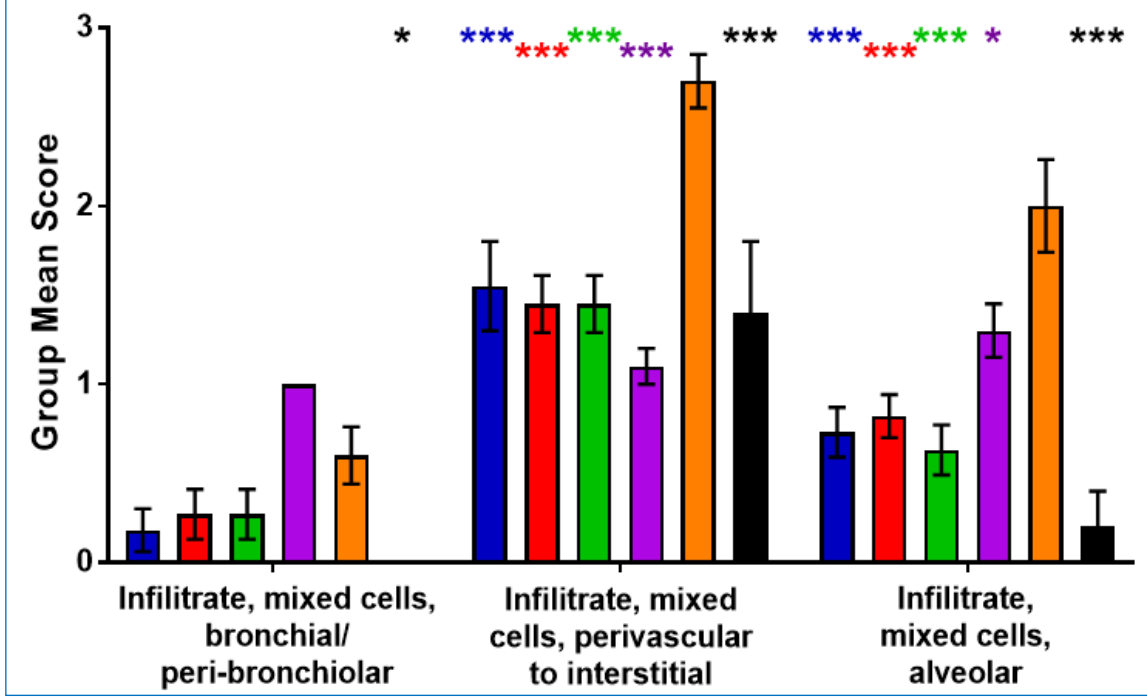
EDP-323 Impairs Viral-Induced Cytokine Responses in Mice



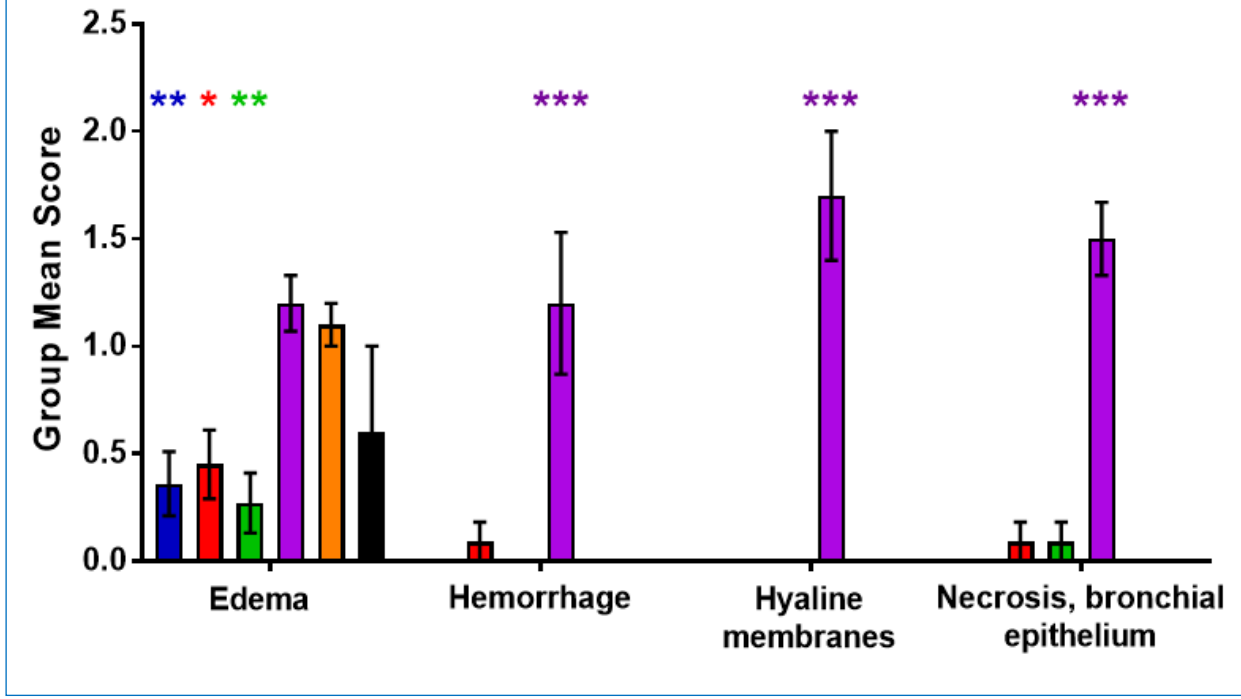
- EDP-323 inhibits RSV-induced increases in pro-inflammatory cytokine biomarkers

EDP-323 Reduces Viral-Induced Pathology in Mice

Mouse Lung Mean Inflammatory Cell Infiltrate Scores



Mouse Lung Mean Histopathology Scores



50 mg/kg EDP-323 (BID) | 150 mg/kg EDP-323 (BID) | 400 mg/kg EDP-323 (BID)
Ribavirin 90 mg/kg QD | Vehicle | Naive

- All EDP-323 cohorts significantly reduced viral-induced pathology vs vehicle treated infected animals

***p<0.001, **p<0.01 by one one-way ANOVA followed by Dunnett's multiple comparison test with infected, vehicle-treated animals.

EDP-323, A Potent L-Protein Inhibitor for the Treatment of RSV

EDP-323…

- is a non-nucleoside inhibitor of the RSV RdRp protein inhibiting both viral replication and transcription
- is active against both RSV-A and RSV-B subtypes with low picomolar EC_{50}s in multiple primary human cell culture systems
- maintains efficacy and potency across a range of viral loads and times post-infection
- protects mice in a dose-dependent manner from RSV infection both prophylactically and therapeutically as quantified by both virological and pathological endpoints
- demonstrates favorable oral bioavailability and PK properties to support once-a-day oral dosing
- Has initiated phase 1 human clinical trials this week

Acknowledgements

Enanta Pharmaceuticals, Inc.'s RSV Team:

- **Chemistry**: Yat Sun Or, In Jong Kim, Jianming Yu, Adam Szymaniak, Kevin McGrath, Tyler Mann
- **Virology**: Nicole McAllister, Nalini Bisht, Rachel Levene, Joyce Sweeney Gibbons, Nathan Manalo, Bryan Goodwin
- **DMPK**: Lijuan Jiang, Indy Zhang
- **Biochemistry**: Anand Balakrishnan, Archie Reyes

External Collaborators/Contributors

- **Boston University MoA Studies**: Barbara Ludeke, Rachel Fearns
- **Mouse Efficacy Studies**: Aragen Life Sciences
- **3D pHAEC-ALI RSV Imaging**: Visikol

Thank you!

Questions?

