Discovery and Development of Novel and Potent Non-Fusion Inhibitors of RSV


11/29/2018

Disclosures: All contributors are employees of Enanta Pharmaceuticals.
RSV Life Cycle and Antiviral Targets

Lung Epithelial Cell

Cytoplasm

Nucleus

RSV mRNAs

Enterovirusds:

(+)

ssRNA+N

Antigenome:

(-)

ssRNA+N

Transcription

Replication

N Inhibitors

L Inhibitors

Fusion Inhibitors

EDP-938

Acts Post Viral Entry

Assembly

Budding

G

M

M2-1

N

L

F

SH

P

RSV Proteins

EDP-938

Release

RDS-938

Inhibitors

Acts Post Viral Entry

RSV Proteins

L Inhibitors

Translation

Budding

RSV Proteins

L Inhibitors

Translation
EDP-938: A Novel Potent RSV N Inhibitor

- **RSV-604**: the previously known RSV nucleoprotein (N) inhibitor*
  - *In vitro* resistance selection mapped to RSV N protein but exact MoA unclear
  - Clinical Proof of Concept efficacy demonstrated: 2.31-log viral load reduction after 5-day treatment in a sub-population of RSV infected stem cell transplantation patients with drug level above EC$_{90}$*

- **EDP-938** has been discovered as a much more potent RSV N inhibitor with no significant cytotoxicity (CC$_{50}>50$ µM)

\[ \text{EDP-938 EC}_{50}=72 \text{ nM} \]
\[ \text{RSV-604 EC}_{50}=1,164 \text{ nM} \]

* Chapman et al 2007 AAC
* Chapman and Cockerill, 2011 Antiviral Drugs

\[ \text{EDP-938 EC}_{50}=52 \text{ nM} \]
\[ \text{RSV-604 EC}_{50}=1,451 \text{ nM} \]
EDP-938 Potently Inhibits All RSV Lab and Clinical Strains Tested *in vitro*

### RSV laboratory strains

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Strain</th>
<th>Cell</th>
<th>Assay</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV-A</td>
<td>M37</td>
<td>HBEC</td>
<td>PCR</td>
<td>23 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEp-2</td>
<td>PCR</td>
<td>54 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEp-2</td>
<td>CPE</td>
<td>28 ± 4</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>HBEC</td>
<td>PCR</td>
<td>20 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEp-2</td>
<td>PCR</td>
<td>89 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEp-2</td>
<td>CPE</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>RSV-B</td>
<td>A2</td>
<td>HEp-2</td>
<td>PCR</td>
<td>59 ± 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEp-2</td>
<td>CPE</td>
<td>28 ± 4</td>
</tr>
<tr>
<td></td>
<td>Wash</td>
<td>HBEC</td>
<td>PCR</td>
<td>62 ± 32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A549</td>
<td>PCR</td>
<td>83 ± 38</td>
</tr>
</tbody>
</table>

### Clinical isolates from the Netherlands

(mostly pediatrics)

<table>
<thead>
<tr>
<th>Subtype (# of isolates)</th>
<th>Cell</th>
<th>Assay</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV-A (n=10)</td>
<td>HEp-2</td>
<td>ViroSpot</td>
<td>43 ± 8</td>
</tr>
<tr>
<td>RSV-B (n=10)</td>
<td>HEp-2</td>
<td>CPE</td>
<td>51 ± 9</td>
</tr>
</tbody>
</table>

### Clinical isolates from the US

<table>
<thead>
<tr>
<th>Subtype (# of isolates)</th>
<th>Cell</th>
<th>Assay</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV-A (n=12)</td>
<td>HEp-2</td>
<td>CPE</td>
<td>68 ± 26</td>
</tr>
<tr>
<td>RSV-B (n=10)</td>
<td>HEp-2</td>
<td>CPE</td>
<td>116 ± 4</td>
</tr>
</tbody>
</table>

CPE: Cytopathic Effect  
HBEC: primary Human Bronchial Epithelial Cells
EDP-938 Shows *in vitro* Efficacy Post Viral Infection

Time of EDP-938 Addition Post Infection

Cell Viability (%)

EDP-938 [μM]

- 2 hr (n=6)
- 2 hr (n=6)
- 6 hr (n=6)
- 24 hr (n=3)

RSV-A Long, MOI = 0.1
CPE readout, 5 days post infection endpoint
Combinations of EDP-938 with other RSV Inhibitors Result in Moderate Synergy

EDP-938 + EDP-938

EDP-938 + Fusion Inhibitor

EDP-938 + L Inhibitor

EDP-938 + Nuc

Conditions
• RSV-A Long
• HEp-2 cells
• MOI=0.1
• CPE assay

Analysis using Loewe additivity model

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ave. Combination Index (CI) at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50}</td>
</tr>
<tr>
<td>EDP-938 + EDP-938</td>
<td>0.8</td>
</tr>
<tr>
<td>EDP-938 + ALS-8112</td>
<td>0.7</td>
</tr>
<tr>
<td>EDP-938 + AZ-27</td>
<td>0.8</td>
</tr>
<tr>
<td>EP-938 + GS-5806</td>
<td>0.9</td>
</tr>
</tbody>
</table>

CI <0.9 = synergy
CI >1.1 = antagonism
CI 0.9 - 1.1 = additivity
EDP-938 Demonstrates *in vivo* Efficacy in the African Green Monkey Model

N=4 per group, dosing:100mg/kg BID compound, LOD (limit of detection) = 100 copies/mL, virus: RSV-A2
RSV Rapidly Develops Resistance to Fusion and L Polymerase Inhibitors

**Fusion Inhibitor**

Mutations in F: L141V/N197T
>40,000-fold EC$_{50}$ shift

- Resistance mutations also emerged quickly in the human challenge study and in patients treated with fusion inhibitors.

**L Polymerase Inhibitor**

Mutations in L: Y1631H/R/C
>1,000-fold EC$_{50}$ shift

- 10X EC$_{50}$ starting concentration
- RSV-A Long strain
- 0.1 MOI initial infection
EDP-938 Displays a High Barrier to RSV-A Resistance Selection \textit{in vitro}

- Exposing RSV-A to \geq4\times EC_{50} EDP-938 resulted in complete elimination of the virus rather than selection of resistance
- A slow, stepwise increase in EDP-938 concentration, starting with 1\times EC_{50}, eventually led to viral populations surviving up to 64\times EC_{50} of EDP-938

\textit{Note:} Black filled markers indicate failure of the virus to survive at any concentration level tested at or after this collection. All cultures initiated with a viral MOI of 0.1 using RSV-A Long.
EDP-938 Displays a High Barrier to RSV-B Resistance Selection *in vitro*

- Exposing RSV-B to ≥4xEC\(_{50}\) EDP-938 resulted in complete elimination of the virus rather than selection of resistance.
- A slow, stepwise increase in EDP-938 concentration, starting with 1xEC\(_{50}\), eventually led to viral populations surviving up to 32xEC\(_{50}\) of EDP-938.

**Note:** Black filled markers indicate failure of the virus to survive at any concentration level tested at or after this collection. All cultures initiated with a viral MOI of 0.5 – 1 using RSV-B VR-955.
## RSV Resistance Mutations Against EDP-938

**RSV Genome**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutations in RSV Proteins</th>
<th>EDP-938 EC$_{50}$ Fold Change vs. WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-Type (WT) A / B</td>
<td>N</td>
<td>G</td>
</tr>
<tr>
<td>RSV-A</td>
<td>#1 M109K</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>#2 Q102L M109T I129M</td>
<td>K205G K213G T219A</td>
</tr>
<tr>
<td></td>
<td>#3 V90A S134T</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>#4 T29S S134T</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>#5 M109I</td>
<td>R8H</td>
</tr>
<tr>
<td></td>
<td>#6 K136R</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>#7 S134T</td>
<td>-</td>
</tr>
<tr>
<td>RSV-B</td>
<td>Population 1</td>
<td>L139Q*</td>
</tr>
<tr>
<td>Population 2</td>
<td>M109T</td>
<td>E226G*</td>
</tr>
</tbody>
</table>

- Of note: N is the most conserved RSV gene while G is the least.
Location of Mutations Found in the RSV N Protein of RSV-A & -B

RSV-A N Protein (a.a. 32-253)

Red = RSV A drug resistant mutation
Blue = RSV-B drug resistant mutation
Purple = Both
# RSV-A Reverse Genetics System: Fold Resistance Contribution by Mutation

<table>
<thead>
<tr>
<th>RSV-A Virus</th>
<th>Mutations in RSV N</th>
<th>EDP-938 EC₅₀ Fold Change vs. WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Mutant Clones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M109K</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Q102L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M109T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I129M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V90A</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>S134T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T29S</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>S134T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M109I</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>K136R</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>S134T</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

Assay MOI = 0.1
WT = 45 ± 21 nM

Individual Fold Resistance Contribution

N Protein
(a.a. 32-253)
Fitness of Mutants Inversely Correlates with Resistance

- Cytopathic effect and infectivity of mutant virus decreases with increased resistance to EDP-938
- The 2 most resistant mutants are also the least fit (100 times less than wild-type)

<table>
<thead>
<tr>
<th>RSV-A&lt;sup&gt;R&lt;/sup&gt; Clones: Mutations in Proteins</th>
<th>EDP-938 EC&lt;sub&gt;50&lt;/sub&gt; Fold Change vs. WT</th>
<th>Average % Cell Viability Days 4-5 Post Infection</th>
<th>% Mutant PFU vs WT AUC days 1-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M109K</td>
<td>-</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Q102L M109T I129M</td>
<td>K205G K213G T219A</td>
<td>50</td>
<td>106</td>
</tr>
<tr>
<td>V90A S134T</td>
<td>-</td>
<td>3.8</td>
<td>66</td>
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<td>T29S S134T</td>
<td>-</td>
<td>3.3</td>
<td>69</td>
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<tr>
<td>M109I R8H</td>
<td>3.1</td>
<td>23</td>
<td>54</td>
</tr>
<tr>
<td>K136R</td>
<td>-</td>
<td>2.7</td>
<td>48</td>
</tr>
<tr>
<td>S134T</td>
<td>-</td>
<td>2.6</td>
<td>24</td>
</tr>
</tbody>
</table>
EDP 938-001: Phase 1 Study, First-In-Human (FIH) 
Overall Safety Data During SAD and MAD

• In the EDP 938-001, a randomized, double-blind, placebo-controlled study:
  - A total of 90 subjects enrolled (N = 50 in SAD/FE; N = 40 in MAD)

• All randomized subjects completed the study in both SAD and MAD phases

• EDP-938 was generally safe and well-tolerated across all cohorts
  • Adverse events (AEs) were of mild intensity
    • Headache was the most frequent AE in the SAD and MAD with the majority reported as possibly related to EDP-938 or placebo, and with no relationship to dose
  • No SAEs or AEs that led to study drug discontinuation were reported

EDP 938 absorbed rapidly with dose dependent exposure
- Median $T_{\text{max}}$ ranged from 2.0 – 5.0 hr across all cohorts
- Little accumulation from Day 1 to Day 7 with a mean accumulation index of 1.1 to 1.4 QD, 1.5 BID.

PK suitable for once or twice daily oral dosing regardless of food intake
- Mean half-life ranged from 12.9 – 17.6 hr across all cohorts

Mean EDP-938 exposures were approximately 7-31x higher than the EC$_{90}$ against RSV-infected human cells
- Mean $C_{24}$ ranged from 146-610 ng/mL following 100 mg to 600 mg QD dosing (Day 7)
- Mean $C_{12}$ was approximately 618 ng/mL following 300 mg BID dosing (Day 7)
Highly active against all RSV-A and B laboratory strains and clinical isolates tested

Excellent *in vivo* efficacy in the African green monkey model

High barrier to resistance
- Unlike fusion and L polymerase inhibitors, difficult to select resistance *in vitro*
- EC$_{50}$ shift <100-fold vs. >1,000-40,000 fold with fusion and L polymerase inhibitors
- The most significant resistance mutants >100 times less fit than the wild-type

Phase 1 study in healthy subjects
- Safe and well tolerated after a broad range of single and multiple ascending doses
- Exhibited PK suitable for once or twice daily oral dosing, without regard to food

Currently being evaluated in a Phase 2 Proof of Concept Challenge Study
Acknowledgements

Enanta Pharmaceuticals, Inc.:

- Nicole McAllister, Jonathan Castillo, Nalini Bisht, Susan Clugston, Nathan Manalo, Bryan Goodwin, Kai Lin (Virology/Biology)
- In Jong Kim, Jianming Yu, Adam Szymaniak, Tom Blaisdell, Kevin McGrath, Solymar Negretti-Emmanuel, Kaicheng Zhu, Brian Shook (Chemistry)
- Falguni Gadkari, Andrew Hague, John Zhao, Matthew Ronsheim (CMC)
- Xiang Luo, Susanne Fyfe, Khanh Hoang (In Vivo Pharmacology)
- Nathalie Adda, Alaa Ahmed, Kajal Larson, Kristin Sanderson (Clinical)
- Pallabi De (Medical Writing)

Collaborators:

- AGM Study: Bioqual, Inc.
- N Protein Structure: Evotec AG
- RSV Reverse Genetics: Martin Moore (Emory U.)
- Statistical Support: Jeff Sorbel (Triangle Biostatistics, LLC)
- Lijuan Jiang, Sean Liu, Lisha Xu, Jonathan Kibel (DMPK)
- Brenda Yamamoto, Sokleang Koy, Kellye Daniels (Toxicology)
- Yat Sun Or (SVP R&D, CSO)
- Clinical Investigator: Daniel Dickerson (PRA)
- PK Support: Mohit Gandhi (PRA)
- Clinical Isolates: Pedro Piedra (Baylor U.)
  Kelly J. Henrickson (Med. College Wisconsin)
  Viroclinics Bioscience BV

We extend our thanks to the subjects who participated in this study and the PRA team and site personnel for their involvement in the study
Thank you!

Questions?