Blockage of Cathepsin L Activity by SLV213, a Novel Cysteine Protease Inhibitor, Prevents SARS-CoV-2 Viral Entry

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Chief Science Officer
Selva Therapeutics
**Host Protease Activity Is Needed for SARS-CoV-2 Cell Entry**

- **Activation of the SARS-CoV-2 spike protein can be mediated via two host proteases:**
  - Cathepsin L, a cysteine protease
  - TMPRSS2, a serine protease

- **In vitro experiments have shown that:**
  - Blocking Cathepsin L can shut down viral entry completely,
  - Blocking TMPRSS2 partially shuts down viral entry,
  - Potential for synergy of blocking Cathepsin L and TMPRSS2

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### SLV213 is a Potent Inhibitor of Cathepsin L

<table>
<thead>
<tr>
<th></th>
<th>Cruzain (nM)</th>
<th>Cathepsin L (nM)</th>
<th>Cathepsin B (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki [nM]*</td>
<td>0.23</td>
<td>0.25</td>
<td>14.4</td>
</tr>
<tr>
<td>IC50 [nM]**</td>
<td>0.2</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>IC50 [nM]***</td>
<td>&lt;0.2</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

* Selva internal data

** Chen et al. (2010, PLoS Neglected Tropical Diseases 4(9):e825)

*** Ndao et al. (2014, Antimicrobial Agents and Chemotherapy 58(2):1167-1178)
• Protein activity experiments demonstrate that Cathepsin L is cleaving the Spike protein in a dose-dependent manner (Cath L titration against fixed S protein)

• SLV213, when added, prevents Cathepsin L from cleaving the Spike protein

• Cathepsin B does NOT cleave the Spike protein

Thomas Meek, Texas A&M, pers. communication
CATHEPSIN L VS. TMPRSS2 EXPRESSION IN HUMAN LUNG

https://jvi.asm.org/content/87/23/12552
## Calu-3 Data for SLV213 and Camostat

<table>
<thead>
<tr>
<th>qRT-PCR cycle count</th>
<th>Vero E6</th>
<th>A549</th>
<th>Calu-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin L</td>
<td>n/a</td>
<td>13.9</td>
<td>20.2</td>
</tr>
<tr>
<td>TMPRSS2</td>
<td>n/a</td>
<td>34.0</td>
<td>24.7</td>
</tr>
</tbody>
</table>

Selva (unpublished, data included in IND), Tseng et al. Univ. Texas Medical Branch
## SLV213 In Vitro Activity Against SARS-CoV-2

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cell Type</th>
<th>Inhibition of Viral Infectivity EC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero E6</td>
<td>African Green Monkey kidney epithelial</td>
<td>0.62</td>
</tr>
<tr>
<td>A549</td>
<td>Human lung carcinoma transformed with ACE2 receptor</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Calu-3</td>
<td>Human lung carcinoma cells</td>
<td>~5</td>
</tr>
</tbody>
</table>

CPE-based assays
No cytotoxicity at >10µM

Selva (unpublished, data included in IND), Tseng et al. Univ. Texas Medical Branch
• Pilot study to determine the potential for efficacy of SLV213 against SARS-CoV-2 in a non-human primate model (African Green Monkey)
• N=6 (2 controls, 4 on drug)
• Simple design:
  – Pretreatment of animals with SLV213 (100mg/kg) or placebo
  – Inoculation with virus at $C_{\text{max}}$ (3-4 hours after first SLV213 dose)
  – Treatment of animals with SLV213 for 7 days (100mg/kg/day)
  – Necropsy on day 7
• Daily measurement of viral titers via BAL, nasal swabs, pharyngeal swabs
• Additional exams and organ assessment included
NHP Study: PK Model for QD vs. BID (100 mg/kg/day)

Key Observations:
- PK simulation based on data from cynomolgus monkey study (200 mg/kg) assuming linear model
- EC50 values based on SARS-CoV-2 activity in Vero E6 cells

- QD dosing results in Cmax >> 2x EC50, whereas BID does not
- QD dosing has significantly higher AUC above 2x EC50 compared to BID dosing
- BID dosing results in Cmax > EC50 twice per day, whereas QD does not
- BID dosing maintains concentrations above EC50 for a longer duration than QD

Selva data, unpublished
## Primate Study: Preliminary Findings from Gross Pathology

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Weight [kg]</th>
<th>Left lung [g]</th>
<th>Right lung [g]</th>
<th>Total [g]</th>
<th>% of weight</th>
<th>Lymph enlargement [fold]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>6.00</td>
<td>29.02</td>
<td>29.79</td>
<td>58.81</td>
<td>0.98%</td>
<td>3</td>
</tr>
<tr>
<td>54</td>
<td>5.75</td>
<td>14.83</td>
<td>23.42</td>
<td>38.25</td>
<td>0.67%</td>
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</tr>
<tr>
<td>SLV213</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>6.55</td>
<td>19.06</td>
<td>14.43</td>
<td>33.49</td>
<td>0.51%</td>
<td>2.5</td>
</tr>
<tr>
<td>50</td>
<td>6.70</td>
<td>21.72</td>
<td>16.84</td>
<td>38.56</td>
<td>0.58%</td>
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</tr>
<tr>
<td>52</td>
<td>5.80</td>
<td>13.06</td>
<td>17.22</td>
<td>30.28</td>
<td>0.52%</td>
<td>2</td>
</tr>
<tr>
<td>53</td>
<td>6.05</td>
<td>12.79</td>
<td>16.99</td>
<td>29.78</td>
<td>0.49%</td>
<td>2</td>
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</tbody>
</table>

![Graph 1](https://www.nature.com/articles/s41586-020-2423-5)

![Graph 2](https://www.nature.com/articles/s41586-020-2423-5)

*Selva data, unpublished*
SLV213 Preclinical Safety Assessment Is Complete

- **Pharmacokinetics/ADME**
  - Readily absorbed after oral dosing ($T_{\text{max}}$ ~1-4 hrs; bioavailability ~15-20%)
  - Half-life in monkeys (2-6 hrs) supports daily administration in humans
  - Liver is major organ of distribution/metabolism, with excretion primarily via feces

- **Safety Pharmacology**
  - hERG channel inhibition $EC_{50}$ (18.3 µM) is 10 to 100-fold greater than concentrations expected *in vivo*
  - No significant effects on ECG, blood pressure, or body temperature.
  - No effects on pulmonary function or neurobehavioral assessment in rats (15, 50 or 150 mg/kg)

- **Toxicology**
  - Liver is target organ for toxicity – reversible elevation of transaminases observed at high doses
  - Emesis (dog, monkey) and effects on body weight (rat, dog) observed at high doses only
  - No genotoxicity signal in 3 different GLP studies
  - No unexpected observations in ongoing 28-day GLP oral toxicity study in dog (in-life completed)
SUMMARY

• SLV213 is a potent inhibitor of human Cathepsin L
• Strong in vitro inhibitory effect preventing SARS-CoV-2 viral infection
• In vivo non-human primate study ongoing; promising early results
• Clean safety profile
• IND filed
• Phase 1 clinical trial to start in late October
• Potential for synergistic effect with other host protease inhibitor (e.g. camostat)
• Potential for synergistic effect with antivirals targeting viral proteins (e.g. remdesivir)
Thank You.

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