Identification and Characterization of PPI-461, a Potent and Selective HCV NS5A Inhibitor with Activity Against all HCV Genotypes

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Disclosure: All authors are employees of Presidio Pharmaceuticals
HCV NS5A Protein

- Functions as a dimer (via Domain 1)
- Interacts with membranes and is required for formation of viral replication complex, particle assembly and pathogenesis
- Zn$^{++}$ binding protein that binds ssRNA at dimer interface
- Phosphorylated, with degree of phosphorylation regulating replication levels
- Numerous reported interactions with cellular proteins involved in signaling, lipid transport, transcription, etc.
- Plays a key role in IFN response and tumor induction
- No known cellular homologs
- Binding site of inhibitors likely resides within the N-terminal 100 aa
Advantages of NS5A Inhibitors

• Exhibit excellent potency
  – HCV 1a and 1b EC$_{50}$s in pM range in replicon assays
  – 3-log viral load drop within 16 hr following single 10 mg dose of BMS-790052$^1$ in human clinical trials

• Target a protein with a range of critical functions - potential to simultaneously inhibit multiple stages of viral replication

• Broad-spectrum coverage of all major HCV genotypes

• Potential for low dose, once daily dosing in man

• Low probability of drug-drug interactions

• Appear to have good safety profiles

• Potential to be core component of combination regimens

$^1$Nettles et al. Poster 893, AASLD 2008
Presidio NS5A Inhibitor Program

• Extensive medicinal chemistry effort culminated in the synthesis of >1,600 proprietary compounds

• Several chemical series pursued in parallel with all compounds screened in HCV 1a and 1b replicon assays

• Over 650 compounds achieved virologic selection criteria of <1 nM EC\textsubscript{50} vs. HCV 1a and 1b, and >80 were further characterized for ADME and PK properties

• Multiple compounds from distinct chemical series selected as potential clinical candidates and profiled extensively

• The most advanced is PPI-461, which was nominated as our first clinical candidate in June 09
PPI-461 Virology Profile

- PPI-461 EC₉₀ levels are 0.62 nM (1a) and 0.022 nM (1b)
- 6-fold EC₅₀ increase observed in serum shift studies (40%/2% human serum)
- Inactive in BVDV, HRV-16, Flu-A, HIV-1, RSV and HSV-1 cell protection assays (EC₅₀ >10,000 nM)
- CC₅₀ >10,000 nM in 3-day cytotoxicity assays using 7 human cell types

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Replicon Assay EC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCV 1a</td>
</tr>
<tr>
<td>PPI-461</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>Telaprevir (PI)</td>
<td>500 ± 196</td>
</tr>
<tr>
<td>ITMN-191 (PI)</td>
<td>5.5 ± 1</td>
</tr>
<tr>
<td>HCV-796 (NNuc)</td>
<td>14.6 ± 6</td>
</tr>
<tr>
<td>PSI-6130 (Nuc)</td>
<td>565 ± 82</td>
</tr>
</tbody>
</table>

N ≥ 3 independent assays
HCV Spectrum of PPI-461

- Panel of HCV 1b replicons constructed that contain the NS5A gene of each of the other major genotypes (exchanged gene segments included all of Domain 1 and ranged from 175-424 aa in length)

<table>
<thead>
<tr>
<th>HCV Genotype</th>
<th>Replicon Assay</th>
<th>EC_{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Stable Cell Line</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>1a</td>
<td>Transient Transfection</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>1b</td>
<td>Stable Cell Line</td>
<td>0.01 ± 0.002</td>
</tr>
<tr>
<td>1b</td>
<td>Transient Transfection</td>
<td>0.02 ± 0.003</td>
</tr>
<tr>
<td>2a</td>
<td>Stable Cell Line</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>3a</td>
<td>Stable Cell Line</td>
<td>9.3 ± 1.7</td>
</tr>
<tr>
<td>4a</td>
<td>Transient Transfection</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>5a</td>
<td>Transient Transfection</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>6a</td>
<td>Transient Transfection</td>
<td>6.1 ± 0.11</td>
</tr>
<tr>
<td>7a</td>
<td>Transient Transfection</td>
<td>0.6 ± 0.04</td>
</tr>
</tbody>
</table>

N ≥ 3 independent assays
# PPI-461 Combination Studies

**Stable HCV 1b Replicon Cell Assay**

<table>
<thead>
<tr>
<th>Compound 1 (Range)</th>
<th>Compound 2 (Range)</th>
<th>Combination Index (CI)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI-461 (0.0025 – 0.2 nM)</td>
<td>ITMN-191 (PI) (0.062 - 5 nM)</td>
<td>EC\text{50} 1.10 EC\text{75} 0.92 EC\text{90} 0.82</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>HCV-796 (NNuc) (1.23 - 100 nM)</td>
<td>EC\text{50} 0.90 EC\text{75} 0.65 EC\text{90} 0.47</td>
<td>Additive to Synergistic</td>
</tr>
<tr>
<td></td>
<td>2’-C methyl Adenosine (Nuc) (10 - 833 nM)</td>
<td>EC\text{50} 0.95 EC\text{75} 0.94 EC\text{90} 0.93</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>IFN-α (0.08 – 6.7 IU)</td>
<td>EC\text{50} 0.92 EC\text{75} 0.83 EC\text{90} 0.75</td>
<td>Additive to Synergistic</td>
</tr>
</tbody>
</table>

CI values < 0.8 indicate synergy, >1.2 indicate antagonism

- No evidence of antagonism observed
- PPI-461 can likely be combined with all classes of HCV inhibitors
Resistance

• Resistance is the potential Achilles heel of all antivirals and antimicrobials

• Critical to understand resistance patterns using multiple viral genotypes for more comprehensive assessment of anticipated resistance profile in patients

• Comprehensive analysis conducted on PPI-461
  – Multiple cell passage studies using HCV 1a and 1b replicon cell lines
  – Colony formation assays with HCV 1b (3a NS5A) cell line
  – Genotypic and phenotypic analysis, along with determination of replicative capacity of all emerging variants
  – Extensive panel of resistant variants generated in various genetic backbones
  – Cross-resistance studies
Selection of PPI-461 Resistant Variants (Cell Passage Studies)

- **HCV 1b**
  - EC$_{50}$ 0.01 nM
  - **Early Passages**
    - EC$_{50}$ 3 - 4 nM
    - 31V, 93H
  - **Extended Passage**
    - EC$_{50}$ 1,067 nM
    - 31V+93H

- **HCV 1a**
  - EC$_{50}$ 0.13 nM
  - **Early Passages**
    - EC$_{50}$ 22 - 160 nM
    - 28T, 30K, 31V, 93H/N/C, 30K+81S, 24T+30R
  - **Extended Passage**
    - EC$_{50}$ 319 - 4,101 nM
    - 24R+30K, 28T+30R, 24Q+93N

- Multiple pathways to resistance depending on viral genotype
- High level resistance requires multiple substitutions
- Resistance maps to Domain 1, with greatest decreases in susceptibility associated with substitutions at amino acid residues 24-31 and 93
**Lack of Cross Resistance**

<table>
<thead>
<tr>
<th>HCV 1a Variant</th>
<th>HCV 1a EC$_{50}$ (nM)</th>
<th>HCV 1b EC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPI-461</td>
<td>ITMN-191</td>
</tr>
<tr>
<td>WT</td>
<td>0.13</td>
<td>5.4</td>
</tr>
<tr>
<td>NS5A L31V+Y93H</td>
<td><strong>3,825</strong></td>
<td>4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>NS3 D168A</th>
<th>NS5B C316Y</th>
<th>NS5B S262T</th>
<th>NS5A 262Q+318W+320E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI-461 (NS5A)</td>
<td>0.01</td>
<td><strong>0.01</strong></td>
<td>0.02</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>ITMN-191 (PI)</td>
<td>0.5</td>
<td>66</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>HCV-796 (NNuc)</td>
<td>6.9</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSI-6130 (Nuc)</td>
<td>754</td>
<td>3,512</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsA (NS5A)</td>
<td>112</td>
<td></td>
<td></td>
<td>567</td>
</tr>
</tbody>
</table>

*N ≥ 3 independent assays

- No evidence of cross resistance between NS5A and other classes of inhibitors
Combination Resistance Studies
(21-Day Colony Formation Assay)

- Combination treatment prevents emergence of resistant variants
- No evidence of dual resistant colonies following genotypic characterization

EC₅₀ concentrations: PPI-461 (NS5A) 10 nM, PSI-6130 (Nuc) 1,000 nM, HCV-796 (NNuc) 15 nM and IFNα 2 IU/mL
PPI-461 ADME and PK Profile

- Highly stable in liver microsomal extracts from human, monkey, dog and rat
- Protein binding (equilibrium dialysis): 94%
- No significant inhibition against a panel of major CYP450 isozymes (IC50 >10,000 nM)
- PK profile determined in rats, monkeys and dogs
  - Good oral bioavailability (29-86%) across species
  - Elimination half-lives predictive of once daily dosing in humans
  - Observe good volume of distribution across species
  - Dose dependent increase in plasma exposure (Cmax, AUC) up to levels exceeding HCV 1a replicon EC50 by >100,000-fold
  - Enhanced liver exposure vs. plasma, with comparable half-lives in both and no evidence of accumulation with repeated dosing
PPI-461 GLP Toxicology Studies

• No significant inhibition in hERG assay ($IC_{50} > 10 \mu M$)

• Negative in panel of genotoxicity assays
  – Bacterial Reverse Mutation (Ames) Assay
  – Mammalian Chromosome Aberration in Human PBL
  – Rat Bone Marrow Micronucleus Assay

• Well tolerated in pivotal 14-Day studies
  – No drug-associated deaths in any study
  – Rat NOAEL observed at plasma $C_{\text{max}}$ levels of 19-32 µM
    (90,000 to 152,000-fold HCV 1a EC$_{50}$)
  – Monkey NOAEL observed at plasma $C_{\text{max}}$ levels 7-17 µM
    (33,000 to 81,000-fold HCV 1a EC$_{50}$)

• Well tolerated in panel of pharmacology studies
  – Rat CNS
  – Rat Respiratory
  – Monkey CV
Summary

• HCV NS5A inhibitors belonging to several distinct chemical series identified following an extensive medicinal chemistry effort

• PPI-461 nominated as first clinical candidate
  – Highly potent and selective inhibitor in HCV 1a and 1b replicon assays ($EC_{50} \leq 0.2$ nM)
  – Active against other major genotypes ($EC_{50}$s 0.1-9 nM)
  – Possesses desirable ADME and PK properties predictive of once daily dosing in humans
  – Well tolerated in a battery of GLP toxicology studies

• Enabling studies completed and regulatory documents filed

• Anticipate initiation of clinical studies in near future
Additional Acknowledgements

- Presidio Team
- Chemistry CROs
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  - Aptuit
  - ChemPartner
  - Sygnature
  - Syngene
- Biology CRO
  - ImQuest
- ADME/PK CROs
  - Cerep
  - ChemPartner
  - Ricerca
  - XenoTech
- Toxicology CROs
  - BioReliance
  - ChanTest
  - Ricerca