HCV Drug Resistance Advisory Group
HCV DRAG: Goals

• Produce consensus recommendations of appropriate methodology for HCV resistance testing
  – For drug/biologic development
  – For clinical practice

• Provide scientific guidance to facilitate discussion between industry and regulatory agencies in areas of HCV drug resistance
**HCV DRAG**: Methods

- Facilitated discussion between representatives from pharmaceutical/biotech companies, academic institutions and regulatory agencies
  - Two meetings per year
  - Teleconference/email
  - Working groups dedicated to specific topics
    - Sequencing: Ann Kwong
    - Phenotype: Neil Parkin
    - Clinical: Chip Schooley
    - Database: tbd
**HCV DRAG**: Measurables

- Presentations/abstracts
  - HCV Resistance Workshops, HepDART
  - AASLD, EASL

- White papers/manuscripts
  - Consensus recommendations
HCV DRAG: 1\textsuperscript{st} Meeting (18 May 07)

- Identified issues
- Classified issues:
  - Sequencing
  - Phenotype
  - Clinical
  - Other
- Formed working groups
- Defined timetables for action items
**HCV DRAG**: Process

- Within each working group, define issue categories
  1. Immediate recommendation
  2. Recommendation based on discussions within working groups and between WG’s and DRAG
  3. Multiple possibilities to be described by working group with pro/con and context
  4. Insufficient data/methodology at this time, noted as issue
HCV DRAG: Sequencing questions

- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?
Example of resistance mutations in on-treatment rebound and at end of follow-up

200 mg boceprevir + peginterferon-α2b

Population Sequencing
- WT
- T54S
- V36M
- R155K

Q41H and A156V variants detected at pre-treatment
R155K mutation detected in follow-up
HCV DRAG: Sequencing questions

- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?
- What gene segments should be sequenced?
Whole genome sequencing of a subset of PCR positive samples is currently under evaluation.

Start-up of development and optimisation of this near-full genome genotypic resistance assay for other HCV geno- and subtypes.

Advantage in clinical studies and in clinic:

⇒ near-full genome HCV genotypic resistance test can assess resistance in multiple combination therapies in one run.

S. Van Dooren: sequence whole genome for combinations?
HCV DRAG: Sequencing questions

• Which samples should be sequenced?
• At what viral load do you try to obtain a sequence?
• What gene segments should be sequenced?
• Sequencing technologies
• Clonal vs population sequencing?
• Minority species detection?
# Boceprevir resistance mutations

<table>
<thead>
<tr>
<th>Genotype 1 WT residue</th>
<th>Mutations reported or observed in clinic</th>
<th>Mutations reported or observed in replicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>V36</td>
<td>A, M, L, T</td>
<td></td>
</tr>
<tr>
<td>Q41</td>
<td>R, H, L</td>
<td>R</td>
</tr>
<tr>
<td>F43</td>
<td>S, C, L, V</td>
<td>S, C</td>
</tr>
<tr>
<td>T54</td>
<td>A, S, P</td>
<td>A, S</td>
</tr>
<tr>
<td>R155</td>
<td>K, Q, T, M, S, G</td>
<td></td>
</tr>
<tr>
<td>A156</td>
<td>S, T, V</td>
<td>S, T</td>
</tr>
<tr>
<td>V170, I170</td>
<td>A, T, F</td>
<td>A, T</td>
</tr>
</tbody>
</table>

Blue: observed with clonal sequencing but not population sequencing
Less abundant variants show complex pattern of resistance mutations

Population sequencing:
- T54A, A156S, V170A
- V36M, R155K
- V36M, T54S, R155K
- V36M, T54S, R155K

Variant frequency (percent)

- T54A
- R155K
- T54A+R155K
- V36M+R155K

~LOD pop.
LOQ clonal
LOD clonal
Quantification of resistant variants by allele-specific PCR

V36I

T54A

V170A

mutant percent

IFN

IFN+PI

PI

(pre)

F/U

TaqMAMA

clonal seq

TaqMAMA

clonal seq

TaqMAMA

clonal seq
How would combination of antivirals affect the selection of resistance?

Targeting different sites of the polymerase compromises resistance selection.

I. Najera: can this assay be used to assess synergy/antagonism?
HCV DRAG: Sequencing questions

- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?
- What gene segments should be sequenced?
- Sequencing technologies
- Clonal vs population sequencing?
- Minority species detection?
- Nomenclature
- Mutation vs polymorphism
- Do we compare isolates to baseline or to consensus sequences?
HCV NS5B: 4 allosteric binding sites identified

THUMB II
Benzimidazoles (BI)
Indoles (Merck)

THUMB I
Thiophene-COOH (Shire)
Pyranoinoles (Wyeth-ViroPharma)

PALM I
Benzofurans, HCV-796
(Wyeth-ViroPharma)

PALM II
Benzothiadiazines
(GSK), (A-848837 Abbott)
Acylpyrrolidines, GSK625433
**Effect of L419I/I482L Substitutions on the Susceptibility of the Con-1 Replicon to NNPIs**

<table>
<thead>
<tr>
<th>Construct</th>
<th>Benz-imidazole NNPI1</th>
<th>Thiophene carboxylic acid NNPI2</th>
<th>Benzo-thiadiazine NNPI3</th>
<th>Benzofuran NNPI4</th>
<th>IFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>L419I</td>
<td>4</td>
<td>326</td>
<td>0.2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>I482L</td>
<td>3</td>
<td>201</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L419I/I482L</td>
<td>8</td>
<td>2238</td>
<td>0.2</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

S. Shi: pol binding sites?
Activity Spectrum of HCV NNPIs

S. Shi: NNI or NNPI?

Fold Change in EC₅₀ over Con-1 (log10)

-1.5 -1 -0.5 0 0.5 1 1.5 2 2.5 3 3.5

NNPI1 NNPI2 NNPI3 NNPI4

Inhibitor Binding Site

-1.5 -1 -0.5 0 0.5 1 1.5 2 2.5 3 3.5

NNPI1 NNPI2 NNPI3 NNPI4

Inhibitor Binding Site

S. Shi: NNI or NNPI?
**HCV DRAG**: Phenotype questions

- Methodology
  - Replicon vs full-length infectious system
  - Replicon systems
  - Chimeric cell-based systems
  - Enzyme (cell-free system)
- What should be amplified?
Co-expression in *E. coli* of a recombinant λ cI repressor containing an HCV cleavage-site with a HCV NS3/4A protease results in the induction of the phage’s lytic functions
A chimeric reporter system with desirable attributes

NS3 activity liberates a secreted reporter from intracellular membrane tether

Technical advantages relative to HCV replicon
- No “cassette” issues
- Transfection of plasmid DNA, not transcribed RNA
- Robust suspension cells used, not Huh7
- Homogenous assay for NS3 activity
- Same reporter plasmid used for clonal sequencing

S. Seiwert: enzyme vs replicon?
Phenotyping assay faithfully reports potencies against variants identified using in vitro resistance studies

> **Previous results from in vitro selections**
>  - Substitution at D168 appears to be fundamental to ITMN-191 resistance
>    - All replicons carry substitution at D168
>    - As selection pressure increases additional substitutions are required

> **Characterization of potency against in vitro identified substitutions by clonal analysis (duplicate measurement)**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Phenotyping assay</th>
<th>HCV replicon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC\textsubscript{50} (nM)</td>
<td>Fold Change</td>
</tr>
<tr>
<td>Wild Type</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>D168V</td>
<td>33</td>
<td>22-fold</td>
</tr>
<tr>
<td>D168A</td>
<td>134</td>
<td>90-fold</td>
</tr>
<tr>
<td>D168V + A156V</td>
<td>~3,700</td>
<td>~2,467-fold</td>
</tr>
<tr>
<td>D168A + F43S</td>
<td>&gt;6,000</td>
<td>&gt;4,000-fold</td>
</tr>
</tbody>
</table>

Similar rank order potency reported by replicon and phenotyping assay

S. Seiwert: enzyme vs replicon?
HCV DRAG: Phenotype questions

• Methodology
  – Replicon vs full-length infectious system
  – Replicon systems
  – Chimeric cell-based systems
  – Enzyme (cell-free system)
• What should be amplified?
• Standardization
• Minority species
• Interpretation
## Summary of *In Vitro* Activity Spectrum of PF-00868554

<table>
<thead>
<tr>
<th>Genotype of NS5B</th>
<th>Mean EC$_{50}$ (µM)</th>
<th>Range EC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (24 strains)</td>
<td>0.059</td>
<td>0.0088 - 0.087 (H77 = 0.39)</td>
</tr>
<tr>
<td>2 (4 strains)</td>
<td>15</td>
<td>11 - 17</td>
</tr>
</tbody>
</table>
Susceptibility of Chimeric Replicons Carrying Patient Polymerase Gene to GS-9190

![Graph showing the EC50 values for GS-9190 for 1b and 1a isolates.](image-url)
**HCV DRAG**: Phenotype questions

- Methodology
  - Replicon vs full-length infectious system
  - Replicon systems
  - Chimeric cell-based systems
  - Enzyme (cell-free system)
- What should be amplified?
- Standardization
- Minority species
- Interpretation
- Replication capacity
Replication capacity can affect the ease of selection of the resistant mutants

C316Y confers high level resistance and has good replication capacity
S365T confers high level of resistance but has low replication capacity
M414I has good replication capacity but confers low level resistance
A balance between replication capacity and level of resistance is required for selection

2nd International workshop on Hepatitis C resistance and new compounds Boston 31 October-1 November 2007 Roche Palo Alto
Effect of NS5B mutations on the replication capacity

W.-R. Jiang: relevance of RC in replicon?

- S282T co-exists with K81R, I239L, L320F, A421V, Y586C under high selective pressure with PSI-6130
- Combination of S282T with K81R, I239L, L320F, A421V, Y586C increased replication capacity from 16% to 51%
- The 6-mutant replicon exhibits similar sensitivity to PSI-6130 and NM107 as compared with the WT replicon

### Transient replicon assay mean EC$_{50}$ ± SEM (μM)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>S282T</th>
<th>6-mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSI-6130</td>
<td>0.82 ± 0.04</td>
<td>2.51 ± 0.29</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>NM107</td>
<td>2.12 ± 0.19</td>
<td>46.3 ± 17.2</td>
<td>1.62 ± 0.53</td>
</tr>
</tbody>
</table>
Relative replication capacity of 1b-N D168 mutant replicons without drug treatment

Relative Replication Capacity (WT = 100%)

Relative R.C. = (Mut replication signal / WT replication signal)
(Mut input signal / WT input signal)

D. He: relevance of RC in replicon?
HCV DRAG: Clinical questions

- Standard repositories
  - Clinical strain library
  - Compounds?
- Early clinical trial design recommendations to assess resistance
- How do we promote combination of investigational agents?
- Database
- Correlation between baseline resistance and SVR
- Clinical cutoffs
- Genetic barrier
HCV DRAG: Today’s Agenda

HCV DRAG/HCNG Group
Jean-Michel Pawlotsky

Sequence Analysis Working Group
Ann Kwong

Phenotype Working Group
Neil Parkin

Clinical Working Group
Chip Schooley

Moving forward/Next steps Group