Use of Resistance Testing in the Management of Chronic HCV Infection

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Dr Wyles has reported the following financial relationships with commercial firms:

- **Consultant**: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, Merck & Co, Inc, and Janssen Therapeutics, Inc

- **Payments to the Regents of the University of California San Diego for the conduct of clinical research**: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, Merck & Co, Inc, and Tacere Therapeutics, Inc
Outline

1. Background and terminology

2. Overview of DAA specific resistance
   - NS3 PI resistance
   - NS5A resistance

3. Guidance on the practical management of resistance
   - What resistance tests are available
   - When should they be used

4. Treatment approaches in patients with HCV resistance
Antiviral Resistance Concepts

- Resistant variant (or resistance mutation)
  - Amino acid change responsible for the phenotype change (change in EC$_{50}$)
- Viral fitness
  - The “cost” the mutation imposes on the virus
- Resistance barrier
  - Multiple components which together determine the “ease” with which mutants are selected and propagate
- Clinical resistance
  - Failure to achieve the treatment goal in the patient

All DAAs can be expected to select for resistant variants.
The HCV Lifecycle Favors Resistance Development…But Not Persistence

**Favors Resistance**
- High viral turnover rate
  - $10^{12}$ virions/day
- Error-prone RNA polymerase
  - ~1 error per 10,000 bases
  - Involved twice in replication
- No overlapping reading frames
- Moderate rate of infected hepatocyte turnover

**Lack of Persistence**
- No DNA intermediate
  - Contrast to integrated HIV
  - Contrast to HBV cccDNA
- No long-lived cellular reservoir known
  - Latently infected HIV + CD4 cells
  - HBV cccDNA
- There are exceptions!

Resistant variants pre-exist in all patients\(^1\)

## Characteristics of HCV antiviral classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Antiviral Potency</th>
<th>Genotype Activity</th>
<th>Resistance barrier</th>
<th>FDA Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS3 Protease Inhibitors</td>
<td>+++ to ++++</td>
<td>1 (and 4)</td>
<td>Low to moderate</td>
<td>Simeprevir (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paritaprevir (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grazoprevir (2016)</td>
</tr>
<tr>
<td>NS5B Nucleoside/tide</td>
<td>++ to ++++</td>
<td>1-6</td>
<td>Very High</td>
<td>Sofosbuvir (2013)</td>
</tr>
<tr>
<td>NS5B Non-nucleoside</td>
<td>++ to +++</td>
<td>1</td>
<td>Low</td>
<td>Dasabuvir (2014)</td>
</tr>
<tr>
<td>NS5A Inhibitors</td>
<td>++++</td>
<td>1, 4-6 (+/- 2,3)</td>
<td>Very Low</td>
<td>Ledipasvir Ombitasvir (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Daclatasvir (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Elbasvir (2016)</td>
</tr>
</tbody>
</table>
NS3 PI Resistance

NOT A MAJOR CONSIDERATION WITH CURRENT DAA THERAPIES
The saving grace with PI resistance?

91% of nonSVR with resistance

1a: R155K +/- Q80K
1b: D168V

Lenz O. EASL 2014.
Real World Data: Impact of prior PI therapy?

- PI failure = PEG/RBV + PI
- Resistance testing results not available
  - Majority did not have baseline testing
- Prior PI failure was associated with a decreased SVR rate
  - OR: 0.4 (0.2-0.9)

Lack of Q80K impact with the “appropriate” duration of therapy

**OPTIMIST-1**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Q80Q</th>
<th>Q80K</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>97%</td>
<td>96%</td>
</tr>
<tr>
<td>8 weeks</td>
<td>84%</td>
<td>73%</td>
</tr>
</tbody>
</table>

**OPTIMIST-2**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Q80Q</th>
<th>Q80K</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>92%</td>
<td>74%</td>
</tr>
</tbody>
</table>

Data are lacking with 24 weeks of SOF/SMV therapy.

Kwo P. #14 EASL 2015. Lawitz E. #LP04 EASL 2015
**NS3 Resistance testing—where does it fit?**

- **Significant** baseline NS3 RAVs are rare
  - Routine baseline testing not needed

- There is no clear impact of Q80K on SOF+SMV when using approved durations
  - Data are lacking with 24 weeks in cirrhotics

- Well studied non-PI containing options are available

- If you need to use an NS3 PI soon after PI failure
  - NS3 resistance testing makes sense
    - Marks K. #644 CROI 2015.
  - Determine duration in re-treatment with triple DAA regimens?
NS5A Resistance

IMPACT ON DAA TREATMENT RESPONSES
AND MOST LIKELY AREA FOR CLINICAL
UTILITY OF RESISTANCE TESTING
Baseline polymorphisms associated with resistance are relatively prevalent (~10%) - They impact responses in *select settings*

Currently available NS5A inhibitors suffer from broad cross-resistance at key positions - M28, Q30, L31, and Y93

NS5A variants persist for prolonged periods

Selected NS5A RAVs impact re-treatment responses
## Broad cross-resistance with “early generation” NS5As

<table>
<thead>
<tr>
<th>Fold-change</th>
<th>1a</th>
<th>1b</th>
<th>1a</th>
<th>1b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M28T</td>
<td>Q30R</td>
<td>L31M/V</td>
<td>Y93H/N</td>
</tr>
<tr>
<td>LDV</td>
<td>20x</td>
<td>&gt;100x</td>
<td>&gt;100x/1000x</td>
<td>1000x/10000</td>
</tr>
<tr>
<td>Ombitasvir</td>
<td>&gt;1000x</td>
<td>&gt;100x</td>
<td>&lt;3x</td>
<td>10000x/10000x</td>
</tr>
<tr>
<td>DCV</td>
<td>&gt;100x</td>
<td>&gt;1000x</td>
<td>&gt;100x/10000x</td>
<td>10000x/10000x</td>
</tr>
<tr>
<td>Elbasvir</td>
<td>20x</td>
<td>&gt;100x</td>
<td>&gt;100x</td>
<td>10000x/10000x</td>
</tr>
<tr>
<td>Velpatasvir</td>
<td>&lt;10x</td>
<td>&lt;3x</td>
<td>20x/50x</td>
<td>10000x/10000x</td>
</tr>
<tr>
<td>ACH-3102</td>
<td>30x</td>
<td>20x</td>
<td>&lt;10x</td>
<td>10000x/10000x</td>
</tr>
<tr>
<td>ABT-530</td>
<td>&lt;3x</td>
<td>&lt;3x</td>
<td>&lt;3x</td>
<td>&lt;10x/&lt;10x</td>
</tr>
<tr>
<td>MK-8408</td>
<td>&lt;10x</td>
<td>&lt;10x</td>
<td>&lt;10x</td>
<td>&lt;10x</td>
</tr>
</tbody>
</table>

Deep sequencing analysis of baseline samples (n=1904) in phase 2/3 SOF/LDV studies

GT1 (n=2137)

- No RAVs 84%
- NS5A RAVs 16%
Baseline NS5A resistance and SOF/LDV

- Deep sequencing analysis of baseline samples (n=1904) in phase 2/3 SOF/LDV studies

GT1 (n=2137)

- No RAVs 84%
- NS5A RAVs 16%
- 93% SVR12
- 97% SVR12

Sarrazin C. #1926 AASLD 2014.
Baseline NS5A resistance and SOF/LDV

- Deep sequencing analysis of baseline samples (n=1904) in phase 2/3 SOF/LDV studies

**GT1 (n=2137)**

- 97% SVR12
- 93% SVR12

**GT 1a (n=1602)**

- 96% SVR12
- 16% NS5A RAVs
- 84% SVR12

**GT 1b (n= 529)**

- 98% SVR12
- 16% SVR12
- 84% SVR12
- 95% SVR12

Sarrazin C. #1926 AASLD 2014.
Baseline NS5A resistance and SOF/LDV

- Deep sequencing analysis of baseline samples (n=1904) in phase 2/3 SOF/LDV studies

GT1 (n=2137)

- 97% SVR12 with no NS5A RAVs
- 93% SVR12 with NS5A RAVs

GT 1a (n=1602)

- 96% SVR12 with no NS5A RAVs
- 92% SVR12 with NS5A RAVs

GT 1b (n=529)

- 98% SVR12 with no NS5A RAVs
- 95% SVR12 with NS5A RAVs

SOF/LDVx12wks (GT1a): 98% no NS5A RAVs vs 91% with NS5A RAVs - Zeuzem S. #91 AASLD 2015.
Impact of baseline NS5A RAVs in patients with cirrhosis treated with SOF/LDV

Impact of subtype and fold-change

SVR12 (%)

1a

98

263

154

85

40

1b

97

100

70

3

96

52

193

11

SVR12 combined: 98% no RAVs vs 89% RAVs [@15% level]

Sarrazin C. #P0773. EASL 2015.
Impact of baseline NS5A RAVs in patients with cirrhosis treated with SOF/LDV

Impact of duration and RBV usage

Sarrazin C. #P0773. EASL 2015.
Impact of baseline NS5A RAVs in GT1a patients treated with GZP/EBR

Population Sequencing

**EBR RAVs**
- No RAVS: 414/438 (95%)
- 5%

**NS5A Class RAVs**
- No RAVS: 432/438 (80%)
- 20%

Next Generation Sequencing (1% level)

**EBR RAVs**
- No RAVS: 396/439 (90%)
- 10%

**NS5A Class RAVs**
- No RAVS: 289/439 (65%)
- 35%

Regimen: GZP/EBR x 12 weeks

<table>
<thead>
<tr>
<th>SVR12 (%)</th>
<th>EBR RAVs</th>
<th>NS5A Class RAVs</th>
<th>EBR RAVs</th>
<th>NS5A Class RAVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without RAVs</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>Patients with RAVs</td>
<td>405/414</td>
<td>345/352</td>
<td>389/396</td>
<td>284/289</td>
</tr>
</tbody>
</table>

GT1a naïve/relapsers

<table>
<thead>
<tr>
<th>SVR12 (%)</th>
<th>EBR RAVs</th>
<th>NS5A Class RAVs</th>
<th>EBR RAVs</th>
<th>NS5A Class RAVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients without RAVs</td>
<td>58</td>
<td>72</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Patients with RAVs</td>
<td>14/24</td>
<td>31/43</td>
<td>136/150</td>
<td></td>
</tr>
</tbody>
</table>

EBR RAVs = RAVs with >5x fold shift in EBR EC50

Impact of baseline NS5A RAVs in patients treated with GZP/EBR

- Impact of NS5A RAVs on TE (non-responder) GT1a treated with GZP/EBR x 12 wks
  - EBR RAVs (population): 97% vs. 29% (No RAVs vs. RAVs)

- Extension to 16-18 weeks with RBV appears to negate the impact of NS5A RAVs

- Baseline RAVs have no significant impact in GT 1b

- Population sequencing identifies the vast majority of significant RAVs
Persistence of selected NS5A resistance

- The majority of patients failing an NS5A-containing DAA regimen will have NS5A RAVs
  - ~75% to >95%
- NS5A RAVs persist for >2 years

Wyles D, Dvory-Sobol H. Abstract O059 EASL 2015
NS5A RAVs are associated with retreatment failure

N=41

SOF/LDV

weeks

0 12 24

SVR12 (%)

Combined  No RAVs RAVs

71 60

100 100

Q30R or M28T  L31M  Y93H/N

5/5  4/5  2/6

SVR12

Lawitz E. #O005 EASL 2015.
Is resistance a unique consideration in DAA failures? **YES.**

1. DAA resistance is frequently selected on failure
2. Resistance mutations to some DAA classes (NS5A) persist for prolonged durations
3. RAVs are associated with retreatment failure

What we don’t know for sure is:

Selection of retreatment therapy based on resistance testing (selection of non-cross resistant regimens) will result in improved treatment success.
Commercial HCV Resistance Assays

- Genotypic resistance testing is clinically available
- Ultra-deep (or NGS) vs population (Sanger)
  1. LabCorp/Monogram Biosciences
     - NGS with 10% detection level reported
     - Regions: NS3/4a, NS5A, NS5B
  2. Quest Diagnostics
     - RT-PCR with DNA sequencing
     - Regions: NS3/4a, NS5A, NS5B
     - HCV viral load ≥ 2000 IU/mL
       - Currently limited to GTs 1 and 3 (NS5A only)
- Separate tests (must request each region you want)
- Cost: $700/region (estimated)

http://www.monogrambio.com/content/hcv-ns5a-testing
### Examples: NS3 resistance genotyping

<table>
<thead>
<tr>
<th>Drug</th>
<th>HCV GenoSure® NS3/4A</th>
<th>Assessment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generic Name</td>
<td>Region</td>
<td>Drug Resistance Associated Mutations Detected</td>
<td>Drug</td>
</tr>
<tr>
<td>Boceprevir</td>
<td>NS3</td>
<td>None</td>
<td>BOC Sensitive</td>
</tr>
<tr>
<td>Paritaprevir</td>
<td>NS4A</td>
<td>None</td>
<td>PTV/r Resistance Possible</td>
</tr>
<tr>
<td>Simeprevir</td>
<td>NS3 Q80K</td>
<td>None</td>
<td>SMV Resistant</td>
</tr>
<tr>
<td>Telaprevir</td>
<td>NS3 Q80K</td>
<td>None</td>
<td>TVR Sensitive</td>
</tr>
</tbody>
</table>

#### Important Definitions
- All mutations are reported relative to the HCV genotype/subtype specific reference H77.
- Assessment of drug susceptibility is based on detected mutations and is interpreted using a rules-based algorithm (version 3).
- Protease inhibitors are indicated by the abbreviation PI.
- Resistance to NS3 protease inhibitors may be inherited or acquired during therapy. The treatment-naive patient may respond better to therapy than the treatment-experienced patient.

#### Region Genotype Summary of All Mutations Observed

<table>
<thead>
<tr>
<th>Region</th>
<th>Genotype</th>
<th>Summary of All Mutations Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS4A</td>
<td>1a</td>
<td>T40A, V78I, S91A, L104L/F, P192L/S, Q46R</td>
</tr>
<tr>
<td>NS3</td>
<td>1a</td>
<td>T40A, V78I, S91A, L104L/F, P192L/S, Q46R</td>
</tr>
</tbody>
</table>

Comments: Q80K NOT DETECTED.
Examples: NS5A resistance genotyping

<table>
<thead>
<tr>
<th>Drug</th>
<th>HCV GenoSure®</th>
<th>Assessment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ledipasvir</td>
<td>NS5A</td>
<td>Q30R</td>
<td>LDV</td>
</tr>
<tr>
<td>Ombitasvir</td>
<td>NS5A</td>
<td>Q30R</td>
<td>OBV</td>
</tr>
</tbody>
</table>

**Important Definitions**

- All mutations are reported relative to the HCV genotype/subtype specific reference H77
- Assessment of drug susceptibility is based on detected mutations and is interpreted using a rules-based algorithm (version 3)
- Hepatitis C virus resistance-associated polymorphisms identified at baseline may impact sustained virologic response rates if the treatment regimen, or adherence, is suboptimal. The impact of these polymorphisms may vary in treatment-naive compared to treatment-experienced populations and according to disease status.

**Summary of All Mutations Observed**


**Test Procedure**

| Hepatitis C genotype 3 NS5A drug resistance |

| HCV NS5a Subtype: | 3a |
| Daclatasvir resistance: | PREDICTED |

Mutations Detected: Y93H

**Reference Ranges**

- HCV NS5a Subtype: NOT DETECTED

This test was developed and its performance characteristics have been determined by Focus Diagnostics. Performance characteristics refer to the analytical performance of the test.

This test utilizes RT-PCR and DNA sequencing to detect the presence of treatment-emergent HCV genotype 3 NS5a variants associated with NS5a inhibitor antiviral therapy.

This assay is designed to amplify HCV Genotype 3 and may not successfully amplify other HCV genotypes.
Additional considerations in patients who failed a DAA-based regimen

- Was initial therapy sub-optimal (or sub-maximal)?
  - Duration
  - RBV use
- What specific medication classes were used
  - What role does resistance play?
- Stage of liver disease/host characteristics
- Indications of other problems
  - Adherence?
  - Significant drug interactions? PPI use?
    - Terrault N. #94 AASLD 2015.
  - Immunosuppression?
When to do NS5A resistance testing?

DAA naïve patients:
- Baseline testing should be performed prior to use of GZP/EBR in GT 1a patients.
  - 12 weeks without RBV can be used in 1a patients without NS5A RAVs (including cirrhosis and treatment-experienced)
  - Alternative regimens or 16 weeks + RBV if EBR RAVs* found at baseline

DAA-experienced patients (IFN-free DAA failures)
- Resistance testing recommended (my opinion)
- Based on the results…

* RAVs at positions 28, 30, 31, and 93
**DAA failure**

**Genotypic resistance testing**

- **No NS5A RAVS**
  - SOF/LDV + RBV 24 weeks

- **No Q80K (or other PI RAVs)**
  - SOF + SMV + RBV 24 weeks

- **+ NS5A RAVs (Q30, L31, H58, Y93)**
  - SOF + SMV + RBV 24 weeks (even if Q80K)

- **+NS5A RAVs + NS3 RAVs (R155, A156, D168)**
  - Desperation time
    - PrOD + SOF (LB-20)
    - SOF + SMV + DCV + RBV
    - SOF/LDV + RBV

- Investigational Triple Regimens
Role of Resistance in GT3 Responses

- **SOF+DCV x 12 weeks (ALLY-3)**
  - 54% SVR12 with baseline Y93H (7/13)
  - ALLY-3+ SVR12: 93% (38/41) vs. 88% (7/8) (No RAVs vs. RAVs)
    
    Leroy V. LB-3 AASLD 2015

- **SOF+VEL x 12 weeks (ASTRAL-3)**
  - SVR12: 97% (225/231) vs. 88% (38/43) (No RAVs vs. RAVs)
  - Y93H: 84% SVR12 (21/25)
    
    Mangia A. NEJM 2015.

- **GZP/MK-3682/MK-8408 (C-CREST 1 & 2):**
  - 45% (5/11) vs. 97% (72/74) [NS5A RAVs vs not]
    
    Gane EJ. LB-15. AASLD 2015
Summary

- The NS3 Q80K RAV does not appear to impact responses
- Currently NS5A RAVs are the most clinically important
- Baseline NS5A RAVs impact treatment response
  - Should be viewed as another negative predictor
  - Baseline testing recommended for GZP/EBR in GT1a
    - Other vulnerable/difficult to treat populations?
      - GT3
- Resistance testing should be done in patients failing a DAA regimen
  - Selection of re-treatment regimens may be tailored based on resistance testing
  - Prospective trials demonstrating the validity of this approach are not available.