Disclosures

Christos J Petropoulos is:
- The Chief Scientific Officer of Monogram Biosciences, a member of the LabCorp Specialty Testing Group
- Monogram Biosciences markets services for the diagnosis and treatment of viral diseases including HIV and HCV infection
- An Officer (Vice President) of Laboratory Corporation of America
- A shareholder of Laboratory Corporation Holdings (LH)
Acknowledgements

- Monogram R&D
  - Special thanks to Jackie Reeves and Wei Huang
  - Alicia Newton, Jen Cook, Arne Frantzell, Elizabeth Anton, Kristi Strommen, Sam Jauregui,
- Monogram PDO (Yuping Tan, Jeff Larson)
- Monogram Bioinformatics (Agnes Paquet, Mojgan Haddad)
- Monogram Clinical Reference Laboratory Personnel
  - Special thanks to Jeannette Whitcomb (GM, VP Ops)
- Our many industry, government and academic collaborators
- Patients that have donated samples for testing
• HCV DAA resistance perspective

• Landscape of NS5A/B DAA susceptibility
  – NS5A
  – NS5B NNI
  – NS5B NI (RBV, IFN)

• Preliminary observations from NS5A and NS5B inhibitor resistance testing
HIV Drug Development and Resistance Testing

- **1983**: zidovudine, first ARV, approved by the FDA
- **1987**: didanosine
- **1989**: lamivudine, saquinavir
- **1991**: zalcitabine, ritonavir, indinavir, nevirapine
- **1994**: stavudine, efavirenz, abacavir
- **1996**: nelfinavir, delavirdine, enfuvirtide, atazanavir, emtricitabine, fosamprenavir
- **1998**: amprenavir, tipranavir, darunavir, maraviroc, raltegravir
- **2001**: lopinavir, tenofovir, etravirine
- **2004**: Monogram launches GeneSeq®
- **2005**: Monogram launches PhenoSense® Entry
- **2009**: Monogram launches Trofile®DNA and GenoSure MG®
- **2011**: Monogram launches PhenoSense® Integrate and GenoSure Archive
- **2013**: Monogram launches PhenoSense GT® Plus Integrate and GenoSure Archive

- **1983**: HIV, the virus responsible for AIDS, is identified
- **1987**: Resistance to ARVs is first documented and published
- **2001**: Monogram develops PhenoSense GT®, first combo geno/pheno resistance assay
- **2004**: Monogram (ViroLogic) commercializes PhenoSense® HIV
- **2009**: Monogram launches Trofile® Co-Receptor Tropism Assay
- **2013**: Monogram launches GenoSure PRime®
HCV Drug Development and Resistance Testing

- **1989**: Hepatitis C virus identified
- **1991**: Standard interferon (IFN)
- **1998**: Ribavirin
- **2001**: Pegylated IFN
- **2010**: Boceprevir, telaprevir, (DAAs)
- **2012**: Simeprevir, sofosbuvir
- **2015**: Harvoni® (ledipasvir + sofosbuvir)
- **2015**: Viekira Pak™ (paritaprevir/r + ombitasvir + dasabuvir)

Monogram launches HCV GenoSure® NS3/4A, the first resistance test for hepatitis C DAA treatment

Monogram adds Q80K callouts to HCV GenoSure® NS3/4A report

Monogram launches NS5A and NS5B resistance tests
If everything seems under control, you're just not going fast enough.

Mario Andretti, 02/28/1940 - Italian automobile racer
Routine HCV NS3/4A Testing

FDA approval

BOC
TVR

SOF
SMV

SOF/LDV
SOF+SMV
OMV/PTV/r +DSV

NS5A Inhibitor Resistance
Phylogeny of HCV NS5A Sequences Derived from DAA Naïve Subjects

GT-1a sequences

GT-1b sequences

J Cook et al., CROI 2015
Replication of HCV Replicons Containing Patient-derived NS5A Sequences

<table>
<thead>
<tr>
<th></th>
<th>Median (range) RC (% of Con1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a+1b</td>
<td>20% (1-164%)</td>
</tr>
<tr>
<td>1a</td>
<td>11% (1-56%)</td>
</tr>
<tr>
<td>1b</td>
<td>57% (19-164%)</td>
</tr>
</tbody>
</table>

- **1a+1b**: N=109
- **1a**: N=71
- **1b**: N=38

**Chart Description**
- **Y-axis**: RC (% reference)
- **X-axis**: Genotypes
- **Legend**
  - Red squares: 1a+1b
  - Yellow diamonds: 1a
  - Orange circles: 1b

**Median (range) RC (% of Con1)**
- 1a+1b: 20% (1-164%)
- 1a: 11% (1-56%)
- 1b: 57% (19-164%)
Susceptibility of HCV Replicons Containing Patient-derived NS5A Sequences

Median FC (range) relative to Con1
1a IC50 FC = 1.38 (0.52->200)
1a IC95 FC = 2.90 (1.12->200)
1b IC50 = 0.71 (0.49-2.50)
1b IC95 = 0.95 (0.54->200)

Samples with NS5A DRMs (red)
1 (1a). M28T
2 (1a). Q30H
3 (1a). Q30H
4 (1a). Q30H, Y93H
5 (1a) Q30Q/H, Y93Y/H/N
6 (1a). L31L/M
7 (1b). L31L/M, Y93Y/H
8 (1b). Y93Y/H
Sample 7 Pool (1b): L31M + Y93Y/H

Sample 8 Pool (1b): Y93Y/H

Sample 7 clone-1: L31M + Y93 (30/39)

Sample 8 clone-1: Y93 (24/41)

Sample 7 clone-2: L31M + Y93H (9/39)

Sample 8 clone-2: Y93H (17/41)
GT1a GT1b DRM Distinctions

J Cook et al., CROI 2015
**NS5A Resistance Summary**

- **Phenotype:**
  - Replicons containing GT1 NS5A sequences exhibit variability in replication capacity and NS5A inhibitor susceptibility (*compared to NI susceptibility*).
  - Pre-existing NS5A DRMs in DAA naïve viruses confer large reductions in NS5A susceptibility.
  - Reductions in NS5A inhibitor susceptibility may manifest as reductions in the % inhibitory maximum rather than increases in IC\(_{50}\)/IC\(_{95}\).
  - NS5A DRMs introduced onto an GT1a backbone generally conferred larger reductions in susceptibility than DRMs introduced into an GT1b backbone.

- **Genotype (sequence):**
  - GT1 NS5A sequences exhibit a relatively large degree of sequence diversity.
    - Including two distinct clusters within GT1a.
  - The prevalence and diversity of NS5A RAV/DRM was higher in GT1a viruses versus GT1b viruses.

- **Implication:** *The combination of multiple resistance pathways, lower genetic and resistance barriers may provide advantages for GT1a viruses to escape NS5A inhibition.*
NS5B  Non-nucleoside Inhibitor Resistance
Phylogeny of HCV NS5B Sequences Derived from DAA Naïve Subjects
The vast majority of recombinant replicons exhibited a replication capacity sufficient for evaluating inhibitor susceptibility.
NNI Susceptibility of Replicons Containing GT1, 2, 3, 4 NS5B Regions

- Replicons exhibit large variation in NNI susceptibilities
  - NNI-A inhibitors have GT1, 3 and 4 activity
  - NNI-D inhibitors have pan-genotypic (GT1-4) activity

### IC FC Range
- **NNI-A:** 827
- **NNI-B:** >8.2
- **NNI-D:** 152

### Susceptibility
- **NNI-A:** 1,3,4>2
- **NNI-B:** (1)>4>2,3
- **NNI-D:** 1,3>2,4
### DRM Distinctions: Subtype and Binding Site Group

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Inhibitor</th>
<th>Phenotypic Data</th>
<th>% Mutant detected*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC50FC</td>
<td>IC95FC</td>
</tr>
<tr>
<td>Con1 L392I</td>
<td>NNI-A</td>
<td>7.60</td>
<td>5.82</td>
</tr>
<tr>
<td>Con1 P495A</td>
<td>NNI-A</td>
<td>21.67</td>
<td>&gt;19</td>
</tr>
<tr>
<td>H77 P495L</td>
<td>NNI-A</td>
<td>128.95</td>
<td>&gt;27</td>
</tr>
<tr>
<td>Con1 M423T</td>
<td>NNI-B</td>
<td>&gt;410</td>
<td>&gt;51</td>
</tr>
<tr>
<td>Con1 Y448H</td>
<td>NNI-C</td>
<td>16.39</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Con1 C316Y</td>
<td>NNI-C</td>
<td>8.15</td>
<td>6.61</td>
</tr>
<tr>
<td></td>
<td>NNI-D</td>
<td>116.91</td>
<td>&gt;53</td>
</tr>
<tr>
<td>Con1 S282T</td>
<td>NI</td>
<td>15.64</td>
<td>19.36</td>
</tr>
<tr>
<td>H77 S282T</td>
<td>NI</td>
<td>18.27</td>
<td>&gt;22</td>
</tr>
</tbody>
</table>

- NNI susceptibility can vary based on GT1 subtype (H77-1a vs Con1-1b)
- NNI susceptibility can vary across NNI binding site groups (NNI-C vs NNI-D)
NS5B NNI Resistance Summary

- Phenotype:
  - Variation in replication capacity and NS5B NNI susceptibility across GT1-4 NS5B replicons was comparable to the variability within GT1 NS5A replicons.
  - Pre-existing NS5A DRMs in DAA naïve viruses confer large reductions in NS5B NNI susceptibility.
  - NS5B NNI susceptibility varied significantly across HCV genotypes based on the NNI-binding site group.
    - The NNI-D inhibitor exhibited pan-inhibitory activity across GT1,2,3,4
    - NS5B NNI DRMs introduced into GT1 replicons exhibited subtype and NNI-binding site group differences

- Genotype (sequence):
  - GT1 NS5B sequences exhibit a relatively large degree of sequence diversity
    - Less diversity than NS5A
  - The prevalence and diversity of NS5B RAV/DRM was equivalent in GT1a and GT1b viruses, however sample numbers were small.

- Implication: Accurate determination of HCV genotype-subtype may become important for the appropriate use of NS5B NNI inhibitors.
NS5B  Nucleoside Inhibitor Resistance
IFN, RBV and NI Susceptibility Replicons Containing GT1, 2, 3, 4 NS5B Regions
NS5B NI Resistance Summary

- **Phenotype:**
  - IFN susceptibility was equivalent across replicons containing GT1-4 NS5B
  - RBV susceptibility was greater for replicons containing GT2,3,4 NS5B sequences compared to GT1 NS5B sequences
  - NS5B NI (SOF) susceptibility was greater for replicons containing GT1,2 NS5B sequences compared to GT3,4 NS5B sequences
  - Replicons containing the S282T mutations exhibits severely impaired replication capacity….and increased susceptibility to RBV (data not shown)
  - IC_{95} or inhibition slope are more accurate determinants of NS5B NI resistance (data not shown)

- **Genotype (sequence):**
  - The prevalence of NS5B NI DRM is low DAA naïve and routine resistance testing samples

- **Implication:** Genotype specific differences in NI and/or RBV susceptibility may contribute to improved SVR rates with NI and/or RBV containing regimens
Preliminary Observations from NS5A and NS5B Inhibitor Resistance Testing
Routine HCV NS5A/B Testing

Sample volume over time for NS5A and NS5B from 3/29 to 5/17.
**NS5A-I RAV Prevalence: NGS 10% Threshold**

### Comparison of DAA naïve viruses and routine testing viruses

<table>
<thead>
<tr>
<th>AA positions</th>
<th>K24R</th>
<th>M28T/V</th>
<th>Q30H/R/L/S</th>
<th>L31M/V</th>
<th>H58D</th>
<th>A92T</th>
<th>Y93C/H/N/R/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAA naïve 1a = 15/71 (21%)</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>routine testing 1a = 26/164 (16%)</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

### DAA naïve viruses

<table>
<thead>
<tr>
<th>No.</th>
<th>RAVs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a+1b viruses</td>
<td>109</td>
<td>17</td>
</tr>
<tr>
<td>1a viruses</td>
<td>71</td>
<td>15</td>
</tr>
<tr>
<td>1b viruses</td>
<td>38</td>
<td>2</td>
</tr>
</tbody>
</table>

### routine testing viruses

<table>
<thead>
<tr>
<th>No.</th>
<th>RAVs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a+1b viruses</td>
<td>203</td>
<td>36</td>
</tr>
<tr>
<td>1a viruses</td>
<td>164</td>
<td>26</td>
</tr>
<tr>
<td>1b viruses</td>
<td>39</td>
<td>10</td>
</tr>
</tbody>
</table>

**NS5A-I RAVs of DAA naïve viruses (%)**

- 1a+1b viruses: 15.6%
- 1a viruses: 21.1%
- 1b viruses: 5.3%

**NS5A-I RAVs of routine testing viruses (%)**

- 1a+1b viruses: 17.7%
- 1a viruses: 15.9%
- 1b viruses: 25.6%
**DSV/SOF RAV Prevalence: NGS 10% Threshold**

### Comparison of DAA naïve and routine testing viruses

**AA position**
- **G307E/K/R**: 1
- **C316Y**: 1
- **S556G/N**: 1

<table>
<thead>
<tr>
<th>AA position</th>
<th>G307E/K/R</th>
<th>C316Y</th>
<th>S556G/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAA naïve 1a = 1/25 (4.0%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>routine testing 1a = 10/122 (8.2%)</td>
<td>7</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>DAA naïve 1b = 2/25 (8.0%)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>routine testing 1b = 1/26 (3.8%)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

### DSV/SOF RAVs of DAA naïve viruses (%)

<table>
<thead>
<tr>
<th>DAA naïve viruses</th>
<th>No.</th>
<th>RAVs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a+1b viruses</td>
<td>50</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>1a viruses</td>
<td>25</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>1b viruses</td>
<td>25</td>
<td>2</td>
<td>8.0</td>
</tr>
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### DSV/SOF RAVs of routine testing viruses (%)

<table>
<thead>
<tr>
<th>routine testing viruses</th>
<th>No.</th>
<th>RAVs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a+1b viruses</td>
<td>148</td>
<td>11</td>
<td>7.4</td>
</tr>
<tr>
<td>1a viruses</td>
<td>122</td>
<td>10</td>
<td>8.2</td>
</tr>
<tr>
<td>1b viruses</td>
<td>26</td>
<td>1</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Future Considerations

• Patient group:
  – Genotype/subtype
  – Disease stage
  – Co-morbidities
  – Co-infection
  – Re-infection

• Treatment:
  – Tx duration
  – Rx composition
  – Baseline RAV
  – RAV persistence
  – 1\textsuperscript{st} vs 2\textsuperscript{nd} line
Acknowledgements

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