Hepatitis C Virus Resistance Testing

Mark Douglas
About 170 Million People Worldwide Have Chronic HCV Infection

- 27% of cirrhosis and 25% of HCC worldwide
- Main cause of cirrhosis, liver transplant and HCC in Australia, USA, UK
- Causes more deaths than HIV in Australia and USA

Prevalence of infection:
- >10%
- 2.0–10%
- 1.0–1.9%
- <1.0%

Evolution of SVR rates

*Range of values reported; lower bar represents lower value

Adapted from Manns, Foster et al., Nature Reviews Drug Discovery 2007
Potential New Drug Targets

- Entry inhibitors
  - Neutralizing antibodies

- RNA interference
  - Antisense oligos
  - Ribozymes

- IRES inhibitors

- Protease inhibitors

- α-glucosidase inhibitors

- Interfering peptides and proteins

- Polymerase inhibitors
  - Helicase inhibitors

Direct Acting Antivirals (DAAs)

- NS3/4A protease inhibitors
  - telaprevir, boceprevir, simeprevir, paretaprevir
- NS5B polymerase inhibitors
  - Nucleos(t)ide analogues (sofosbuvir)
  - Non-nucleoside analogues (dasabuvir)
- NS5A inhib. (daclatasvir, ledipasvir, ombitasvir)

Evolution of SVR rates

**SVR (%)**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peg-IFN</td>
<td>IFN + RBV</td>
<td>PEG + RBV</td>
<td>PEG +PEG RBV + SMV</td>
<td>PEG + RBV + SOF (12w)</td>
<td>SOF + RBV (12w)</td>
<td>SOF + RBV (24w)</td>
<td>SOF + LDV or DCV (8-12w)</td>
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<tr>
<td>1998</td>
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<td>35-43</td>
<td>61-79</td>
<td>76-82</td>
<td>63-75</td>
<td>80-81</td>
<td>82-92</td>
<td>93-97</td>
<td>97-99 (86)</td>
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<td>2001</td>
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<td>33-36</td>
<td>61-79</td>
<td>76-82</td>
<td>63-75</td>
<td>80-81</td>
<td>82-92</td>
<td>93-97</td>
<td>97-99 (86)</td>
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<td>2011</td>
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<td></td>
<td>42-46</td>
<td>61-79</td>
<td>76-82</td>
<td>63-75</td>
<td>80-81</td>
<td>82-92</td>
<td>93-97</td>
<td>97-99 (86)</td>
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<tr>
<td>2012</td>
<td></td>
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<td>31-37</td>
<td>61-79</td>
<td>76-82</td>
<td>63-75</td>
<td>80-81</td>
<td>82-92</td>
<td>93-97</td>
<td>97-99 (86)</td>
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<tr>
<td>2013</td>
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<td>35-39</td>
<td>61-79</td>
<td>76-82</td>
<td>63-75</td>
<td>80-81</td>
<td>82-92</td>
<td>93-97</td>
<td>97-99 (86)</td>
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<tr>
<td>2014</td>
<td></td>
<td></td>
<td>37-40</td>
<td>61-79</td>
<td>76-82</td>
<td>63-75</td>
<td>80-81</td>
<td>82-92</td>
<td>93-97</td>
<td>97-99 (86)</td>
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</tbody>
</table>

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Adapted from Manns, Foster et al., Nature Reviews Drug Discovery 2007

IFN free DAA therapy
But what about resistance?

Resistance associated variants (RAVs)
DAA Resistance - sequencing

Incomplete suppression
- Inadequate potency
- Inadequate drug levels
- Inadequate adherence
- Preexisting resistance

Selection of resistant quasispecies

Market Introduction and Emergence of Resistance (Selected Drugs)

NS3

- Boceprevir, telaprevir - genotype 1
- Simeprevir - genotype 1, 4
- Paritaprevir - genotype 1
- Grazoprevir - pan genotypic

- RAV prevalence 0.1 - 3.1% (likely due to fitness cost)
- except Q80K (5-48%)
- RAV persistence wk48 post treatment 9%
NS5A

- Daclatasvir (BMS) - pan genotypic
- Ombitasvir (AbbVie) - pan genotypic
- Ledipasvir (Gilead) - Genotype 1
- Elbasvir (MSD) - genotypes 1, 3, 4, 6
- Velpatasvir (Gilead) - pan genotypic

- RAV prevalence 0.3-3.5%
- RAV persistence high
  – up to 85% 48 wks post treatment)
NS5B

- **Nucleos(t)ide analogue**
  - Sofosbuvir (Pan genotypic)
  - RAV prevalence 0.0%
  - RAV persistence 0.0% by Wk 4 post treatment

- **Non nucleoside analogue**
  - Dasabuvir (genotype 1)
  - RAV prevalence 0.2-3.1%
  - RAV persistence at wk48 post treatment 57%
HCV Resistance: Does it matter?
QUEST-1/2: PEG-IFN/RBV/Simeprevir (24-48 weeks)

Genotype 1 treatment naïve

Jacobson I et al. AASLD 2013
### Appendix 3: Virology/Resistance in Clinical Studies

#### Table 19: Number (%) of Patients with a Baseline Q80K Polymorphism, by Region; Intent-to-treat – Pooled C205/C206/C208/C216/HPC3007

<table>
<thead>
<tr>
<th>Analysis set: Intent-to-treat</th>
<th>All HCV geno/subtypes</th>
<th>HCV geno/subtype</th>
<th>HCV geno/subtype</th>
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<tbody>
<tr>
<td></td>
<td>2026</td>
<td>926</td>
<td>1100</td>
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<tr>
<td>All Regions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with sequencing data</td>
<td>2007</td>
<td>911</td>
<td>1096</td>
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<tr>
<td>No Q80K</td>
<td>1733 (86.3%)</td>
<td>642 (70.5%)</td>
<td>1091 (99.5%)</td>
</tr>
<tr>
<td>Q80K</td>
<td>274 (13.7%)</td>
<td>269 (29.5%)</td>
<td>5 (0.5%)</td>
</tr>
<tr>
<td>Europe</td>
<td>1267</td>
<td>387</td>
<td>880</td>
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<tr>
<td>Patients with sequencing data</td>
<td>1254</td>
<td>377</td>
<td>877</td>
</tr>
<tr>
<td>No Q80K</td>
<td>1178 (93.9%)</td>
<td>304 (80.6%)</td>
<td>874 (99.7%)</td>
</tr>
<tr>
<td>Q80K</td>
<td>76 (6.1%)</td>
<td>73 (19.4%)</td>
<td>3 (0.3%)</td>
</tr>
<tr>
<td>North America</td>
<td>542</td>
<td>388</td>
<td>154</td>
</tr>
<tr>
<td>Patients with sequencing data</td>
<td>538</td>
<td>385</td>
<td>153</td>
</tr>
<tr>
<td>No Q80K</td>
<td>353 (65.6%)</td>
<td>200 (51.9%)</td>
<td>153 (100%)</td>
</tr>
<tr>
<td>Q80K</td>
<td>185 (34.4%)</td>
<td>185 (48.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>South America</td>
<td>60</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>Patients with sequencing data</td>
<td>60</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>No Q80K</td>
<td>58 (96.7%)</td>
<td>20 (90.9%)</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>Q80K</td>
<td>2 (3.3%)</td>
<td>2 (9.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>United States</td>
<td>415</td>
<td>301</td>
<td>114</td>
</tr>
<tr>
<td>Patients with sequencing data</td>
<td>411</td>
<td>298</td>
<td>113</td>
</tr>
<tr>
<td>No Q80K</td>
<td>268 (65.2%)</td>
<td>155 (52.0%)</td>
<td>113 (100%)</td>
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<tr>
<td>Q80K</td>
<td>143 (34.8%)</td>
<td>143 (48.0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Baseline polymorphisms are defined as changes from Con1 (AJ238799) and H77 (AF009606) for geno/subtype 1b and 1a/other respectively.

Source: data on file, Janssen Research and Development
Q80K in Western Sydney?

- Samples tested 378 (Gt 1a)
- Age: mean 31 yrs
- Gender: Male: 270 (71%)
- Correctional centre (anonymous): 119 (31%)
Of 378 samples:

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Number</th>
<th>%</th>
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<tbody>
<tr>
<td>Q80K</td>
<td>21</td>
<td>5.6</td>
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<tr>
<td>V55A</td>
<td>16</td>
<td>4.2</td>
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<tr>
<td>T54S</td>
<td>10</td>
<td>2.6</td>
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<tr>
<td>Q80L</td>
<td>5</td>
<td>1.3</td>
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<tr>
<td>Q80R</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>R155K</td>
<td>2</td>
<td>0.5</td>
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<tr>
<td>Q80H</td>
<td>1</td>
<td>0.3</td>
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</table>
IFN-free therapy?

- Combination DAA high cure rates
- Failure more likely if baseline RAVs, particularly multiple NS5A RAVs
- Patients failing DAA therapy have high levels of RAVs
Methods of detecting resistance

- Population (Sanger) sequencing
- High throughput (Next generation) sequencing
  - Better sensitivity with NGS - down to <1% (Sanger ~20%)
    » clinical relevance not yet determined
  - Bioinformatics (big data) with NGS
## Raw data - self processed

<table>
<thead>
<tr>
<th>Sequence DNA</th>
<th>Quality control</th>
<th>Map to reference</th>
<th>Call variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche 454 Titanium</td>
<td>BWA Trimmer</td>
<td>SMALT</td>
<td>CASAVA1.8</td>
</tr>
<tr>
<td>Life Technologies SOLiD4 or 5500XL</td>
<td>Trimmomatic</td>
<td>BWA</td>
<td>Samtools</td>
</tr>
<tr>
<td>Illumina Genome Analyzer II or HiSeq</td>
<td>Quake</td>
<td>SSAHA2</td>
<td>GATK</td>
</tr>
<tr>
<td>Pacific Biosciences</td>
<td></td>
<td>Bowtie</td>
<td>SoapSNP</td>
</tr>
<tr>
<td>IonTorrent</td>
<td></td>
<td>Bfast</td>
<td>SSAHA-SNP</td>
</tr>
</tbody>
</table>

With thanks to Grant Hill-Cawthorne
java -Xmx4g -jar snpEff.jar eff -v M62321 HCV9CLCSNV.all.filter.vcf > S9CLC.eff.vcf
Known Resistance Variants

NS3 Protease (180 aa)

boceprevir

36  A
54  A
55  G

155  K
156  T
158  S
I
168  A
170  L

36  A
54  G

132  V
155  G
156  F
168  N

36  A
54  S

155  G
156  M
168  N

36  L
132  I

155  K
156  T
168  T

36  M

155  K
156  T
168  T

43  S

155  G
156  Y
168  V

155  K
168  T

155  A
168  V
Summary

- HCV is curable
- High cure rates with DAAs
- Resistance can emerge and may affect (re)-treatment response
- Reliable, high-throughput assays required to detect RAVs
- Next Generation Sequencing most sensitive, becoming feasible