Should we be doing baseline resistance testing?

Dr Emma Thomson
EHHC 2015
DAA treatment availability in the UK

London 2003-2015
- IFN/RBV
- BOC/IFN/RBV
- SIM/IFN/RBV
- SOF/IFN/RBV
- TEL/IFN/RBV
- GRAZ/ELB
- LED/SOF+RBV
- OMB/PAR/DAS
- SIM/SOF

Glasgow - HCV treatment regimens 2001-2015
- IFN/RBV
- BOC/IFN/RBV
- SIM/IFN/RBV
- SOF/IFN/RBV
- TEL/IFN/RBV
- GRAZ/ELB
- LED/SOF+RBV
- OMB/PAR/DAS

London - NICE guidance

Glasgow - SMC guidance
DAA treatment is highly effective in HIV-co-infected patients: Glasgow Co-infected Cohort

Baseline resistance testing would not have made a difference in managing this cohort – so should we be doing it?
No !
No – not yet and not in the majority of patients at baseline

However, the utility of resistance testing/whole genome sequencing has not been fully figured out....
Why consider resistance testing/full genome sequencing at baseline?

- The presence of baseline resistance associated variants (RAVs) does not strongly impact outcome in most patients. Exceptions: previous treatment failure, cirrhosis and simeprevir.

- However, standardised techniques are being rapidly developed and are cheap.

- Genotyping.

- It may improve the choice of regimen and treatment outcome in some patients.

- We would pick up on transmitted RAVs during DAA roll-out.
HCV clinical research studies investigating resistance at the MRC CVR

- HCV Research UK
- Stratified Medicine to Optimise Treatment for Hepatitis C (STOP HCV)
- Early Access Programme (EAP)
- UK Phyloepidemiology Study (UPS)
- Acute HCV UK
HCV evolution and resistance

• HCV has been evolving for 2000 years

• 7 genotypes with variable response to treatment

• Natural variations within NS3, NS5A and NS5B confer resistance to DAAs
Distribution of HCV genotypes across the UK
HCV is a highly variable virus both within populations and within individual hosts.
HCV replicates inaccurately...

1. Attachment & uncoating
   - Receptor binding
   - Endocytosis
   - Fusion & uncoating
   - Viral RNA targeted to ER

2. Translation & processing
   - Viral proteins translated
   - Polyprotein
   - Viral replication complexes form in a membranous web structure
   - Ribosome

3. Replication
   - Viral RNA
   - NS2
   - NS4A
   - Cytoplasm
   - ER lumen
   - Viral replication complexes form in a membranous web structure

4. Assembly & release
   - Viral assembly
   - Transport through Golgi apparatus
   - Virus particles

DAAs
Multiple resistance mutations have been described within NS3, NS5A and NS5B.
Virological barriers to resistance

- Related to the number of nucleotide changes required for a virus to acquire resistance to an antiviral regimen and replication fitness
- NS5A and NS3 RAVs at baseline are far more common than NS5B RAVs

NS3 Q80K RAV prevalence in genotype 1a

North America 34%
Europe 20%

Sarrazin et al., Antiviral Res 2015
NS5A RAVs occur frequently as natural polymorphisms in G1a

Zeuzem et al., AASLD 2015
Various clinical trials have shown that the presence of RAVs lowers SVR rates at baseline in certain patient groups.
NS3/4A Protease Resistance
OPTIMIST: Baseline NS3 Q80K mutation lowers SVR rates in cirrhotic patients treated with SIM/SOF

<table>
<thead>
<tr>
<th>Treatment History</th>
<th>HCV GT</th>
<th>SVR12 (%)</th>
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<tbody>
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<td>150/155 All pts</td>
<td>97</td>
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<tr>
<td>112/115 Naive</td>
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<td>38/40 Exp’d</td>
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<td>96</td>
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<td>44/46 1a + Q80K</td>
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<td>97</td>
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<tr>
<td>68/70 1a no Q80K</td>
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NS5A Resistance

AVIATOR: No Impact of Baseline RAVs in GT1a Pts Treated With OMV/PTV/RTV + DSV

- Treatment naive pts or null responders to previous pegIFN/RBV
- All differences in SVR24 with vs without baseline RAVs were nonsignificant

The presence of NS5A RAVs impacts on SVR12 in treatment experienced patients treated with LDV/SOF

NS5A RAVs that emerge on treatment persist for at least 96 weeks in the majority of patients.

Persistence of RAVs is clinically important:
24 Wks of LDV/SOF Retreatment After Failure of LDV/SOF-Based Tx

- GT1 HCV–infected pts with and without cirrhosis previously treated with 8 or 12 wks of LDV/SOF ± RBV or LDV/SOF + GS-966

### Table

<table>
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<tr>
<th>Cirrhosis</th>
<th>Previous Tx Duration</th>
<th>BL NS5A RAVs</th>
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</table>

SVR12 (%)

NS5A RAV Persistence After Failure with OMV/PTV/RTV + DSV

PTV-Containing Regimens

- Follow-up Wk 24:
  - Any: 97%
  - D168: 96%
  - R155K: 77%

- Follow-up Wk 48:
  - Any: 93%
  - M28V/T: 89%

OMV-Containing Regimens

- Follow-up Wk 24:
  - Any: 97%
  - M28V/T: 100%

- Follow-up Wk 48:
  - Any: 97%

DSV-Containing Regimens

- Follow-up Wk 24:
  - Any: 75%

- Follow-up Wk 48:
  - Any: 77%

## Table: Fold-Change in EC50

<table>
<thead>
<tr>
<th>Fold-Change in EC50</th>
<th>Genotype 1a</th>
<th>Genotype 1b</th>
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<td>Daclatasvir[^1,^3]</td>
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<td>&lt; 3 x</td>
<td>&lt; 10 x</td>
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Conclusions – what we know

- Baseline RAVs don’t substantially impact treatment naïve SVR rates in non-cirrhotic patients
- NS3 and NS5A mutations do impact response in treatment experienced patients and in cirrhosis
What is not known

- Real life data especially in patients with advanced cirrhosis
- RAVs in genotypes 2, 3, 4, 5, 6, 7
- Many studies have not employed deep/whole genome sequencing
  - Role of minor variant RAVs not clear
  - Genotyping likely to be inaccurate
- Many studies have not sequenced virus longitudinally during treatment
  - Difficulty in sequencing virus at low viral loads
- Will transmitted viruses with NS5A RAVs increase following DAA roll-out?
Stratified Medicine to Optimise Treatment for Hepatitis C Virus Infection (STOP HCV)

- Research grant from MRC (UK); £5 million
- 22 Co-investigators (UK and US)
- Phase I – development of rapid high-throughput sequencing of HCV
STOP HCV sequencing methodology study aims

To develop a robust pipeline for full genome sequencing of HCV

• Varying HCV sequencing and bioinformatics methods

• Test sets x 3 sent to 4 independent centres
  • HCV Research UK panel – 29 samples x 4, varied genotype, viral load
  • Mixed genotype evaluation panel – plasma and RNA transcripts
  • Panel of varying viral loads

• Evaluation criteria
  • Completeness/coverage, accuracy and sequence depth across genome
  • Association with viral load and genotype
  • Population diversity at sites of DAA resistance mutations
Oxford University / Wellcome Trust Centre for Human Genetics

Public Health England

UCL

Great British Sequencing Bake-Off

Oxford University / Wellcome Trust Centre for Human Genetics

MRC Centre for Virus Research, Glasgow

Gordon Ramsay's World Kitchen

Fabulous Fanny

Mary Berry's TV Cook Book
Advances in sequencing technology mean that we can rapidly sequence the whole HCV genome.

**PCR-based**

1. Synthesise Viral cDNA
2. Fragment
3. Ligate adaptors + indexes
4. Sequence

**Metagenomic/RNASEq**

1. Synthesise Viral cDNA
2. Fragment
3. Ligate adaptors + indexes
4. Sequence

**Target enrichment**
Target enrichment is efficient, cheap and unbiased

Genotype 1a sample
Probe set - g1a

Genotype 1b sample
Probe set - g1a

Genotype 4a sample
Probe set - g1a
Double capture increases detection of HCV at low viral loads

- Paired samples sequenced by double capture (VL range, $1 \times 10^2$ – $5.6 \times 10^6$ IU/ml).

- All samples gave >92% coverage of the HCV genome irrespective of VL.
Genotyping using NGS is more accurate than current assays

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<th>Sample</th>
<th>VL-IU/ml</th>
<th>Genotype</th>
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<th>O-Meta</th>
<th>G-SSel</th>
<th>O-Capt</th>
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</table>
NGS allows detection of mixed genotypes

Observed ratios of NGS reads between component genotypes gt A and gt B (listed in Table 1B) and their input ratios (x-axis), plotted on a log/log scale.
Advantages of NGS whole genome sequencing

- Cost – full genome £85-100
- More accurate genotype
- Detection of RAVs
- Detection of minority variants
STOP HCV/HCV Res UK resistance database

Target enrichment methods developed by the STOP HCV consortium now in use in several major studies

STOP HCV
Stratified Medicine to Optimise the Treatment of Patients with Hepatitis C Virus Infection

EAP Early Access Programme
To evaluate the emergence of resistance in patients with advanced HCV infection treated with DAAs - From April 2015, NHS England has provisionally agreed to expand the Early Access Programme to include all UK patients with cirrhosis (n~5000)

BOSON study
Phase III – g2/3 infected patients IFN/RBV/SOF, SOF/RBV (n~600)

Acute HCV UK
To identify novel T cell epitopes in patients with early HCV infection

HCV UK phylogeography study
To characterise HCV risk factors and genotype and strain distribution across the UK
Using sequence technology to assess the impact of resistance in the Early Access Program (EAP)

- 840 patients with advanced cirrhosis

- NHS England - 12 weeks Sofosbuvir/Ribavirin

- BMS/Gilead - Daclatasvir (DCV) and Ledipasvir (LDV)

- 102 patients relapsed; 70% gt3

- Data and samples collected by HCV Research UK
### Baseline Characteristics

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<thead>
<tr>
<th></th>
<th>Total</th>
<th>G1 N = 235</th>
<th>G3 N = 189</th>
<th>Others N = 43</th>
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<tr>
<td><strong>Decompensated cirrhosis (Past or present)</strong></td>
<td>441 (94.4%)</td>
<td>223 (94.9%)</td>
<td>179 (94.7%)</td>
<td>39 (90.7%)</td>
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<tr>
<td>CP-B</td>
<td>309 (66.2%)</td>
<td>161 (68.5%)</td>
<td>121 (64.0%)</td>
<td>27 (62.8%)</td>
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<td>CP-C</td>
<td>46 (9.9%)</td>
<td>19 (8.1%)</td>
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<td>MELD mean (range)</td>
<td>11.9 (6-36)</td>
<td>11.3 (6-24)</td>
<td>12.6 (6-36)</td>
<td>11.9 (6-22)</td>
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<td>Active ascites</td>
<td>178 (38.1%)</td>
<td>97 (41.3%)</td>
<td>67 (35.4%)</td>
<td>14 (32.6%)</td>
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<td>Previous variceal bleed</td>
<td>127 (27.2%)</td>
<td>61 (26.0%)</td>
<td>55 (29.1%)</td>
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<td>Active encephalopathy</td>
<td>80 (17.1%)</td>
<td>41 (17.4%)</td>
<td>34 (18.0%)</td>
<td>5 (11.6%)</td>
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</table>
SVR by genotype

SVR defined as HCV RNA at 12 weeks post-treatment <30 IU/ml
SVR for genotypes 1 and 3 by regimen

SVR defined as HCV RNA at 12 weeks post-treatment <30 IU/ml
EAP preliminary data
RAV detection in genotypes 1 and 3

Genotype 1

Genotype 3

Unknown RAVS?
Rapid reversion?
EAP design – future work

Viral genomics (NGS)

Treatment failures - all relapse (102/840)

Baseline
On treatment
Post treatment
SVR

Emerging RAVs
Pre-existing RAVs
Identification of new and known RAVs
Summary

- Baseline RAVs (especially NS3 and NS5A) are present in treatment-naive pts

- NS3 RAVs at baseline – Q80K
  - SMV + IFN/RBV: Q80K testing is required
  - SMV + SOF: In patients with genotype 1a HCV infection and cirrhosis, test for Q80K

- NS5A RAVs are persistent and a clinical challenge
  - High risk of onward transmission

- Resistance testing is likely to be of benefit in treatment failures

- Advances in sequencing technology and reduction in costs mean we should consider full genome NGS
  - Provides accurate genotype
  - Provides RAV information – to be interpreted carefully
  - Would allow us to pick up any increase in prevalence of RAVs during DAA rollout
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