Debate case based: Do we need resistance testing in HCV therapy to guide decision making? Pro

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Cattedra di Virologia

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Disclosures

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Despite the excellent efficacy of DAA containing regimens reported in clinical trials, virological failures can occur, often associated with development of resistance.

<table>
<thead>
<tr>
<th>IFN-free, DAA-based regimen</th>
<th>Trial(s)</th>
<th>Genotype/subtype</th>
<th>Number of patients with virologic failure analyzed</th>
<th>RASs present at virologic failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1b</td>
<td>9</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1</td>
<td>NA</td>
</tr>
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<td></td>
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<td>5</td>
<td>1</td>
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</tr>
<tr>
<td>Ombitasvir/paritaprevir/ritonavir plus dasabuvir</td>
<td>Integrated analysis of Phase II and III trials\textsuperscript{33}</td>
<td>1a</td>
<td>67</td>
<td>NS3 protease RASs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1b</td>
<td>7</td>
<td>NSSA RASs</td>
</tr>
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<td>4</td>
<td>3</td>
<td>NS5B RASs</td>
</tr>
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<td>PEARL-1\textsuperscript{34}</td>
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<td></td>
<td></td>
<td>1b</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
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</tr>
<tr>
<td></td>
<td>ALLY-3\textsuperscript{20}</td>
<td>3</td>
<td>16</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>ALLY-3\textsuperscript{37}</td>
<td>3</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>Sofosbuvir plus daclatasvir</td>
<td>ALLY-2\textsuperscript{36}</td>
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<td>31</td>
<td>NA</td>
</tr>
<tr>
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<td>COSMOS\textsuperscript{31}</td>
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<td>6</td>
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<td></td>
<td>OPTIMIST-1\textsuperscript{22}</td>
<td>1a</td>
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<tr>
<td></td>
<td>OPTIMIST-2\textsuperscript{23}</td>
<td>1a</td>
<td>16</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Pawlotsky JM, Gastroenterology 2016*
Despite the excellent efficacy of DAA containing regimens reported in clinical trials, virological failures can occur, often associated with development of resistance.

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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS3 protease RASs</td>
</tr>
<tr>
<td>Sofosbuvir plus daclatasvir</td>
<td>ALLY-2[^36]</td>
<td>1D 1</td>
<td>NA</td>
<td>L31M</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALLY-3[^20]</td>
<td>3</td>
<td>16</td>
<td>A30S</td>
</tr>
<tr>
<td></td>
<td>ALLY-3[^17]</td>
<td>3</td>
<td>4</td>
<td>Y93H</td>
</tr>
<tr>
<td></td>
<td>OPTIMIST-1[^22]</td>
<td>1</td>
<td>31</td>
<td>R155K, D168E, I170T</td>
</tr>
</tbody>
</table>

Understanding more about RASs may help us learn why the patients failed, and may allow optimization of retreatment choices.
Even in the era of DAAs, ~47,000 patients would fail to achieve SVR in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>France</th>
<th>Germany</th>
<th>Italy</th>
<th>Spain</th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR (%)</td>
<td>14,411 (14.1%)</td>
<td>3,596 (3.9%)</td>
<td>14,022 (6.7%)</td>
<td>9,023 (5.7%)</td>
<td>9,484 (10.0%)</td>
</tr>
<tr>
<td>NS5A (%)</td>
<td>83,019 (81.0%)</td>
<td>69,771 (75.7%)</td>
<td>158,881 (76.4%)</td>
<td>136,107 (86.7%)</td>
<td>77,785 (81.9%)</td>
</tr>
<tr>
<td>Non-NS5A (%)</td>
<td>5,125 (5.0%)</td>
<td>18,799 (20.4%)</td>
<td>35,014 (16.8%)</td>
<td>11,850 (7.5%)</td>
<td>7,702 (8.1%)</td>
</tr>
<tr>
<td>Treatment failure (%)</td>
<td>13,226 (12.9%)</td>
<td>9,291 (10.1%)</td>
<td>23,224 (11.2%)</td>
<td>15,193 (9.7%)</td>
<td>9,999 (10.5%)</td>
</tr>
</tbody>
</table>

The majority of treatment failures will occur with NS5A inhibitors.

Chhatwal J et al., EASL 2017 poster #FRI-233
DAA-failing patient, who are you?
Many factors contribute to viral response to DAA-treatment

- HCV infection
- immune response
- drug resistance
- inhibition
- Cirrhosis
- IL28B non-CC
- Male gender
- BMI
- Adherence
- Comorbidities
- Failure after multiple DAAs
- Treatment-experienced

- Viral load
- Genotype
- GT 1a
- GT 3
- NS5A RASs
- Q80K

- Herpesvirus
- infection
- immune response
- drug resistance
- inhibition
- potency
- toxicity
- degradation, elimination

- Therapy
- Potency
- Shorter duration of treatment
- +/-RBV
- Genetic barrier
HCV genetic variability is very high....
...higher than HIV’s and HBV’s

31%–33% nucleotide difference among the 7 known HCV genotypes and 20%–25% among the nearly 67 HCV subtypes (Smith et al., 2014).
**Fig. 2. Summary of NS5A substitutions associated with resistance to NS5A inhibitors.** HCV genotypes and subtypes are represented by different colors: 1a-red, 1b-blue, 2-brown, 3a-green, 4-orange. Amino acid substitutions detected in vivo in DAA failing patients are underlined, independently of in vitro data information. In addition, NS5A RASs detected only in vitro but associated with fold-change in drug activity compared to the wild-type replicons $\geq 100$ (1st generation NS5A-inhibitors,) or $\geq 3$ (2nd generation NS5A-inhibitors) are also included in the figures. For 1st generation NS5A-inhibitors, in vivo substitutions with fold-change $\geq 100$, and in vitro substitutions with fold-change $\geq 000$ are represented in bold. For 2nd generation NS5A-inhibitors, in vivo and/or in vitro substitutions with fold-change $>10$ are represented in bold.

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**Not all NS5A RASs are equally clinical relevant**
Useful a HCV sequencing test before starting treatment?
HCV genotype dictates the choice of anti-HCV drugs and can modulate the duration of treatment in infected patients with chronic hepatitis C.

The HCV genotype, including genotype 1 subtype (1a or 1b), should be assessed prior to treatment initiation.

Table 5. IFN-free combination treatment regimens available as valuable options for each HCV genotype.

<table>
<thead>
<tr>
<th>Combination regimen</th>
<th>Genotype 1</th>
<th>Genotype 2</th>
<th>Genotype 3</th>
<th>Genotype 4</th>
<th>Genotypes 5 and 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sofosbuvir + ribavirin</td>
<td>No</td>
<td>Suboptimal</td>
<td>Suboptimal</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sofosbuvir/ledipasvir ± ribavirin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sofosbuvir/velpatasvir ± ribavirin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ombitasvir/paritaprevir/ritonavir + dasabuvir ± ribavirin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ombitasvir/paritaprevir/ritonavir ± ribavirin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Grazoprevir/elbasvir ± ribavirin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sofosbuvir + daclatasvir ± ribavirin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sofosbuvir + simeprevir ± ribavirin</td>
<td>Suboptimal</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
HCV sequencing is useful for identifying RASs but also the “correct” genotype: 16/327 (4.9%) patients were found infected with a different HCV genotype at failure. Notably, 11 patients previously classified as infected with HCV-1 were actually infected with HCV-2 and HCV-3, 9/11 failed a 3D+RBV regimen and all presented RASs at failure.

<table>
<thead>
<tr>
<th>ID</th>
<th>Pre-therapy genotype by commercial assay</th>
<th>Genotype by sequencing at failure</th>
<th>DAA regimen</th>
<th>DAA response</th>
<th>Failure RASs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1497</td>
<td>1a</td>
<td>3a</td>
<td>3D+RBV</td>
<td>Non-responder</td>
<td>Y93H</td>
</tr>
<tr>
<td>2150</td>
<td>1a</td>
<td>3a</td>
<td>3D+RBV</td>
<td>Breakthrough</td>
<td>Q80K, Y93H</td>
</tr>
<tr>
<td>2068</td>
<td>1b</td>
<td>3a</td>
<td>3D</td>
<td>Non-responder</td>
<td>Q80K, Y93H</td>
</tr>
<tr>
<td>1424</td>
<td>1b</td>
<td>3a</td>
<td>3D+RBV</td>
<td>Non-responder</td>
<td>Y93H</td>
</tr>
<tr>
<td>2140</td>
<td>1b</td>
<td>3a</td>
<td>3D+RBV</td>
<td>Non-responder</td>
<td>A30K</td>
</tr>
<tr>
<td>2353</td>
<td>1</td>
<td>3a</td>
<td>3D</td>
<td>Non-responder</td>
<td>Y93H</td>
</tr>
<tr>
<td>1823</td>
<td>1b</td>
<td>2c</td>
<td>3D+RBV</td>
<td>Non-responder</td>
<td>D168V</td>
</tr>
<tr>
<td>2020</td>
<td>1b</td>
<td>2c</td>
<td>3D</td>
<td>Non-responder</td>
<td>D168V, F28C</td>
</tr>
<tr>
<td>2623</td>
<td>1b</td>
<td>2c</td>
<td>3D</td>
<td>Relapse</td>
<td>F28C</td>
</tr>
<tr>
<td>2890</td>
<td>1b</td>
<td>2c</td>
<td>SMV+SOF</td>
<td>Relapse</td>
<td>L31M</td>
</tr>
<tr>
<td>2222</td>
<td>1b</td>
<td>3a</td>
<td>LDV+SOF+RBV</td>
<td>Relapse</td>
<td>R30Q+L31I+Y93H, C316N</td>
</tr>
<tr>
<td>2204</td>
<td>2</td>
<td>1b</td>
<td>LDV+SOF+RBV</td>
<td>Relapse</td>
<td>R30Q+L31I+Y93H, C316N</td>
</tr>
<tr>
<td>2886</td>
<td>2</td>
<td>1b</td>
<td>SOF+RBV</td>
<td>Relapse</td>
<td>Y56F, C316N</td>
</tr>
<tr>
<td>2153</td>
<td>2</td>
<td>3a</td>
<td>SOF+RBV</td>
<td>Relapse</td>
<td>A30K+L31F</td>
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<tr>
<td>1111</td>
<td>4</td>
<td>1a</td>
<td>2D+RBV</td>
<td>Breakthrough</td>
<td>V36M+Y56H, M28T</td>
</tr>
<tr>
<td>45</td>
<td>4</td>
<td>3a</td>
<td>SMV+SOF</td>
<td>Relapse</td>
<td>D168K</td>
</tr>
</tbody>
</table>

Di Maio VC et al., EASL 2017; Update Di Maio VC et al., ICAR 2017 Oral #78; Aragri et al., ICAR 2017 OC 51
HCV Resistance Testing Prior to (First-Line) DAA Therapy

Available, reliable, Interpretable, understandable*

Presence of NS5As RASs conferring high-level resistance (pop seq or >15%)

Add ribavirin and/or increase treatment duration

Not available

Optimize therapy to avoid treatment failure

- Add RBV in TE patients with SOF/LDV, SOF/DCV, SOF/SMV
- With 3D, use RBV in GT1a, treat NR cirrhotics 24 weeks
- Use GZR/EBR 16 weeks with RBV in GT1a patients

*Recommended for GZR/EBR for patients with GT1a but also for LDV/SOF for patients with GT1a and DCV/SOF and VPV/SOF patients with GT3

Pawlotsky et al, Gastroenterology 2016
NEW EASL Guidelines Sept 2016
Clinically relevant NS5A RASs that can be used to guide treatment decisions in GT1a and GT3 patients

If present: add RBV and/or increase treatment duration

EASL Guidelines Sept 2016, J of Hepatology 2017
The prevalence of pre-treatment NS5A RASs in GT-1 is different across different countries, ranging from 6% to 25%, and different according to subtype.

The Italian experience: different natural NS5A RASs prevalence according to genotype and subtype in DAA naive patients

Cento V, et al AASLD 2016, Update Ceccherini Silberstein CROI 2017
Impact of baseline NS5A RASs with $>100$ fold-resistance can be reduced by changing the regimen (longer duration of treatment and inclusion of RBV) in HCV-1 patients treated with Ledipasvir/Sofosbuvir.

First-line DAA regimen

![Graph showing SVR12 by Level of NS5A RASs in those Treated with Ledipasvir/Sofosbuvir.](image)

Figure 2. SVR12 by Level of NS5A RASs in those Treated with Ledipasvir/Sofosbuvir.

Patient baseline sequences generated by population and deep sequencing were pooled (using 1 percent cutoff for deep sequencing and population sequencing with a substitution detection of ~15 percent). (c) SVR12 for patients with NS5A RASs with $>100$-fold-resistance to ledipasvir in treatment-naive patients treated for 8 or 12 weeks and treatment-experienced patients treated for 12 or 24 weeks with and without ribavirin. * One patient experienced breakthrough due to documented noncompliance during the dosing period. LDV, ledipasvir. SOF, sofosbuvir. RBV, ribavirin

Sarrazin et al Gastroenterology 2016
Different impact according to specific baseline NS5A RASs in HCV-1 patients treated with Ledipasvir/Sofosbuvir

First-line DAA regimen

1% cutoff
No RAS, SVR:
GT1a: 98.3%
1306/1329 pts
GT1b: 98.6%
1741/1770 pts

15% cutoff
No RAS, SVR:
GT1a: 98%
1416/1445 pts
GT1b: 98.7%
1880/1915 pts

Figure 4. Treatment Outcome in Patients with NS5A RASs. Substitution analyses were conducted on deep sequencing data (population sequences were not included). (a) SVR12 by specific baseline NS5A RASs and cutoff (1 percent and 15 percent) in patients treated with ledipasvir/sofosbuvir.

Sarrazin et al Gastroenterology 2016
SVR rates were reduced in **GT-3 patients** with natural NS5A RAVs treated with grazoprevir, MK-3682 (NS5B), and MK-8408 (NS5A inhibitor) for 8 weeks.

**First-line DAA regimen**

**ASTRAL-3**: phase 3 study of SOF + VEL for 12 weeks in **GT 3 patients**

SVR12 84% (21/25) in patients with Y93H

Mangia A, AASLD 2015

Better results if longer duration of treatment and/or inclusion of RBV?
**First-line DAA regimen**

**Individualization of treatment yielded very high SVR rates among patients with HCV-3 infection**

- By adding RBV in patients with cirrhosis and/or with BL Y93H RAS: 100% SVR in patients treated with velpatasvir/sofosbuvir-based regimens

**VEL/SOF +/- RBV for 12 weeks – Results**

<table>
<thead>
<tr>
<th>GT3 patients n=72</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEL/SOF 12 wks.</td>
</tr>
<tr>
<td>VEL/SOF+RBV 12 wks.</td>
</tr>
<tr>
<td>Cirrhosis</td>
</tr>
<tr>
<td>BL Y93H</td>
</tr>
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</table>

**SVR (%)**

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Naive</th>
<th>Tx-exp.</th>
<th></th>
<th>All</th>
<th>Naive</th>
<th>Tx-exp.</th>
<th></th>
<th>All</th>
<th>Naive</th>
<th>Tx-exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Cirrhosis</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>No Cirrhosis</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>No Cirrhosis</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BL Y93H</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>BL Y93H</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>BL Y93H</td>
<td>100</td>
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</tr>
<tr>
<td>Cirrhosis</td>
<td>100</td>
<td>100</td>
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<td>Cirrhosis</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>Cirrhosis</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

- Baseline A30K, n (%) = 3 (4)
- Baseline Y93H, n (%) = 4 (6)

*includes 6 patients with SVR4; all other patients with SVR12

Vermehren J et al., EASL 2017
First-line DAA regimen

The presence of A30K or Y93H in NS5A at baseline can have an impact on short treatments of GT-3 infected patients treated with Glecaprevir/Pibrentasvir

**Table 6. Impact of A166S or Q168R in NS3 and/or A30K or Y93H in NS5A at Baseline on Treatment Outcome in GT3-Infected Patients**

<table>
<thead>
<tr>
<th>Baseline Polymorphism</th>
<th>No Cirrhosis</th>
<th>Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment-naïve</td>
<td>Treatment-experienced</td>
</tr>
<tr>
<td></td>
<td>8 Weeks</td>
<td>12 Weeks</td>
</tr>
<tr>
<td><strong>OVERALL SVR12</strong>a</td>
<td>97 (175/181)</td>
<td>99 (254/257)</td>
</tr>
<tr>
<td>With A166S</td>
<td>82 (14/17)b</td>
<td>100 (21/21)</td>
</tr>
<tr>
<td>Without A166S</td>
<td>98 (161/164)</td>
<td>99 (231/234)</td>
</tr>
<tr>
<td>With Q168R</td>
<td>(0/1)b</td>
<td>50 (1/2)d</td>
</tr>
<tr>
<td>Without Q168R</td>
<td>97 (175/180)</td>
<td>99 (251/253)</td>
</tr>
<tr>
<td>With A30K</td>
<td>73 (14/18)b</td>
<td>93 (13/14)c</td>
</tr>
<tr>
<td>Without A30K</td>
<td>99 (161/163)</td>
<td>99 (241/243)</td>
</tr>
<tr>
<td>With Y93H</td>
<td>100 (10/10)</td>
<td>91 (10/11)e</td>
</tr>
<tr>
<td>Without Y93H</td>
<td>97 (165/171)</td>
<td>99 (244/246)</td>
</tr>
</tbody>
</table>

aSVR12 rate in patients with available NS3/4A and/or NS5A baseline sequence.

bOne patient experiencing virologic failure had NS3-A166S/Q168R + NSSA-A30K (though NS3-A166S/Q168R were not detectable at the time of failure in this patient).
cOne virologic failure patient had NS3-A166S + NSSA-A30K; two SVR12 achieving patients had NS3-A166S + NSSA-A30K at baseline.

dOne patient experiencing virologic failure had NS3-Q168R + NSSA-A30K/Y93H at baseline.
eOne patient experiencing virologic failure had NS3-A166S + NSSA-A30K at baseline.

Better results if longer duration of treatment and/or inclusion of RBV?  

Krishnan P et al., EASL 2017
Italian real-life from 2 clinical centers within VIRONET-C network:

Proof-of-concept for a “Tailored” Resistance Guided Treatment

93 patients candidate to start a FIRST all-oral anti-HCV treatment

Baseline NS3 & NS5A GRT (optional NS5B)

36% GT-1a and 17% GT-3 (plus GT-1b, GT-2c, GT-4a/d)
50% previously treated (10% PI-experienced)
74% cirrhotics (4.3% decompensated)
Overall median (IQR) liver stiffness: 14.0 (11.2-26.3) kPa

Cento V, et al. unpublished
Italian real-life from 2 clinical centers within VIRONET-C network:

**Proof-of-concept for a “Tailored” Resistance Guided Treatment**

93 patients candidate to start a FIRST all-oral anti-HCV treatment

Baseline NS3 & NS5A GRT (optional NS5B)

- At least 1 RAS, N=30
  - 32.2%
- No RASs, N=63

36% GT-1a and 17% GT-3 (plus GT-1b, GT-2c, GT-4a/d)
50% previously treated (10% PI-experienced)
74% cirrhotics (4.3% decompensated)
Overall median (IQR) liver stiffness: 14.0 (11.2-26.3) kPa

NS5A RASs for daclatasvir/elbasvir/ledipasvir/ombitasvir/velpatasvir were found in 15/93 patients (16.1%), including 2/33 GT-1a (6.1%), 7/26 GT-1b (26.9%), 2/7 GT-2c (28.6%), and 4/16 GT-3a (25.0%). Double RAS were detected in 1 GT-1a, 2 GT-1b, and 1 GT-2c. Most frequently detected NS5A RASs were Y93H in GT-1b (3/26, 11.5%) and GT-3a (2/16, 12.5%); and F28C in GT-2c (2/7, 28.6%).

Cento V, et al. unpublished
Italian real-life from 2 clinical centers within VIRONET-C network:

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The appropriateness of treatment and adherence to guidelines has been evaluated according to: treatment duration, ribavirin, genotype and subtype, presence of compensated or decompensated cirrhosis, previous experience in IFN (or PI), eventual kidney failure

Other options

- RBV, and/or 12 weeks

2016

Other options

N=32

N=31

N=2

+ RBV, and/or 24 weeks

2015

+ RBV

24 weeks

N=16

N=7

2015

N=9

N=11

2016

N=30

N=19

Cento V, et al. unpublished
Optimal cure rate (100%) by personalized HCV regimens in real-life practice: a proof-of-concept study

Fig. 1. SVR rates according to treatment regimen. Histograms represent individual SVR rates for each DAA regimen, categorized according to current EASL recommendations 2016 and HCV genotype/subtype. Within each regimen category, the percentages of patients receiving a 12-week regimen is reported, along with percentages of patients with baseline resistance associated substitution for the selected regimens, and of patient with cirrhosis. 3D, paritaprevir/ritonavir, ombitasvir and dasabuvir; BL, baseline; DAA, direct antiviral agent; DAC, daclatasvir; HCV, hepatitis C virus; LDV, ledipasvir; RAS, resistance associated substitution; RBV, ribavirin; SIM, simeprevir; Sof, sofosbuvir; SVR, sustained virological response.

Cento V, et al unpublished
Virological issues in the DAAs Era

After treatment failure: useful / recommended the resistance test?

The utility of HCV resistance testing prior to retreatment in patients who failed on any of the DAA-containing treatment regimens is unknown. If reliable resistance testing is performed, retreatment can be guided by probabilities of response according to the resistance profile observed in the context of an experienced multidisciplinary team (B2).

For patients with cirrhosis or other patients who require retreatment urgently, testing for RAVs that confer decreased susceptibility to NS3 protease inhibitors (eg, Q80K) and to NS5A inhibitors should be performed using commercially available assays prior to selecting the next HCV treatment regimen.
Management of patients with treatment failure to IFN-free combinations

Statements:

• Resistance testing after treatment failure in all 3 genes (NS3, NS5A, NS5B [for the two different classes of nucleoside and non-nucleoside inhibitors] independently from the failure regimen) is mandatory in order to optimize retreatment strategy*.

• According to resistance results, current re-treatment strategies for patients failing a first course with DAAs should include at least 2 active drug classes, with a preferential use of one drug with high genetic barrier to resistance, and with extended treatment durations and addition of Rbv, otherwise waiting for better future options.

* NOTE: It would be desirable to preserve a sample before starting treatment with DAA, because in case of failure and presence of RAVs, the study of the baseline sample may help to distinguish if the resistance occurred during failure or it was already present as natural resistance before treatment. This information may help to set to the best the next regimen.
In our real-life experience, 88% of DAA-failures were relapsers

Overall, 327 failures following six different DAA-based therapies were analyzed

- SMV+SOF+/-RBV (N=85)
- 3D+/-RBV (N=48)
- 2D+/-RBV (N=2)
- LDV+SOF+/-RBV (N=97)
- DCV+SOF+/-RBV (N=67)
- SOF+RBV (N=28, HCV-2)

\[ \rightarrow 24.3\% \text{ of breakthroughs and non-responses were related to wrong GT assignments} \ldots \]
RASs prevalence at failure was high in almost all HCV genotypes/subtypes

RASs prevalence was significantly higher in breakthrough/non-responders (97.3%) than in relapsers (78.2%)

Di Maio VC et al., EASL 2017 & ICAR 2017
RASs prevalence was found in all genes tested: 
**NS5A very frequent (92%), NS3 frequent (67%), NS5B less common (24-39%)**

Y93H was the most frequent NS5A-RAS detected at failure (90% GT1b; 87.2% GT3a)

* NS5B RAS S282T and L159F were detected in 3.2% and 15.5% of sofosbuvir failures, respectively

Di Maio VC et al., EASL 2017 & ICAR 2017
Notably, 127/299 (42.5%) patients treated with ≥2 DAA classes showed RASs on ≥2 DAA-targets at failure

- 26.6% DAA-failing patients showed double-class NS3+NS5A RASs
- Among 113 patients treated without NS5A-inhibitors, 31.8% showed extra-target NS5A-RASs, more frequently in HCV-1b (36.1%) and HCV-2a/b/c (55.5%)
A NS5A RAV is forever

→ analysis of 36 GT1 patients who failed an elbasvir-regimen in phase 2/3 clinical trials.

Both pre-existing and treatment-emergent NS5A RAVs show persistence >2 years post-treatment.

Lahser F. et al., AASLD 2016
“Difficult-to-Retreat” Patients

NS5A inhibitor-containing regimen failures

Retreatment options are currently limited in patients who have failed HCV treatment with NS5A-containing regimens

Retreatment of genotype-3 infected patients who previously failed a recommended 24-weeks SOF + DCV + Rbv regimen with Y93H RAV should not be considered for retreatment with SOF + VEL + Rbv for 24 weeks, but rather wait for new combination, whether retreatment can be delayed.

Viganò M, et al DLD 2017

EASL Recommendations on Treatment of Hepatitis C 2016
SOF/VEL + RBV for 24 weeks may be an effective retreatment strategy for patients who had failed prior NS5A-containing regimens

No impact of RAVs on treatment outcomes in GT1 & GT2

Lower SVR among patients with NS5A RAVs in GT3

- 1/13 GT 3 patients with RAVs had Y93H; 9/11 (82%) achieved SVR12
- 5 patients had 2 NS5A RAVs; all 5 achieved SVR12
- 3 patients had NS3 RAVs; all 3 achieved SVR12

1% deep sequencing cut-off. *1 patient could not be sequenced

Gane EJ et al., EASL 2016
Y93H RAS commonly observed after daclatasvir failure, and retreatment of these patients is challenging...

Retreatment of GT3 patients who failed a first course of DCV+SOF therapy

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Cirrhosis</th>
<th>Prior PEG/R experience</th>
<th>Y93H</th>
<th>Retreatment</th>
<th>Outcome</th>
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</thead>
<tbody>
<tr>
<td>DCV+SOF, 24 wks.</td>
<td>male</td>
<td>yes</td>
<td>yes</td>
<td>Yes</td>
<td>DCV+SOF+RBV, 24 wks.</td>
<td>REL</td>
</tr>
<tr>
<td>DCV+SOF, 12 wks.</td>
<td>male</td>
<td>no</td>
<td>yes</td>
<td>Yes</td>
<td>DCV+SOF+RBV, 24 wks.</td>
<td>SVR12</td>
</tr>
<tr>
<td>DCV+SOF, 24 wks.</td>
<td>male</td>
<td>yes</td>
<td>yes</td>
<td>Yes</td>
<td>LDV+SOF, 24 wks.</td>
<td>REL</td>
</tr>
<tr>
<td>DCV+SOF, 12 wks.</td>
<td>male</td>
<td>yes</td>
<td>yes</td>
<td>Yes</td>
<td>VEL+SOF+RBV, 24 wks.</td>
<td>Pending</td>
</tr>
<tr>
<td>DCV+SOF, 12 wks.</td>
<td>male</td>
<td>no</td>
<td>yes</td>
<td>Yes</td>
<td>VEL+SOF+RBV, 24 wks.</td>
<td>SVR4</td>
</tr>
<tr>
<td>DCV+SOF, 12 wks.</td>
<td>male</td>
<td>no</td>
<td>yes</td>
<td>Yes</td>
<td>VEL+SOF, 12 wks.</td>
<td>REL</td>
</tr>
</tbody>
</table>

Retreatment of GT3 patients who failed a first course of SOF/RBV therapy

Retreatment with an NS5A-Inhibitor (n=40)

Interim analysis: SVR 88% (n=21/24)
80.4% (74/92) of patients achieved SVR$_{12}$ after retreatment of a IFN-free DAA failure

101 IFN-free DAA failures started a 2$^{nd}$ line regimen

To date, virological failure was observed in 19 patients: 16 patients relapsed, 2 experienced a virological breakthrough and 1 was a non-responder.

Cento V et al., update from EASL 2017; ICAR 2017 OC 53
80.4% (74/92) of patients achieved SVR$_{12}$ after retreatment of a IFN-free DAA failure

Retreatment choice based on

P-value was calculated by Fisher exact test. BL, baseline; GRT, genotypic resistance test; SVR, sustained viral response

Cento V et al., update from EASL 2017 & ICAR 2017
80.4% (74/92) of patients achieved SVR$_{12}$ after retreatment of a IFN-free DAA failure

<table>
<thead>
<tr>
<th>Retreatment choice based on</th>
<th>EASL guided</th>
<th>GRT guided</th>
<th>Not EASL, not GRT guided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sofosbuvir + ribavirin</td>
<td>49/56</td>
<td>18/22</td>
<td>7/14</td>
</tr>
<tr>
<td>N=59</td>
<td>P=0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sofosbuvir + simeprevir +/- ribavirin</td>
<td>32 with BL, GRT</td>
<td>8/12</td>
<td>4/6</td>
</tr>
<tr>
<td>N=22</td>
<td>P=0.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS5A inhibitors +/- ribavirin</td>
<td>14 with BL, GRT</td>
<td>11/15</td>
<td>4/4</td>
</tr>
<tr>
<td>N=11</td>
<td>P=0.033</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value was calculated by Fisher exact test.
BL, baseline; GRT, genotypic resistance test; SVR, sustained viral response

Cento V et al., update from EASL 2017 & ICAR 2017
Efficacy of NS5A-retreatment is reduced by baseline presence of NS5A-RASs (natural or derived by NS5Ai-exp)

Double NS5A RASs reduced SVR rates to 50% even in GT-1b patients.

Only 3/7 patients with Y93H/C RASs reached SVR.

19 patients failed retreatment, and all those tested showed NS5A RASs and 67% multiple and/or multiclass RASs …

P-value was calculated by Fisher exact test.

NS5Ai, NS5A inhibitor; RAS, resistance associated substitution; SVR, sustained viral response

Cento V et al., update from EASL 2017; ICAR 2017 OC 53
MAGELLAN-I: efficacy of glecaprevir/pibrentasvir in DAA-experienced patients can be reduced by **double-class RASs**

SVR\textsubscript{12} rate by prior DAA-experience

SVR\textsubscript{12} rate by baseline RASs

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Pilot-Matias T. et al., EASL 2017 poster #SAT-204
Retreatment may require «unconventional» approaches with multiple DAAs

DAA failure

Genotypic resistance testing
NS3+NS5A+NS5B

No NS5A RAVs

SOF/LDV ± RBV
SOF + DCV ± RBV
SOF/VPV ± RBV
GZR/EBV ± RBV
16/24 weeks

No NS3 RAVs

SOF + SIM + RBV
3D + RBV
24 weeks

NS5A RAVs (Q30, L31, H58, Y93)

SOF + SIM + RBV
24 weeks
(even if Q80K)

NS5A and NS3 RAVs (R155, A156, D168)

Desperation time

3D + SOF* or 2D++SOF
SOF + GZR/EBV + RBV*
SOF + SIM + DCV + RBV*
12/24 weeks

Investigational dual/triple regimens

* AASLD/IDSA HCV guidance, update of April 2016
EASL September 2016

Modified by Wyles D, AASLD 2015

Viganò M, et al DLD 2017
Do we need resistance testing in HCV therapy to guide decision making?

✓ Prior to first-line treatment (DAA-naive patient):
  – It is mandatory to assess HCV-genotype and GT-1 subtype

HCV sequencing is useful for identifying RASs but also the “correct” genotype (recommended in all rare cases where genotype or subtype cannot be determined by the commercial assays)
  – Natural resistance to NS3, NS5B and NS5A inhibitors has a substantial prevalence and a recognized role in treatment failure. Although genotypic resistance testing at baseline is not yet recommended (exception in GT1a: NS3-Q80K for SIM and NS5A-RASs for EBV), it remains a factor to be discussed in subgroups of patients with unfavourable BL factors (i.e. cirrhosis, TE, GT1a, GT3, high VL) or to allow more “sure” short treatments

✓ Baseline HCV-RNA quantification, along with HCV-GT and natural resistance interpretation, can be used together with clinical and host factors to optimize treatment choice and duration: PERSONALIZED THERAPY

✓ After failure prior to retreatment (DAA-experienced patient):
  – HCV resistance testing prior to retreatment can be helpful to make a decision if reliable resistance testing is performed. The resistance test should be performed in all 3 genes NS3 + NS5A + NS5B to understand if it will be better waiting for better future options

✓ Retreatment can be guided by probabilities of response according to the resistance profile observed in the context of an experienced multidisciplinary team (complexity: virus, host, clinical aspects, previous treatment outcome, DAA, RAS pattern)
HCV Virology Italian Resistance Network: VIRONET-C

VIRONET-C BOARD: F Ceccherini-Silberstein (Co-president), A Craxì (Co-president), M Andreoni, CF Perno, M Puoti, M Zazzi

STEERING COMMITTEE: S Bonora, M Brunetto, AP Callegaro, MR Capobianchi, V Cento, GB Gaeta, G Raimondo, T Santantonio

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GRT VALIDATION: + MSilvestri, MZazzi (Coordinator); AP Callegaro, VCento, NCoppola, ARGarbuglia, TRuggiero