#2 - HCV Resistance: Barriers, Selection and Monitoring of Resistance
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Many factors contribute to response

The symbol in the top-right hand corner of successive slides denotes whether the content refers to patient, virus, regimen or some combination thereof.

Sensitive virus
Resistant virus
Direct-acting antiviral (DAA)
Peg-IFNa/ribavirin (P/R)
Slide Set #2 Key points

1. There are genetic and replication fitness barriers to viral resistance
2. Resistant variants are present before treatment and can be selected during treatment
3. Frequent monitoring of HCV RNA during treatment can detect treatment failure and resistance
Virologic barriers to resistance

Antiviral

Genetic barrier
- Number and type of nucleotide changes required for a virus to acquire clinical resistance to an antiviral regimen\(^1,2\)

Viral fitness
- Relative capacity of a viral variant to replicate in a given environment
- Some resistance mutations can compromise viral enzyme function and thus reduce viral replication ability compared to wild-type in a drug-free environment

Genetic Barrier: Multiple nucleotide changes may be required to create a single amino acid change.

Example: Codon 155 of the HCV Protease

Subtype 1a – WT AGG → AAG

\[
\text{AGG} \rightarrow \text{AGG} \\
\text{R155} \quad \text{K155}
\]

Requires 1 step

Subtype 1b – WT CGG → AAG

\[
\text{CGG} \rightarrow \text{AGG} \rightarrow \text{AAG} \\
\text{R155} \quad \text{R155} \\
\text{AGG} \rightarrow \text{AAAG} \\
\text{R155} \quad \text{K155}
\]

Requires 2 steps
Impact of viral genotype on genetic barriers to resistance

Acquisition of protease inhibitor resistant variant V36M+R155K is more likely with subtype 1a than 1b

**Subtype 1a:** R155K+V36M variant observed clinically\(^1,2\)

\[
\begin{align*}
\text{AGG} &\rightarrow AGG \\
\text{R155} &\rightarrow K155 \\
\text{GTG} &\rightarrow ATG \\
\text{V36} &\rightarrow M36
\end{align*}
\]

Requires 2 steps

**Subtype 1b:** V36M+R155K variant not observed clinically

\[
\begin{align*}
\text{CGG} &\rightarrow AGG \\
\text{R155} &\rightarrow R155 \\
\text{GTG} &\rightarrow GTG \\
\text{V36} &\rightarrow V36
\end{align*}
\]

\[
\begin{align*}
\text{AGG} &\rightarrow AGG \\
\text{R155} &\rightarrow R155 \\
\text{GTG} &\rightarrow ATG \\
\text{V36} &\rightarrow M36
\end{align*}
\]

Requires 4 steps
Changes in drug susceptibility: Detection of resistance

- Sequence analysis and phenotype analysis are used in combination to identify/discover resistance pathways

  - **Sequence Analysis**: Detects specific amino acid substitutions relative to a pre-treatment or standard reference sequence that are known to decrease susceptibility to antiviral agents
    - Can identify substitutions known to impact drug susceptibility
    - Can identify novel drug resistance pathways associated with treatment failure

  - **Phenotypic Analysis**: Determines drug concentrations needed to inhibit viral replication
    - Effective concentration (EC): drug concentration required to inhibit viral replication by 50% or 90% (EC\textsubscript{50} or EC\textsubscript{90})
    - Less susceptible (resistant) viruses will require *more* drug to be inhibited, thus an *increase* in EC\textsubscript{50} or EC\textsubscript{90}
Resistant variants are present before treatment

- In every patient, HCV exists as a population mixture of genetically distinct but closely related virions\(^1\) (\textit{i.e.} quasispecies)
  - \(\sim 10^{12}\) viruses produced per day
  - \(\sim 1\) nucleotide mutation per virus produced
  - All possible single nucleotide-mutant viruses, and all combinations of double nucleotide-mutant viruses, are thought to preexist before treatment in most patients\(^2\)

- Most resistant variants are relatively unfit and may be not be detectable prior to therapy with current technology\(^3,4\)

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1. Pawlotsky JM. 	extit{Clin Liver Dis.}, 2003; 7:45-66
2. Rong L. 	extit{Sci Transl Med.}, 2010; 2 (30):30ra32
Resistant variants can be selected during treatment

Potent antiviral therapy eliminates sensitive variants

Resistant variants are uncovered which can then expand

Antiviral

Sensitive virus

Resistant virus
Drug resistance arises when a specific amino acid change occurs at a position that modifies the interaction with a drug.

- Decreased binding of a drug results in decreased inhibition of viral replication
- Decreased binding to the natural ligand results in decreased viral replication

(A) Telaprevir bound to the wild-type protease with the substrate envelope in blue. Intra and inter-molecular hydrogen bond interactions are marked as red and grey dashed lines. Telaprevir is also shown bound to the drug-resistant variants (B) R155K, (C) D168A and (D) A156T with the transparent coordinates representing the wild-type structure to better highlight the molecular changes of each mutation. In all cases, catalytic residues are depicted in yellow, the P2 subsite in pink, and the drug molecules in orange.

Romano KP, PLOS Pathogens 2012; 8(7):e10028232
Certain resistance mutations can reduce viral fitness

The A156T variant is less fit than WT

Steric hindrance due to the longer sidechain of Thr156 prevents the substrate from efficiently binding to the mutant protease active site

However, the impact on viral fitness depends on the specific resistance mutation

Clinically relevant NS3-4A resistance mutations, *in vivo*

**NS3 Protease (180 aa)**

- **simeprevir**: Q80 K, S122 I, R155 I, A156 S, D168 N
- **vaniprevir**: R155 I, A156 S, D168 N
- **faldaprevir**: R155 I, A156 S, D168 N
- **asunaprevir**: V36 M, R155 I, D168 N

Legend:
- Red aa = gt1a
- Blue aa = gt1b
- Purple aa = gt2

Amino acid positions where substitutions were detected in at least 10% of treatment failure patients:

Amino acid positions where substitutions were detected in less than 10% of treatment failure patients:

Amino acid substitutions identified in at least 10% of treatment failure patients:

Amino acid substitutions identified in less than 10% of treatment failure patients:
Clinically relevant NS5A resistance mutations, *in vivo*

**NS5A Domain 1 (213 aa)**

- **Daclatasvir**
  - M28: T
  - Q30: RE
  - L31: MM
  - Y93: CH

- **Ledipasvir**
  - M28: T
  - Q30: RH
  - L31: M
  - H58: D
  - Y93: HH

Legend:
- Red aa – gt1a
- Blue aa – gt1b
- Purple aa – gt2

Amino acid positions where substitutions were detected in at least 10% of treatment failure patients.

Amino acid positions where substitutions were detected in less than 10% of treatment failure patients.

Clinically relevant NS5B resistance mutations, \textit{in vivo}

\textbf{NS5B Polymerase (591 aa) - Non-nucleoside Analog}

deleobuvir

\textbf{NS5B Polymerase (591 aa) - Nucleoside Analog}

sofosbuvir

- Amino acid positions where substitutions were detected in at least 10% of treatment failure patients
- Amino acid positions where substitutions were detected in less than 10% of treatment failure patients
- Red aa – gt1a
- Blue aa – gt1b
- Purple aa – gt2
Frequent monitoring of HCV RNA levels can detect treatment failure and resistance.
Resistant variants selected during IFN-free, 2-DAA treatment associated with virologic failure

Resistance to both drugs detected in all 7 subjects with breakthrough or relapse*

NS5A
- Q30R
- L31M,V
- Y93C,N

NS3
- R155K
- D168A,E,T,V,Y

*The relapper had NS3 R155K detected at baseline, with emergence of NS5A Q30E at time of relapse
Say “NO” to CRAP therapy

Continued Replication under Antiviral Pressure

- Continued replication in the presence of drug will likely lead to further evolution of the viral population.
- In theory, further evolution can result in a more fit, drug-resistant viral population that may remain enriched in the patient, even in the absence of drug pressure.
- As known for HIV, though not yet shown for HCV, evolution of viral resistance may include secondary mutations that improve viral fitness.
- This should be prevented by discontinuing the direct acting antiviral if a patient has a confirmed increase in HCV RNA levels while adhering to therapy.
Potential fate of resistant variants after treatment

Sensitive virus

Resistant virus

HCV RNA

Treatment

Return to pre-treatment state

Post-treatment

Treatment

Persistence of resistant virus
Long-term follow-up of patients with resistant variants after failing treatment

- HCV population and clonal amino acid analyses in patient plasma suggest that PI-resistant viral populations *may* return to pre-treatment levels over time.

Resistant viral populations may return to pre-treatment (WT-dominant) state over time

- For boceprevir, 66 – 96% of patients no longer had detectable resistant variants after a median time of 1.11 year (13 months). These patients were followed up for 1.7 year (20 months).

- For telaprevir, 60-89% of patients no longer had detectable resistant variants after a median follow-up time of 10.6 months for genotype 1a, and 0.92 months for genotype 1b. These patients were followed for 1.3 year (16 months).

- Understanding the clinical significance of treatment-acquired resistance requires studies in which patients who experienced virologic failure while on a direct acting antiviral (DAA), are retreated with a DAA regimen.