Generic HCV Replication Cycle: A Few Facts About The Virus

- avrg. viremia (GE/ml): $10^5$
- virus production/d: $10^{12}$
- t1/2: 3h
- error rate RdRp: $2 \times 10^{-5}$
- variants/d: $\sim 10^{11}$

Bartenschlager et al., J Hepatol. 2010
HCV Replication Cycle: Entry

- EGF-Receptor
- Niemann-Pick C1–like 1 (NPC1L1) cholesterol uptake receptor

Lupberger et al., 2011; Sainz et al., 2012
HCV Entry into the Host Cell

Somewhat different requirements for entry molecules in case of cell-to-cell spread and less susceptible to anti-Env antibodies

Adapted from Gerold and Rice, 2011
HCV Genome Organization

Internal ribosome entry site
**Important Role of miR-122 for HCV**

- Increases HCV RNA stability
- Stimulates RNA translation
- Enhances RNA replication
- Stimulates mevalonate pathway
- Masking 5’ triphosphate end?

Honda et al., J. Virol. 1999

Machlin et al. PNAS 2011

Jopling et al., 2005, 2008; Henke et al.; 2008; Machlin et al., PNAS 2011; Shimakami et al., PNAS 2012
Functions of HCV Proteins

Structural proteins:
- Virus particle
- Ion channel
- Assembly
- Protease
- Membrane vesicles
- Phosphoprotein

Non-structural proteins:
- Protease/Helicase
- NS3-cofactor
- RNA Binding
- Replicase module
- Assembly module

IRES

5'

C E1 E2 2 3 4B 5A 5B 3'

p7

4A

4B

RdRp

Phosphoprotein

Assembly module

Replicase module
Properties of NS3/4A

- chymotrypsin-like enzyme
- activation by NS4A
- cleaves viral and cellular proteins
  - Polyprotein processing
  - innate responses (TLR3, RIG-I)

Brass et al. PNAS 2008
Properties of NS5A

- RNA-binding phosphoprotein
- assumed to regulate replication vs. assembly
- interaction with multiple viral and cellular partners
- Formation of oligomers?
- no known enzymatic activity
- difficult to identify inhibitors
Some Properties of NS5B RdRp

- 3 structural domains (palm, fingers, thumb)
- Fully encircled active site
- Initiation of RNA synthesis de novo
- Tail anchored protein
- RNA binding groove stacked to membrane interface
  - conformational change for activation
- Preferential binding to poly(U) and 5BSL3.2
- Regulation of activity by viral and cellular factors
  - NS5A
  - cyclophilin?

Appel et al., JBC, 2005
HCV Replication Cycle:
Formation of MW & RNA Amplification
Cyclophilin A
- binds to NS5A
- activates the RC

PI4Kinase III-alpha
- required for web formation
Essential Role of PI4KIIIα for Membranous Web Integrity

Normal conditions

PI4KIIIα depleted cell

HCV replication

Reiss, Rebhan et al., CHM 2011
HCV Replication Cycle:

Assembly and Release of Infectious Particles

Bartenschlager et al., J.Hepatol., 2010
Antiviral Therapy of Chronic Hepatitis C:
HCV-Specific Drug Targets

Viral Targets

- NS3/4A protease
- NS5A
- NS5B RdRp
The NS3/4A Protease

- Chymotrypsin-like enzyme
- Activation by NS4A
- Shallow and exposed substrate binding pocket
- Cleavage of viral and cellular proteins
  - Polyprotein processing
  - Inhibition of innate immunity (TLR3, RIG-I)
- Substrate specificity D/E - X₄ - C/T  S/A - X₃
- End product inhibition
  - Peptidomimetics
Development of NS3/4A Protease Inhibitors

Ingallinella et al., Biochemistry 1998

Telaprevir
Structures of NS3-Specific Drugs

Telaprevir (Incivek®, Incivo®)

Boceprevir (Victrelis™)

MK5172

TMC435
Mode-of-Action of NS3-specific DAAs

Blockage of polyprotein cleavage
- block of new formation of replication vesicles
- no effect on established replication vesicles

 Restoration of innate immune response?
- RIG-I (MAVS)
- TLR3 (TRIF)
HCV-Specific Drug Targets

Viral Targets
- NS3/4A protease
- NS5A
- NS5B RdRp

Host Cell Targets
- miR-122
- Cyclophilin A
- PI4Kinase-IIIα
Two Classes of Compounds Assumed to Target NS5A

Resistance mutations in NS5A domain I:
L31V, Y93H (gt 1b)
M28T, Q30H/R, L31M/V, Y93C (gt 1a)

target NS5A

Resistance mutations in NS5A domain I, II and III:
L199F, T200P, E212D, P299L, I302T,
V362A, S370P, V388D, S390G
NS4B: S258T
NS5B: S76A

target PI4K-IIIα

BMS -790052
(Daclatasvir)

A-831:4-NH₂-quinazolines (Arrow/AZ)

Schmitz & Tan, Rec Pat Anti Infect Drug Discov, 2008; Delang et al., Viruses 2010; Bianco et al., Plos Path, 2012
BMS-79005: The most potent DAA

- discovered via high-throughput screen with subgenomic HCV replicons
- active against genotype 1 – 6 replicons (chimeras)

<table>
<thead>
<tr>
<th>HCV subtype</th>
<th>Replicon EC$_{50}$ (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>50</td>
</tr>
<tr>
<td>1b</td>
<td>9</td>
</tr>
<tr>
<td>2a</td>
<td>12-63</td>
</tr>
<tr>
<td>3a</td>
<td>127</td>
</tr>
<tr>
<td>4a</td>
<td>12</td>
</tr>
<tr>
<td>5a</td>
<td>33</td>
</tr>
</tbody>
</table>

Possible Mode-of-action of Daclatasvir

NS5A inhibitors are dominant negative (1 inhibitor per 100 – 1,000 NS5A molecules)

- mistargeting of NS5A
- block of 5A oligomerization?
- block NS5A hyperphosphorylation?
HCV-Specific Drug Targets

Viral Targets
- NS3/4A protease
- NS5A
- NS5B RdRp

Host Cell Targets
- miR-122
- Cyclophilin A
- PI4Kinase-IIIα
Nucleosidic and Non-nucleosidic NS5B-Specific Drugs

Non-nucleosidic Inhibitors

Benzimidazole derivative
Thiophene derivative
Thiadiazine derivative

Nucleosidic Inhibitors

PSI-7977
INX-189
2'-C-methyl cytidine
Mode-of-Action of NS5B-Specific Inhibitors

1 (+) RNA $> 1.000$ polyproteins

Block of RNA synthesis
- direct effect also on established replication complexes
- direct inhibition of NS5B by induction of conformational change (non-nucs)
- block of elongation, i.e. chain termination (nucs)
HCV-Specific Drug Targets

Viral Targets

NS3/4A protease

NS5A

NS5B RdRp

Host Cell Targets

miR-122

Cyclophilin A

PI4Kinase-IIIα
Mode-of-action of Miravirsen

Proof-of-Concept Study in Chimpanzees

• Long-lasting suppression of viremia
• No resistance
• No side-effects
• Down-regulation of ISGs
• Improvement of liver histology

Lanford et al., Science 2010
Structure of CsA and CsA-Derivatives

adapted from Galley, Clin Liv Dis 2009
### Structure of Cyclophilin Inhibitors

<table>
<thead>
<tr>
<th>Structure</th>
<th>Immunosuppression Activity (IL-2 inhibition)</th>
<th>CypA PPlase Inhibition (Kᵢ)</th>
<th>Anti-HCV Activity (EC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIM811</td>
<td>&gt;10 µg/mL</td>
<td>2.11 nM</td>
<td>0.06 µM</td>
</tr>
<tr>
<td>Alisporivir</td>
<td>21.5 µg/mL</td>
<td>0.34 nM</td>
<td>0.04 µM</td>
</tr>
<tr>
<td>SCY-635</td>
<td>13 µg/mL</td>
<td>1.8 nM</td>
<td>0.1 µM</td>
</tr>
<tr>
<td>Sanglifehrin</td>
<td>?</td>
<td>0.3 nM</td>
<td>0.02 µM</td>
</tr>
</tbody>
</table>

Adapted from Gallay, Clin Liv Dis 2009
Mode-of-Action of Cyp Inhibitors?

HCV replication

HCV replication
If I decide to become a scientist, eventually a virologist, and will work on hepatitis C virus what will my future be?
Department for Infectious Diseases
Molecular Virology

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