HIV, HBV, HCV
Virology

Anna Maria Geretti
Institute of Infection & Global Health
University of Liverpool
• Many similarities
• Several fundamental differences
HIV

RNA virus

- Chronic infection
- Without treatment, most people develop AIDS and die within ~10 years (7.5 to 11.6)\(^1,2\)
- Non-AIDS HIV-related disease

- Latent reservoir as integrated provirus
- Antiviral therapy controls but does not eradicate HIV
- Life-long therapy required to suppress virus replication
- PrEP and PEP

# HBV

| DNA virus | • Vaccine  
|-----------|--------------------------------------|
|           | • Chronic infection in >90% children, <5% adults  
|           | • Cirrhosis (~30%)  
|           | • Hepatocellular carcinoma (with/without cirrhosis)  
|           | • Extra-hepatic disease  
|           | • Persistence as cccDNA, may integrate  
|           | • Several replicative states  
|           | • Antiviral therapy not always required, controls but does not eradicate HBV, can be stopped in some cases  
|           | • Antivirals work as PrEP  

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[Image of liver and hepatitis B virus particles]
# HCV

**RNA virus**

- Chronic infection ~80%
- Cirrhosis (41% over 30 years), hepatocellular carcinoma
- Extra-hepatic disease increasingly recognised\(^1,2\)

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>No stable or latent reservoir\</td>
</tr>
<tr>
<td></td>
<td>Curable with antiviral therapy\</td>
</tr>
</tbody>
</table>

Attachment                      Fusion                      Release of RNA

Reverse transcription           Integration             Transcription

HIV replication

Assembly

Maturation & budding
Targets of therapy

- Attachment
- Fusion
- Release of RNA

- Reverse transcriptase inhibitors
- Integrase inhibitors
- RT inhibitors
- Pro tease inhibitors
- CCR5 antagonists
- Pro tease inhibitors

Maturation & budding
Maturation & budding

Protease

Polyprotein
HIV Reverse transcriptase/Polymerase

Two mechanisms of inhibition
• Competitive – NRTIs
• Allosteric – NNRTIs
DNA chain terminated

Template strand

Primer strand

NRTI

DNA chain terminated

5'

3'

5'
HIV DNA forms

- HIV RNA
- Linear HIV DNA
- Integration
- Proviral DNA
- Host DNA
- HIV RNA

Nucleus

Host cell
Effect of ART duration on virological & immunological parameters

- **sCD14**: $p=0.19$
- **CD8$^+$HLA-DR$^+$**: $p=0.17$
- **CD8$^+$CD38$^+$**: $p=0.01$
- **CD4$^+$CD69$^+$**: $p=0.22$
- **CD4$^+$CD38$^+$**: $p=0.01$
- **CD4$^+$CD26$^+$**: $p=0.69$
- **CD4 count**: $p=0.05$
- **Integrated HIV-1 DNA**: $p=0.28$
- **Total HIV-1 DNA**: $p=0.60$
- **2-LTRc DNA**: $p=0.01$
- **Residual plasma HIV-1 RNA**: $p=0.08$

Mean difference per 10 years of suppressive ART

Log-transformed variables
Virus replication resumes if therapy is stopped

- Antiretroviral therapy cannot achieve HIV eradication
- After stopping therapy HIV replication resumes to pre-treatment levels
Mechanisms of HIV genetic evolution

1. Errors by viral reverse transcriptase
   - \(~1\) mis-incorporation per genome round
2. Errors by cellular RNA polymerase II
3. APOBEC-driven G\(\rightarrow\)A hypermutation
   - *Deamination of cytosine residues in nascent DNA*
4. Recombination between HIV strains
• Rapid replicating virus (~$10^{10}$ particles/day)
• Rapid clearance of newly produced virus
• Highly error prone polymerase $\Rightarrow$ High mutation rate
• Some mutations detrimental, some allow escape

Quasispecies

- rapid turnover
- rapid adaptation

Escape

Fitness
Plasma HIV RNA

Viral gene (e.g., RT)

PCR

HIV RNA

Plasma

Sequencing

Mutations

RT M184V
Methionine → Valine
@ codon 184 of RT
ATG / AUG → GTG / GUG
Limit of detection of conventional sequencing

Detected by deep sequencing

Detected by conventional sequencing

Natural background

~10-20%
Emergence & evolution of HIV drug resistance

Single mutant  Double mutant  Triple mutant
The genetic barrier to resistance is expression of multiple interacting factors

- Virus sequence
- Phenotypic effect of individual mutations
- No. of mutations required to reduce drug susceptibility
- Fitness cost of the mutation
- Ease of emergence of compensatory adjustments

- Drug potency
- Mode of interaction between drug and target
- Drug concentration
- Drug combination
- Antagonism or synergism between resistance pathways

- Viral load
- Host genetics
- Host immune function
- Reservoirs of replications

More than the sum of each drug in a regimen
Mechanisms of NRTI resistance

- T215Y (AZT, ABC, ddl, d4T, TDF)
- M184V (3TC, FTC)
Mechanisms of NRTI resistance

- **T215Y**
- **M184V**

Antagonised by M184V
Dissociation time of integrase inhibitors

![Graph showing dissociation time of integrase inhibitors](image)

Dissociation time of integrase inhibitors

Replicative capacity of integrase mutants

Dissociation time of integrase inhibitors

![Graph showing dissociation time of integrase inhibitors with curves for DTG, RAL, and EVG.](image)

- **GGT or GGC**: Glycine
- **AGT or AGC**: Serine
- **GGA or GGG**: non-B subtypes

Replicative capacity of integrase mutants

![Bar graph showing replicative capacity of integrase mutants.](image)

- **E92Q**: 75
- **Y143**: 72
- **Q148**: 75
- **N155**: 75
- **R263K**: 70
- **R263K/Q148**: 95
- **R263K/H51Y**: 25
- **R263K/E138K**: 25

Codon usage at integrase position 140 in B vs. non-B subtypes

- **GGT or GGC**: Glycine
- **AGT or AGC**: Serine
- **GGA or GGG**: non-B subtypes

References:
- Hightower Antimicrob Agents Chemother 2011
- Quashie J Virol 2012
- Wainberg ISHEID Conference 2014
- Doyle JAC 2015
HBV replication
HBV drug targets

Nucleoside and nucleotide analogues
- Lamivudine*
- Adefovir
- Entecavir*
- Telbivudine
- Tenofovir*
- Emtricitabine*
HCV replication

Receptor binding and endocytosis

Transport and release

Fusion and uncoating

(+) RNA

RNA replication

Virion assembly

Translation and polyprotein processing

Cleavage

NS3/4A Protease

NS5A

NS5B

HCV Replicase

Replicated HCV RNA

NSSB Polymerase

Brett Nature 2005
HCV drug targets

- Receptor binding and endocytosis
- Fusion and uncoating
- Transport and release
- NS5A Inhibitors
- NS5B Inhibitors
- RNA replication
- Virion assembly
- Translation and polyprotein processing
- NS3 Protease Inhibitors
- NS5A Inhibitors

HCV enzymes provide good targets for drug development. RNA Polymerase C... 
• Rapid replicating virus (HIV $10^{10}$ - HBV $10^{11}$ - HCV $10^{12}$ particles/day)
• Rapid clearance of newly produced virus
• Highly error prone polymerase ➔ High mutation rate
• Some mutations are detrimental, some allow escape

Quasispecies

Escape
- rapid turnover
- rapid adaptation

Fitness
Incidence of HBV drug resistance

Years 1-5; first-line therapy

- 1st generation
  - LAM: 24, 38, 49, 67, 70%

- 2nd generation
  - ADV: 0, 3, 11, 18, 29%
  - LdT: 4, 17, 34%

- 3rd generation
  - ETV: 0.2, 1.2, 1.2, 1.2
  - TDF: 0.2, 1.2, 1.2, 1.2

LAM = Lamivudine
ADV = Adefovir
LdT = Telbivudine
ETV = Entecavir
TDF = Tenofovir
HCV genetic variability

- **NS3**: 42% of amino acid conserved among all genotypes
- **NS5A**: 46% of amino acid conserved among all genotypes
- **NS5B**: 55% of amino acid conserved among all genotypes
Prevalence of NS3 resistance mutations in naïve patients with HCV Gt1a

- HCV treatment-naïve subjects
- Tested by Conventional Sequencing (CS) and Deep Sequencing (DS, Illumina)
- Most mutations detected by CS (= dominant)
- No difference in HCV RNA load in samples with vs. without resistance (= preserved fitness)

<table>
<thead>
<tr>
<th>RAMs</th>
<th>CS &amp; DS &gt;10% n 238</th>
<th>DS 1-10% n 178</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 M</td>
<td>1 (0.4)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>36 L</td>
<td>5 (2.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>54 S</td>
<td>9 (3.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>55 A</td>
<td>10 (4.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>80 K</td>
<td>44 (18.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>168 E</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>170 A</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>170 T</td>
<td>0 (0)</td>
<td>2 (1.1)</td>
</tr>
</tbody>
</table>

RAMs = Resistance-associated mutations
# Profile of HCV treatment options

<table>
<thead>
<tr>
<th>TARGET of THERAPY</th>
<th>Protease 1&lt;sup&gt;st&lt;/sup&gt; gen</th>
<th>Protease 2&lt;sup&gt;nd&lt;/sup&gt; gen</th>
<th>NS5A</th>
<th>NS5B NAs</th>
<th>NS5B non-NAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype coverage</td>
<td>![Red]</td>
<td>![Blue]</td>
<td>![Blue]</td>
<td>![Green]</td>
<td>![Blue]</td>
</tr>
<tr>
<td>Potency</td>
<td>![Blue]</td>
<td>![Green]</td>
<td>![Green]</td>
<td>![Green]</td>
<td>![Blue]</td>
</tr>
</tbody>
</table>

- ![Red] Least favourable profile
- ![Blue] Average profile
- ![Green] Good profile
Key points: Drug resistance with HIV, HBV, HCV

- Drug-resistant mutants emerge “spontaneously” during virus replication
- HIV and HBV mutants exist as rare species prior to therapy
- HCV single/double mutants are often dominant in naïve patients (NS3 and NS5A)
- Virus replication under drug pressure drives expansion of the mutants – *Natural evolution* ➞ *increasing resistance & fitness*
- If therapy is stopped, drug susceptible virus tends to outgrow resistant mutants selected by therapy – *mutants persist as enriched minority species*
- Mutants are archived in HIV DNA provirus and HBV cccDNA
Your turn 😊
Which of the following correctly describes HIV?

1. RNA virus, high replication during AIDS phase only
2. RNA virus, high replication, stable genetic make-up
3. RNA virus, high replication, rapid genetic evolution
Your turn 😊
Which of the following correctly describes HIV?

1. RNA virus, high replication during AIDS phase only
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3. RNA virus, high replication, rapid genetic evolution
Your turn 😊

Which of the following correctly describes HBV?

1. HBV polymerase lacks reverse transcriptase activity
2. The genomic structure favours rapid emergence of resistance
3. Resistance is less of a problem with 3rd gen drugs
Your turn 😊
Which of the following correctly describes HBV?

1. HBV polymerase lacks reverse transcriptase activity
2. The genomic structure favours rapid emergence of resistance
3. Resistance is less of a problem with 3rd gen drugs
Your turn 😊

Which of the following correctly describes HCV?

1. Resistance is created by suboptimal therapy
2. Resistance is selected by suboptimal therapy
3. Resistance is archived in the nucleus of hepatocytes
Your turn 😊
Which of the following correctly describes HCV?

1. Resistance is created by suboptimal therapy
2. Resistance is selected by suboptimal therapy
3. Resistance is archived in the nucleus of hepatocytes
Well done!
The HIV virology timeline

1982

HIV-1 isolated

1985

HIV-1 genome sequenced

1991

HIV replicates at high levels throughout the infection

1995

HIV replication drives immune compromise

1996

Plasma HIV RNA (‘viral load’) suppression as goal of therapy

2009

Highly active antiretroviral therapy

2010

HIV eradication research

HIV replication causes disease through immune activation & inflammation
HIV tropism defined by co-receptor use

- Naive CD4 cells
- Memory CD4 cells
- Macrophages

Must be activated to memory phenotype to become target of R5

Esté Lancet 2007
HIV DNA load during antiretroviral therapy

- HIV DNA quantified in PBMC

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## Genetic barrier and cross-resistance

<table>
<thead>
<tr>
<th>Class</th>
<th>ARVs</th>
<th>Genetic Barrier</th>
<th>Cross Resistance</th>
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<tbody>
<tr>
<td><strong>NRTIs</strong></td>
<td>ZDV/3TC, d4T/3TC</td>
<td>+/++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>ABC/3TC, TDF/3TC</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>TDF/FTC</td>
<td>+/++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>NNRTIs</strong></td>
<td>EFV, NVP, RPV</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>ETR</td>
<td>+/++</td>
<td>++(+)</td>
</tr>
<tr>
<td><strong>PIs</strong></td>
<td>Unboosted</td>
<td>+/++</td>
<td>++/++++</td>
</tr>
<tr>
<td></td>
<td>Boosted</td>
<td>+++/+++++</td>
<td>+/++</td>
</tr>
<tr>
<td><strong>Fusion inhibitors</strong></td>
<td>T20</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td><strong>CCR5 antagonists</strong></td>
<td>MVC</td>
<td>+/++</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Integrase inhibitors</strong></td>
<td>RAL, EVG</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>DTG</td>
<td>++/++++ (+)</td>
<td>++(+)</td>
</tr>
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Transmitted Drug Resistance

Relatively stable after transmission
Gradual reversion over time
Persistence at low frequency in plasma
Persistence in latently infected cells