Hepatitis B
Drug Resistance: Navigating the Way Forward

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Head-Research & Molecular Development, VIDRL
The paradigm of antiviral therapy is the suppression and maintenance of viraemia below the limit of detection.
Indications of Emergence of Drug-Resistant Virus

1. Increasing viral load ($\geq 1.0 \log \text{ IU/ml}$)
2. Identification of known genotypic markers of drug resistance within viral polymerase:
   * primary resistance mutations (rtM204I)
   * secondary resistance mutations (rtL180M with rtM204V)
   * compensatory mutations (rtV173L)
3. Increasing serum ALT levels
4. Clinical deterioration

Dynamics of Resistance Emergence
Genotypic Resistance

Lamivudine

ALT (U/L)

PCR assay

HBV DNA log copies/mL

Months

Codon 180
L M M M M M M

Codon 204
L M M M M M M

Codon 207
L M M M M M M

Genotypic resistance

Dynamics of Resistance Emergence
Virologic Breakthrough

Lamivudine

Rebound of serum HBV DNA*

> 1 log_{10} copies/mL

PCR assay

ALT (U/L)

HBV DNA log copies/mL

Months

Codon 180
L L L/M M M M/M/V M M/M/V M M V V

Codon 204
M M M M M M V V V

Codon 207
V V V V V V V V
Dynamics of Resistance Emergence
Clinical Breakthrough

Rise in serum transaminases

Worsening of liver disease

Lamivudine

ALT (U/L)

HBV DNA log copies/mL

PCR assay

Months

Codon 180
L
M
V

Codon 204
L
M
V

Codon 207
L/M
M
V

M/V

M/V

M/V

M/V

M/V

M/V
Drug Potency and Maximal Suppression of Viral Replication

HBV replication

Maximal rapid suppression = minimal risk of resistance

Naturally-occurring variants

Incomplete Suppression of Viral Replication Allows the Selection of Resistant Virus

Viral Replication and Mutational Frequency

- High virion production: $10^{12-13}$ virions per day
- Wild-type HBV Pol lacks proof-reading function
- High mutational rate: $10^{-5}$ substitution/base/cycle
- $10^{10-11}$ point mutations produced per day
- All possible single base changes can be produced per day
- Single / double mutations pre-exist in HBV from patients prior to therapy: WHY MOST MONOTHERAPIES FAIL
- Triple / quadruple mutations require replication in the presence of selection pressure and rarely pre-exist: WHY COMBINATION TREATMENT WORKS

(Colgrone & Japour. 1999. AVR. 41:45)
Primary Resistance Substitutions

Terminal Protein

Spacer

POL/RT

RNaseH

1 183 349 (rt1) 692 (rt 344) 845 a.a.

F__V__LLAQ__YMDD

L-Nucleoside Resistance
LMV
rtA181T/V
rtM204V/I

L-dT
rtA181T/V
rtM204I

Acyclic Phosphonate Resistance
ADV/TFV
rtA181T/V
rtN236T

D-Cyclopentane Resistance
ETV
rtl169
rtL180M
rtS202
rtM250

rtT184
rtM204V/I

Terminal Protein Spacer POL/RT
# Pathways of Antiviral Resistance in Chronic Hepatitis B

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Mutation</th>
<th>Associated Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-nucleoside</td>
<td>rtM204V/I</td>
<td>Lamivudine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Emtricitabine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Telbivudine</td>
</tr>
<tr>
<td>Acyclic phosphonate</td>
<td>rtN236T</td>
<td>Adefovir</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tenofovir</td>
</tr>
<tr>
<td>“Shared”</td>
<td>rtA181T/V</td>
<td>L-nucleosides (see above)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acyclic phosphonates (see above)</td>
</tr>
<tr>
<td>naïve entecavir resistance</td>
<td>rtL180M + rtM204V with one of rtT184, S202 or M250</td>
<td>Entecavir</td>
</tr>
<tr>
<td>Multi-drug resistance</td>
<td>Complex patterns e.g. rtA181T + rtN236T + rtM250L</td>
<td>Multi-drug</td>
</tr>
</tbody>
</table>

Yuen, L. et al 2007. Hepatology vol 46 (No.4, Suppl.1) 659A Abstract #949
Compensatory Substitutions

Relative replication yield of HBV mutants

Multi-Drug Resistance (MDR)

- Sequential addition of resistance mutations to the same viral genome
- **Promoted with sequential monotherapy**, especially by using drugs with similar (structural) characteristics
- **Role of compensatory mutations** virus replication competence (fitness)
- **Need for drug-resistance testing** (Pol sequencing) to determine and monitor therapy:
  - rtA181T (“Shared” Pathway)
  - rtA181T+rtN236T
  - rtA181T+rtI233V+rtN236T+rtM250L
Factors Associated with the Emergence of Resistance to CHB Therapy


- Antiviral potency
- Level of exposure to drug
- Chemical structure
- Number of mutations needed to overcome drug suppression (genetic barrier)

- Prior antiviral exposure
- Adherence
- Immune status
- Persistence of archived mutations as cccDNA
- Metabolism
- Body mass

- Replication rate
- Replication fidelity
- Pre-existing mutations
- Replication fitness and space

Antiviral Drug

Virus

Patient
Genetic barrier increases as the number of specific mutations required for drug resistance increases.

Ways to Prevent Resistance

Maximize antiviral activity
- increase maximum tolerated dose
- select most effective regimen (combination)
- nucleoside analogue potentiation

Maximize genetic barriers to resistance
- avoid sequential monotherapy
- choose drugs requiring multiple resistance mutations (1 or 2 mutations pre-exist vs 3 or 4 require ongoing selection)
- choose drugs where patient is naïve

Increase pharmacologic barriers
- patient compliance
- raising trough levels
- Prior drug experience
- Drug metabolism
- pharmacodynamic issues (eg, cirrhosis)
Impact of Substitutions on Viral Fitness

Resistance substitutions usually impair the ability of the virus to replicate

Entecavir susceptibility varies according to the specific resistance substitution and the proportion of variants in the quasispecies

- Wild-type virus
- LVDr virus (V173L, L180M, M204V)
- ETVr virus (I169T, V173L, L180M, M204V, M250V)

*Transfection of HepG2 cells with recombinant viruses from a patient who was LVD-resistant and experienced virological breakthrough after more than 1 year of ETV therapy

Entecavir susceptibility varies according to the specific resistance substitution and the proportion of variants in the quasispecies

Comparative EC$_{50}$ for HBV Antivirals in Cell Culture

WT: ETV EC$_{50}$ = 5 nM (> 275-fold more potent)

Resistance Rates Through 6 Years Among Nucleos(t)ide-Naïve Patients

Patients with HBV DNA ≥400 copies/mL at Week 72 could add FTC to TDF;

* Cumulative probabilities of resistance taken; † Naïve HBeAg (+); ‡ Naïve HBeAg(-); N/A not available.

Cross-Resistance

• Resistance to drug(s) to which a virus has never been exposed

• Resistance-associated mutations selected by drugs may diminish the antiviral activity of other drugs\(^1\):
  – *this should be considered before any antiviral drug is prescribed*

• Cross-resistance tends to be more common for compounds sharing structural properties\(^2\)

• Any change in therapy, typically combination or add-on strategies, should be made using drugs that lack cross-resistance with the failing agent\(^1\)

## Common Pathways of Antiviral Resistance in CHB: Cross-Resistance

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Resistance Substitutions</th>
<th>Antiviral Agent</th>
<th>Cross Resistance Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Nucleoside (L-NA)</td>
<td>rtM204V/I</td>
<td>Lamivudine (LMV) Emtricitabine (FTC) Telbivudine (LdT)</td>
<td>R S S I</td>
</tr>
<tr>
<td>Acyclic Phosphonate</td>
<td>rtN236T</td>
<td>Adefovir (ADV) Tenofovir (TDF)</td>
<td>S R I S</td>
</tr>
<tr>
<td>&quot;Shared&quot;</td>
<td>rtA181T</td>
<td>L-Nucleoside Acyclic Phosphonate</td>
<td>R R I S</td>
</tr>
<tr>
<td>Naive Entecavir Resistance</td>
<td>rtL180M+rtM204V with one of rtT184, S202 or M250</td>
<td>Entecavir (ETV)</td>
<td>R S S R</td>
</tr>
<tr>
<td>Multi Drug Resistance</td>
<td>Complex patterns e.g. rtA181T+rtL233V+ rtN236T+rtM250L</td>
<td>Multi-Drug</td>
<td>* consult reference laboratory * Role of IFN-α</td>
</tr>
</tbody>
</table>

R = Resistance; S = Sensitive; I = Intermediate

Modified from Zoulim F and Locarnini S. 2009. Gastroenterol;137:1593
Probability of Achieving Complete Virologic Response Under TDF Monotherapy
(HBV DNA levels <400 copies/ml)

Van Bommel F. et al 2010. Hepatol;51:73
Tenofovir Rescue Therapy

rtA181T/V without rtN236T (n=10)

rtN236T patients (n=7, 3 alone, 4 with rtA181T/V)

Patterson, S. et al 2009. Hepatology;50:534A
Patients with LMV Resistant HBV (rtM204I)
• lower serum ALT compared to pre-therapy
• lower HBV DNA elevations compared to pre-therapy

HOWEVER
• marked flares of serum ALT are observed
• acute exacerbations in liver disease can occur
• these flares may be followed by HBeAg seroconversion and/or immune clearance of mutant HBV

BUT
• a new and distinct mutant may be selected and elicit another exacerbation and then select another mutant (rtA181T/V)

• Further compensatory mutations continue to occur, such as rtV173L

and rtV214A/rtQ215S.

and rtT184S

• Which will affect subsequent efficacy of salvage/rescue therapy
LVD-Refractory Cohort (HBeAg[+]): Cumulative Probability of Entecavir Resistance Through 6 Years

- 74/187 (40%) achieved HBV DNA <300 copies/mL
- 5/74 (7%) with HBV DNA < 300 c/mL had subsequent genotypic Entecavir resistance
- Majority of patients in this cohort had confirmed baseline LVDr

ETVr = LVDr (M204V ± L180M) + T184, S202 and/or M250 substitutions

74/187 (40%) achieved HBV DNA <300 copies/mL
5/74 (7%) with HBV DNA < 300 c/mL had subsequent genotypic Entecavir resistance
Majority of patients in this cohort had confirmed baseline LVDr

Entecavir: an Option in LVD-Experienced Patients Without LVD-Resistance

What to do on First Virological Breakthrough/Partial Virological Response

- Repeat HBV DNA testing in a timely manner to confirm VL breakthrough
- If confirmed [HBV VL ≥ 1.0 log IU/ml] THEN perform HBV POL SEQUENCING
- Typical results for the HBV POL:
  i. “WILD-TYPE” SEQUENCE if no known resistance mutations found
  ii. rtM204V/I±rtL180M detected
  iii. rtA181T/V
  iv. rtN236T±rtA181T/V detected
  v. rtT184S/A/I/L+rtS202G/C (and/or rtM250I/V) WITH rtL180M+rtM204V
  vi. Complex pattern(s) detected

Zoulim, F & Locarnini, S. 2009. Gastroenterology; 137:1593
Resistance to Antiviral Therapy can Compromise Treatment Goals

- Development of resistance has a negative impact on CHB therapy:
  - Virological breakthrough\(^1\)
  - ALT elevation or ALT flares\(^1,2\)
  - Decreased HBeAg seroconversion\(^3\)
  - Resistant variants may be archived (cccDNA)\(^4\)
  - Resistant viruses may escape vaccination\(^5\)
  - This may lead to progression of liver disease\(^6\)
    - Hepatic decompensation
    - Hepatocellular carcinoma

Limits Future Treatment Options

Antiviral Effect on Disease Progression* is Reduced when Resistance Develops

Disease progression, defined by the first occurrence of any of the following: an increase of at least 2 points in the Child–Pugh score, spontaneous bacterial peritonitis with proven sepsis, renal insufficiency, bleeding gastric or esophageal varices, the development of hepatocellular carcinoma, or death related to liver disease.

Management Flow Chart for First Virologic Breakthrough/Partial Virologic Response

Repeat HBV DNA testing in a timely manner to confirm virologic breakthrough or partial virologic response

If confirmed THEN perform HBV POL SEQUENCING

Typical results for HBV POL:
- "WILD TYPE" SEQUENCE if no known resistance mutations found
- CHECK PATIENT COMPLIANCE
- rtM204V/I±rtL180M detected
- rtA181T/V
- rtN236T±rtA181T/V detected
- rtT184S/A/I/L+rtS202G/C (and/or rtM250I/V) WITH rtL180M+rtM204V
- Complex pattern(s) detected

Adapt treatment based on cross-resistance profile

Zoulim F & Locarnini S. 2009. Gastroenterol;137:1593
Cross-Resistance Profile

<table>
<thead>
<tr>
<th>Resistance substitution*</th>
<th>LVD/LdT-resistant (L180M +/- M204V/I)</th>
<th>ADV-resistant (N236T)</th>
<th>ADV-resistant (A181T/V)</th>
<th>ADV-resistant (N236T+A181T)</th>
<th>ETV-resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutation confers some degree of reduced sensitivity to listed drugs</strong>1,2†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVD/LdT-resistant (L180M +/- M204V/I)</td>
<td>Entecavir</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
</tr>
<tr>
<td>ADV-resistant (N236T)</td>
<td>Entecavir</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
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<td>Lamivudine/Telbivudine</td>
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<tr>
<td>ADV-resistant (A181T/V)</td>
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<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
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<tr>
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<td>Entecavir</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
</tr>
<tr>
<td>ETV-resistant</td>
<td>Entecavir</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
<td>Tenofovir</td>
<td>Lamivudine/Telbivudine</td>
</tr>
</tbody>
</table>

**Drugs remaining fully active**1,3

<table>
<thead>
<tr>
<th>Entecavir</th>
<th>Tenofovir</th>
<th>Lamivudine</th>
<th>Telbivudine</th>
<th>Entecavir</th>
<th>Entecavir</th>
<th>Adefovir</th>
<th>Tenofovir</th>
</tr>
</thead>
</table>

* First virological breakthrough should be managed with an add-on strategy (combination), not switch (sequential monotherapy).
† Impact on sensitivity variable; results according to laboratory analyses, not patient studies.

Resistance profile of tenofovir not determined due to treatment intensification after Week 72.4,5

Viral Mutational Pathways: rtM204V/I

LAMIVUDINE
• not effective

TELBIVUDINE
• not effective

ENTECAVIR
• higher dose needed
• Reduced sensitivity

ADEFOVIR
• effective

TENOFOVIR
• effective
Viral Mutational Pathways: rtA181T/V

- TENOFOVIR
  - Reduced sensitivity

- ENTECAVIR
  - Effective

- ADEFOVIR
  - Not effective

- LAMIVUDINE
  - Not effective

- TELBIVUDINE
  - Not effective
Viral Mutational Pathways:
rtL180M+rtM204V+T184* or S202* or M250*

**ENTECAVIR**
• not effective

**LAMIVUDINE**
• not effective

**TELBIVUDINE**
• not effective

**ADEFOVIR**
• effective

**TENOFOVIR**
• effective

* other aa changes
Viral Mutational Pathways: rtN236T

- **ADEFOVIR**
  - not effective

- **TENOFOVIR**
  - Reduced sensitivity

- **LAMIVUDINE**
  - effective

- **TELBIVUDINE**
  - effective

- **ENTECAVIR**
  - effective

- **VIDRL**
  - RTN236T

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Viral Mutational Pathways: rtN236T+rtA181T/V

ADEFOVIR
- not effective

TENOFOVIR
- not effective

LAMIVUDINE
- not effective

TELBIVUDINE
- not effective

ENTECAVIR
- effective

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Transmission of LMV-Resistant HBV

Features

- rtL180M plus rtM204V
- lower viral replication level during acute phase
- incubation period of 2-3 months
- typical acute hepatitis
- not previously vaccinated against HBV

- acute hepatitis B caused by LMV-resistant HBV
  - 2/45 cases in Japan

Thibault et al. 2002 AIDS 16:131
The HBV surface gene overlaps completely with the polymerase gene; hence NA-selected changes in the polymerase gene can affect the overlapping surface gene.

### POL and ENV Link #1

#### ENV
- **“a” determinant**
  - PreS1
  - PreS2
  - S

#### Terminal Protein
- Spacer
- G F A B C D E RNASE H

#### POL
- RT Domains

<table>
<thead>
<tr>
<th>Envelope Mutants</th>
<th>Polymerase Mutant*</th>
<th>Ag-Ab Binding [IC50 (μg/ml)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>1.09</td>
</tr>
<tr>
<td><strong>HBIG-Escape</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sG145R</td>
<td>rtW153Q (W499Q)</td>
<td>&gt;55.0</td>
</tr>
<tr>
<td>sD144E/G145R</td>
<td>rtG153E (G499E)</td>
<td>&gt;55.0</td>
</tr>
<tr>
<td><strong>Drug Resistant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sF158Y</td>
<td>rtF166L</td>
<td>1.86</td>
</tr>
<tr>
<td>sE164D</td>
<td>rtV173L</td>
<td>14.86</td>
</tr>
<tr>
<td>sW196S</td>
<td>rtM204I</td>
<td>8.29</td>
</tr>
<tr>
<td>sI195M</td>
<td>rtM204V</td>
<td>5.26</td>
</tr>
<tr>
<td>sM198I</td>
<td>rtV207I</td>
<td>12.5</td>
</tr>
<tr>
<td>sE164D/I195M</td>
<td>rtV173/rtL180M/rtM204V</td>
<td><strong>54.53</strong></td>
</tr>
</tbody>
</table>

*rtL180M (L526M) in Polymerase causes NO change in envelope.

Efficacy of Hepatitis B Vaccine Against Antiviral Drug-Resistant Hepatitis B Virus Mutants in the Chimpanzee Model

Saleem Kamili,1 Vitini Sozzi,2 Geoff Thompson,2 Katie Campbell,3 Christopher M. Walker,3 Stephen Locarnini,2 and Krzysztof Krawczynski1

Individual with chronic HBV treated with NA

Selects for ADAPVEMs

Can infect naïve (anti-HBs-) individuals with ADAPVEMs

Can infect HepB-immunized (anti-HBs+) individuals with ADAPVEMs

ADAPVEM: antiviral drug associated potential vaccine escape mutant

Drug-resistant viruses may evade vaccine protection
Prevention of drug resistance is critical

Strategies to Prevent the Development of Antiviral Resistance

Prevention
- Judicious timing of treatment
- Education regarding adherence to therapy

First line therapy
- High potency drug with high genetic barrier to resistance E.g. Entecavir, Tenofovir
- Consider PEG IFN as an alternative first line therapy (eg. High ALT, Low HBV DNA)

Monitoring
- Regular 3-6 monthly monitoring of viral load with sensitive HBV DNA assay
- Genotypic resistance testing in patients with virological breakthrough

Salvage therapy
- Early initiation of “add on” salvage therapy
- Avoid “switch” sequential monotherapy
- Avoid combination therapy using drugs with similar cross resistance profiles
Summary

Current emerging patterns of antiviral drug resistance to HBV Pol are complex; But four major pathways can be defined

(rtM204V/I;rtN236T;rtA181T/V;ETV [naïve])

Primary resistance mutations across NA groups: A181T/V

Broad clusters of compensatory mutations during Lamivudine therapy (T184G/S202I/M250V Vs rtI169T+rtV173L Vs rtT184S) compromising future salvage therapy options with the newer agents (Entecavir)

Requirement for HBV Pol sequencing to determine profile of antiviral drug resistance

Emergence of MultiDrug Resistance (MDR) clear cause for concern

Public health issues around Pol-Env Overlap and vaccine escape

Need for newer antiviral agents targeted to other sites in the viral life-cycle
Choice of the first line agent is very important in the treatment of CHB!
Questions?