Chronic Hepatitis B
- Antiviral Resistance in Korea -

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HBV Genome

- partially double-stranded DNA genome
- about 3200 nucleotides
- HBV polymerase lacks proof-reading function
- Estimated mutation frequency: $1.4-3.2 \times 10^{-5}$ (approximately 10-fold higher than other DNA viruses)
- HBV production rate: $10^{11}$/day
- dynamic quasispecies
Evolution of HBV Resistance Mutations to Antiviral Drugs

• **Primary resistance mutations**
  mutations directly responsible for the associated drug-resistance

• **Compensatory (Secondary) mutations**
  - mutations that can restore replication fitness of primary resistant mutants
  - ‘fix’ the primary drug-resistant mutations as a genetic archive with quasispecies memory

• **Cross Resistance**
  mutants selected by one agent may also confer resistance to other antiviral agents
Lamivudine-Resistance Mutations at HBV polymerase/RT domain

- primary resistance mutation
- compensatory mutations
- rtM204 mutation confers cross resistance to entecavir, clevudine, telbivudine, emtricitabine
Mechanisms of Resistance

Sensitive (wild type) → Sensitive (rtM204V) → Resistant (rtM204V+rtL180M) → Resistant (rtM204V+rtL180M)

Lamivudine → Discontinuation → Lamivudine

Clevudine
Entecavir
Telbivudine
Emtricitabine
Clinical Definitions
- HBV Resistance to Antiviral Drugs -

• **Genotypic Resistance**
  Detection of mutations in the HBV genome, known to confer resistance

• **Virologic Breakthrough**
  Rebound in serum HBV DNA levels, more than 1 log10 cpm (10 fold)

• **Biochemical (Clinical) Breakthrough**
  Increase of ALT levels (or worsening histology) with virologic breakthrough
Development of Antiviral Resistance

- HBV DNA (Log)
- ALT (IU/L)

Antiviral Drug

Virologic Breakthrough

Development of Genotypic Resistance

Biochemical Breakthrough

1 log

nadir

80

40 (ULN)
Higher Retention of Mutant Virus Go with More Subsequent Occurrence of Viral Breakthrough

Lee CH et. al. Gastroenterology. 2006;130(4)1144
Genotypic Assays for antiviral drug resistance of HBV

- Sequencing (direct or after cloning)
- Hybridization Assay (Line probe assay, LiPA)
- Restriction Fragment Length Polymorphism (RFLP)
- Restriction Fragment Mass Polymorphism (RFMP)
- Allele-specific PCR
- DNA Chip
Genotypic Assays for antiviral drug resistance of HBV

- **Sequencing (direct or after cloning)**
  - the best approach to identify new mutations
  - unable to detect mixed populations of two or more HBV genotypes
  - expensive and time-consuming

- **Hybridization Assay (Line probe assay, LiPA)**

- **Restriction Fragment Length Polymorphism (RFLP)**

- **Restriction Fragment Mass Polymorphism (RFMP)**

- **Allele-specific PCR**

- **DNA Chip**
Genotypic Assays for antiviral drug resistance of HBV

- Sequencing (direct or after cloning)
- Hybridization Assay (Line probe assay, LiPA)
  - more sensitive than direct sequencing
  - most commonly used method in Western countries
- Restriction Fragment Length Polymorphism (RFLP)
- Restriction Fragment Mass Polymorphism (RFMP)
- Allele-specific PCR
- DNA Chip
Genotypic Assays for antiviral drug resistance of HBV

- Sequencing (direct or after cloning)
- Hybridization Assay (Line probe assay, LiPA)
- Restriction Fragment Length Polymorphism (RFLP)
- Restriction Fragment Mass Polymorphism (RFMP)
- Allele-specific PCR
  - can detect minor HBV populations comprising up to 5% of the total viral population (about $10^3$ cpm)
  - robust high throughput manner
  - most commonly used methods in Korea
- DNA Chip
Advances in Management of HBV Infection

1992 - Interferon
1998 - Lamivudine
2003 - Adefovir
2005 - Peg-IFN α-2a
2006 - Entecavir
- - Clevudine
- - Tenofovir
- - Telbivudine
# HBV Therapies Approved in Korea

<table>
<thead>
<tr>
<th>For Naïve CHB</th>
<th>For LAM-Refractory CHB</th>
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<tbody>
<tr>
<td>• Interferon</td>
<td>• Interferon</td>
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<tr>
<td>• Peginterferon $\alpha$-2a</td>
<td>• Peginterferon $\alpha$-2a</td>
</tr>
<tr>
<td>• Lamivudine*</td>
<td>• Adefovir dipivoxil*</td>
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<td>• Entecavir*</td>
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<td>• Clevudine*</td>
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*Approved only as monotherapy and after ALT flare or biochemical breakthrough ($\text{ALT}>80$ IU/L)
New Oral Agents

**Future agents**
- Telbivudine (LdT)
- Tenofovir
- Emtricitabine

**New experimental agents**
- LB80380
- Pradefovir
- Alamifovir
- Valtorcitabine
- Torcitabine
Ideal anti-viral agent

High potency in reducing HBV DNA level
High rate of HBeAg seroconversion
Few adverse events or toxicity
Cost-effective
Long-term, low rate of drug resistance
Lamivudine-Resistance
HBV polymerase/RT domain

Terminal Protein  Spacer  POL/RT  RNaseH

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

I(G)  II(F)  A  B  C  D  E

LMV Resistance  rtL80V/I  rtV173L  rtM204V/I/S

rtL180M

L-dT Resistance  rtM204I

ADV Resistance  rtA181T/V  rtN236T

ETV Resistance  rtT184G  rtS202I  rtM250V

TFV Resistance  rtA194T/rtV214A/rtQ215S
Long-term LAM therapy can lead to the emergence of resistant viruses

2. Yuen et al AASLD 2005
High lamivudine resistance with poor early HBV suppression

Serum DNA at month 6 vs. LAM resistance by month 6

Serum HBV DNA Level at 6 months (copies/mL)

- < 200 (n = 12) 8%
- < 3 log\textsubscript{10} (n = 23) 13%
- < 4 log\textsubscript{10} (n = 41) 32%
- > 4 log\textsubscript{10} (n = 118) 64%

Disease Progression by YMDD Status

Hepatitis flares and Serious Adverse Events with Lamivudine resistance mutations

Clinical consequences of Lamivudine resistance

It is now clear that drug-resistant HBV is not a benign or attenuated virus.

Disease progression, loss of initial benefit, fulminant hepatic failure and death can occur.
Management of Lamivudine-Resistant HBV
Continuation or Discontinuation of Lamivudine

Events within 12 mo. after emergence of YMDD mutations

Continuation or Discontinuation of Lamivudine

• Hepatitis flares and decompensation frequently occur after emergence of YMDD mutations regardless of continuation or discontinuation of lamivudine therapy.

• Compensatory mutations will be selected during continued lamivudine treatment leading to subsequent viral rebound and possibly hepatitis flares.

• Patients with confirmed lamivudine-resistance should receive effective rescue therapy.
Peg-Interferon alfa-2a in Asian patients with NA resistance

12 wks PEGASYS + LAM → 12 wks of PEGASYS monotherapy

HBeAg seroconversion (with HBV DNA <10^5 copies/mL)

- 43.7% at End of treatment (7/16)
- 31.2% at 24 weeks post-treatment (5/16)

In 13% of patients at week 72

Shi et al. APASL 2007
Adefovir alone or in combination with lamivudine in lamivudine-resistant HBV (small, short-term study)

* p<0.001 compared to lamivudine

37% frequency of grade 3 ALT flare in switchover to ADV alone

Peters MG et al, Gastroenterology 2004; 126: 91-101
Increased Risk of Adefovir Resistance in Patients with Lamivudine-Resistant CHB after Adefovir Monotherapy

Incidence of ADV-Resistance at 48 wks of ADV monotherapy

<table>
<thead>
<tr>
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<th>Genotypic mutation</th>
<th>Virologic breakthrough</th>
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<tbody>
<tr>
<td>naive (n=38)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>LMV-resistant (n=57)</td>
<td>18%</td>
<td>7%</td>
</tr>
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</table>

* P < 0.01

Genotypic assay; RFMP

Cumulative Incidence of Adefovir-Resistance in Lamivudine-Resistant CHB patients treated with Adefovir Monotherapy

Genotypic Resistance Virologic Breakthrough

25.4%

Genotypic assay; RFMP

Adding-on vs. Switching-to Adefovir in Lamivudine-Resistant HBeAg(-) CHB

Incidence of Genotypic Resistance

When should we add Adefovir on Lamivudine?

Achievement of PCR Undetectability on Entecavir

- HBeAg - Naïve
  - N = 319
  - 94%

- HBeAg +
  - N = 345
  - 40%

- LVD Refractory
  - N = 178
  - 40%
Genotypic resistance in naive patients
Genotypic resistance in LAM-R patients
Genotypic resistance plus viral rebound in LAM-R patients

Incidence of Entecavir Resistance

ETV Resistance Profile

ETV-R was only observed in patients with preexisting LAM-R virus (M204 V/I and/or L180M).

Resistance to ETV appears to occur through a two-hit mechanism with initial selection of M204V/I mutation followed by amino acid substitutions at rtI169, rtT184, rtS202, or rtM250.

LAM should be discontinued to decrease the risk of entecavir resistance.


**Tenofovir for Lamivudine-Resistant CHB**

- Nucleotide analogue, structurally similar to adefovir, equipotent in wild-type and LAM-R HBV (*In vitro*).
- Because tenofovir appears to be less nephrotoxic, the approved dose is much higher than that of adefovir, 300 mg versus 10 mg daily.
- Thus, tenofovir has more potent antiviral activity than adefovir.
- No randomized controlled trials in HBV.
**Tenofovir vs Adefovir in Patients with Lamivudine Resistance (not randomized)**

*including HIV/HBV coinfection (n=21), and HBV/kidney Tpl (n=5).*


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* HBV DNA (log$_{10}$ copies/mL)

- **Tenofovir**
  - (300 mg/day, $n=35$)

- **Adefovir**
  - (10 mg/day, $n=18$)

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P < 0.001

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Weeks
Recommendations for the Tx of LAM-R CHB

• It is better to start rescue therapy as soon as genotypic resistance is detected before the development of virologic breakthrough.

• Therefore, careful monitoring of genotypic resistance is needed during antiviral therapy for early detection and early rescue of drug resistance.

• Adefovir add-on is preferred over switch (increased rate of adefovir resistance with switch) and preferred over entecavir (high rate of novel mutations in patients with lamivudine resistance).

• If entecavir is used, lamivudine should be stopped as continued presence of lamivudine-resistant mutations will increase the risk of entecavir resistance.

• Tenofovir, 300 mg daily, appears to be superior to ADV, 10 mg daily, in suppressing LAM-resistant strain HBV.
Adefovir-Resistance
HBV polymerase/RT domain

Terminal Protein | Spacer | POL/RT | RNaseH
---|---|---|---
1 | 183 | 349 (rt1) | 692 (rt 344) | 845 a.a.

LMV Resistance | rtL80V/I | rtV173L | rtM204V/I/S
L-dT Resistance | | rtL180M | rtM204I

ADV Resistance | rtA181T/V | rtN236T
ETV Resistance | rtS184G | rtS202I | rtM250V
TFV Resistance | rtA194T/rtV214A/rtQ215S

Terminal Protein: I(G), II(F)
Spacer: A
POL/RT: B, C, D, E
RNaseH: F

HBV polymerase/RT domain:
- 845 amino acids (a.a.)
- 183 amino acids (rt1)
- 692 amino acids (rt 344)

Key regions:
- I(G)
- II(F)
- A: F_V__LLAQ_YMDD
- B: rtL80V/I
- C: rtV173L, rtL180M
- D: rtM204V/I/S
- E: rtM204I

Resistance mutations:
- LMV: rtL80V/I
- L-dT: rtL180M
- ADV: rtA181T/V, rtN236T
- ETV: rtS184G, rtS202I
- TFV: rtA194T/rtV214A/rtQ215S
Incidence of Adefovir Resistance with Adefovir Monotherapy in HBeAg-Negative NA naïve CHB Patients

*Cumulative probabilities calculated by life-table analysis.
†Presence of genotypic resistance plus HBV DNA rebound; confirmed $\geq 1 \log_{10}$ copies/mL increase in HBV DNA from nadir and/or having never achieved HBV DNA suppression $\leq 4 \log_{10}$ copies/mL.

Higher HBV DNA at Year 1 Predictive of Year 3 ADV Resistance

Risk factors
for the development of adefovir resistance

- Old age
- High baseline HBV DNA load
- Suboptimal early viral suppression
- Short-duration of LAM overlap in LAM-R HBV
  (adding-on is better than switching to ADV)
- Presence of LAM-R HBV mutants
  The main LMV resistance mutations rtM204V/I do not confer cross-resistance to adefovir, but the minor LMV resistance mutations rtA181T as well as the rtQ215S are partially cross-resistant to lamivudine in vitro.
Management of Adefovir-Resistant HBV
**In Vitro Cross-Resistance Analysis**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Efficacy against various HBV strains</th>
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<tbody>
<tr>
<td></td>
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<td>Wild type</td>
<td>LAM-R (L180M+M204V)</td>
<td>ADF-R (N236T)</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Emtricitabine</td>
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<td>-</td>
<td>+</td>
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<tr>
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<td>+</td>
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<tr>
<td>Tenofovir</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
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Practical Options for the Tx of ADR-R CHB

• In patients with no prior exposure to other NA
  – add lamivudine
  – add (> or switch to) entecavir

• In patients with prior LAM-R, switched to ADV
  – add lamivudine
  – add (> or switch to) entecavir
  – switch to tenofovir (when approved)
    in combination with lamivudine or entecavir
Case: Adding on LMV for ADV-R

Case: Switching to ETV for ADV-R

Case: Switching to Tenofovir for ADV-R

Ratziu V, et al., Comp Hepatol 2006;5:1.
Prevention of Antiviral Drug Resistance in Patients with CHB

• Current therapy of CHB has limited long-term efficacy.

• Once antiviral-resistant HBV mutants have been selected, they are archived (retained in the virus population) persistently even if treatment is stopped.

• Thus, the best way to reduce the emergence of drug resistance is to select the right patients, right time to start treatment and the right antiviral agent(s).
Prevention of Antiviral Drug Resistance in Patients with CHB

• Select the right patients

Patients with minimal disease and those who are unlikely to achieve sustained response should not be treated with NA

• Select right time to start treatment

• Select the right antiviral agent(s)
Prevention of Antiviral Drug Resistance in Patients with CHB

- Select the right patients
- Select right time to start treatment
  Start therapy at right time with clear indication to maximize antiviral activity
- Select the right antiviral agent(s)
Prevention of Antiviral Drug Resistance in Patients with CHB

• Select the right patients
• Select right time to start treatment
• Select the right antiviral agent(s)

The most potent NA with the lowest rate of genotypic resistance should be administered.
See you in Seoul, Korea
ありがとうございました
Thank you for your attention!!