Is Antiviral Drug-resistant HBV a Problem of the Past?

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Approved HBV Treatments

- Interferon alpha 2b (Intron)
- Pegylated interferon alpha 2a (Pegasys)
- Lamivudine (Epivir)
- Adefovir (Hepsera)
- Entecavir (Baraclude)
- Telbivudine (Tyzeka)
- Tenofovir (Viread)

Treatments approved for HIV with activity against HBV
- Emtricitabine (Emtriva)
- Tenofovir + Emtricitabine (Truvada)
Antiviral Drug-resistant HBV is Inevitable

- High rate of HBV replication
- High error rate in HBV replication – lack of proof reading during reverse transcription of pregenomic RNA to HBV DNA
- Reservoir of covalently closed circular (ccc) DNA in liver refractory to antiviral therapy \(\rightarrow\) prevents viral clearance
- Limitations of approved treatments
  - Incomplete virus suppression with some drugs / in some patients
  - Low genetic barrier to resistance with some drugs
- Realities of life
  - Medication non-compliance
  - Affordability of medications and monitoring
What Causes Antiviral Drug-resistant HBV Variants to become Dominant?

• Antiviral drug-resistant HBV mutations arise spontaneously and are selected during antiviral therapy - survival of the fittest
Factors associated with Antiviral Drug-resistant HBV

VIRUS
- Daily production
- Preexisting mutations

DRUG
- Potency
- Genetic barrier to resistance

HOST
- Prior treatment
- Compliance
- Pharmacogenetics
- Body size
Clinical Potency of Approved Nucleos(t)ide Analogs in HBeAg+ CHB at 1 Year

## Genetic Barriers to Antiviral Resistance

- No. of amino acid changes required to confer resistance
- Decrease in susceptibility (increase in IC$_{50}$) caused by the mutations

<table>
<thead>
<tr>
<th>Nucleosid(t)e analogue</th>
<th>Mutations</th>
<th>Fold-decrease in susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAM/TBV</td>
<td>M 204 V/I</td>
<td>&gt; 1,000</td>
</tr>
<tr>
<td>ADVTDF</td>
<td>A 181 V or N 236 T</td>
<td>3 – 15</td>
</tr>
<tr>
<td>ETV</td>
<td>169 or 202</td>
<td>~ 1</td>
</tr>
<tr>
<td></td>
<td>184 or 250</td>
<td>2 – 10</td>
</tr>
<tr>
<td></td>
<td>M 204 V/I + 1 ETV-R</td>
<td>10 – 250</td>
</tr>
<tr>
<td></td>
<td>M 204 V/I + 2 ETV-R</td>
<td>&gt; 500</td>
</tr>
</tbody>
</table>
Suboptimal Viral Response is Associated with Increased Risk of Drug Resistance

Data from Telbivudine Phase III Trial

Viral resistance defined as confirmed genotypic resistance in patients with HBV DNA >5 log10 copies/mL

Zezem S, J Hepatol 2009
How Common is Antiviral Drug-resistant HBV?

Depends on:

Patient population analyzed
Definition of antiviral resistance
Sensitivity of assay used to detect resistant mutations
Monitoring for Antiviral Resistance

Surveillance for genotypic resistance

Monitoring for virological breakthrough

Serum HBV DNA (log_{10} IU/mL)

TREATMENT

Years

0 1 2

LLOD

TREATMENT

Years

0 1 2

LLOD
Virological Breakthrough Precedes Biochemical Breakthrough

Hepatitis flare

ALT (U/L)

Biochemical breakthrough (clinical resistance)

Virologic breakthrough (viral resistance)

Serum HBV DNA ($\log_{10}$ IU/mL)

Patients

Years

LLOD

ULN

Lok AS, McMahon BJ. Hepatology. 2007;45:507
Cumulative Probability of Antiviral Resistance to Adefovir Therapy of HBeAg-negative Patients

Detection of resistant-mutations by direct sequencing

Year 1 | Year 2 | Year 3 | Year 4 | Year 5
--- | --- | --- | --- | ---
% of Patients

Genotypic resistance
Viral resistance
Clinical resistance

Borroto-Esoda. et al.
Primary Resistance Substitutions

L-Nucleoside
LAM rtA181T/V
TBV rtA181T/V
Acyclic Phosphonate
ADV/TFV rtA181T/V
D-Cyclopentane
ETV rtT184

Terminal Protein
1

Spacer
183 349 (rt1)

POL/RT

RNaseH
692 (rt 344) 845 a.a.

Terminal Protein
 Spacer
 POL/RT
 RNaseH

I(G)
 rtM204V/I
 II(F)
 rtM204I
 A
 rtA181T/V
 rtA181T/V
 B
 rtM204V/I
 rtM204I
 C
 rtA194T?
 rtN236T
 D
 rtT184
 rtS202
 E
 rtM204V/I
 rtM250

Primary Resistance Substitutions
# Assays for Detecting Antiviral Drug-resistant Mutations

<table>
<thead>
<tr>
<th>Assay</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sequencing</td>
<td>Detect all mutations</td>
<td>Least sensitive at detecting minor populations (~20%)</td>
</tr>
<tr>
<td></td>
<td>Most useful with new therapies</td>
<td></td>
</tr>
<tr>
<td>Sequencing of multiple clones</td>
<td>Detect all mutations</td>
<td>Labor intensive</td>
</tr>
<tr>
<td></td>
<td>Sensitivity depends on no. of clones sequenced</td>
<td></td>
</tr>
<tr>
<td>RFLP, Line probe</td>
<td>Sensitive (~5%)</td>
<td>Detect only known mutations</td>
</tr>
<tr>
<td></td>
<td>Early detection of genotypic resistance</td>
<td></td>
</tr>
<tr>
<td>Single genome sequencing</td>
<td>Ultra-sensitive (~0.1%)</td>
<td>Cannot differentiate spontaneous mutations from mutations selected during treatment</td>
</tr>
<tr>
<td>MALDI-TOF mass spectrometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultra-deep pyrosequencing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
<th>Year 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAM¹</td>
<td>23%</td>
<td>46%</td>
<td>55%</td>
<td>71%</td>
<td>80%</td>
<td>–</td>
</tr>
<tr>
<td>ADV‡¹</td>
<td>0%</td>
<td>3%</td>
<td>11%</td>
<td>18%</td>
<td>29%</td>
<td>–</td>
</tr>
<tr>
<td>TBV‡²,³</td>
<td>5%</td>
<td>25%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TDF§⁴</td>
<td>0%</td>
<td>0%§</td>
<td>0%§</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ETV*⁵</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.2%⁶</td>
</tr>
</tbody>
</table>

§ 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.
Is Antiviral Drug-resistant HBV a Problem of the Past in Nucleos(t)ide-naïve Patients?

Very low or no resistance reported, BUT...

• **Entecavir** ~1.2% up to 6 yr
  – A small number of non-responders excluded
  – 1.0 mg dose used from yr 3 onward
  – Data based on 108 patients only

• **Tenofovir** ~0 after 3 yr
  – Most patients with detectable HBV DNA at wk 72 received additional emtricitabine
  – Data on tenofovir monotherapy beyond 72 wk unknown
Is Antiviral Drug-resistant HBV a Problem of the Past in Nucleos(t)ide-naïve Patients?

- Data based on small number of patients in phase III trials, adherence in clinical practice likely lower
- Tenofovir not available in most Asian countries
- Entecavir >10 times the cost of lamivudine, adefovir or telbivudine and not covered by health ministries in many countries
- Lamivudine and adefovir remain the 1st line drug in most countries endemic for HBV
Adherence to HBV Nucleos(t)ide Analogs in Clinical Practice

• Pharmacy claims data base in the U.S.
  – 3 cohorts of CHB patients receiving LAM, ADV, ETV or TDF (2009 cohort only) in Jan 2007, 2008 and 2009 followed for 1 year

• New patients = patients started on treatment in Jan of that year

• Existing patients = patients who had been on treatment in the prior year

• Adherence = % of days during that year in which patient had medications
  – E.g. existing patient who had 11 refills of 30 day supply in the calendar year would have adherence of \((11 \times 30 / 365) \times 100 = 90\%\)
Adherence to HBV Nucleos(t)ide Analogs:
Analysis of pharmacy claims database in 3 cohorts of patients treated in the US in 2007, 2008 and 2009

New Patients (n=458)

- More than 95%: 38.7%
- 91-95%: 18.1%
- 81-90%: 10.5%
- 71-80%: 10.5%
- 61-70%: 8.5%
- 51-60%: 9%
- Less than 51%: 5%

Existing Patients (n=10,295)

- More than 95%: 48.1%
- 91-95%: 18.6%
- 81-90%: 10.5%
- 71-80%: 8.1%
- 61-70%: 3.9%
- 51-60%: 6.1%
- Less than 51%: 3.3%

W Chotiyputta, et al., J Hepatol (in press)
Not All Virologic Breakthroughs Are Caused by Antiviral Resistance Mutations

- 148 CHB patients receiving NUC >1 year
- 39 (26%) had >1 virologic breakthrough (VBT)
  - 15 (38%) VBT not confirmed on retesting 1-3 mo later
  - 13 (33%) no evidence of genotypic resistance (GR) by direct sequencing and line probe assay
- 10 patients with VBT but no confirmed VBT / GR continued on same medications
  - HBV DNA decreased in all 10 and became undetectable in 9
## Cross Resistance Analysis

*(in vitro studies)*

<table>
<thead>
<tr>
<th>Resistantce mutations</th>
<th>M204V/I</th>
<th>N236T</th>
<th>A181V/T</th>
<th>T184, S202, M250</th>
</tr>
</thead>
</table>
| Drugs with marked decrease in activity | ▪ Lamivudine  
▪ Emtricitabine  
▪ Telbivudine | ▪ Adefovir | ▪ Adefovir  
▪ Lamivudine | ▪ Entecavir |
| Drugs with some decrease in activity | ▪ Entecavir | ▪ Tenofovir | ▪ Entecavir  
▪ Telbivudine  
▪ Emtricitabine  
▪ Tenofovir | |
| Drugs remaining fully active | ▪ Adefovir  
▪ Tenofovir | ▪ Lamivudine  
▪ Emtricitabine  
▪ Telbivudine  
▪ Entecavir | ▪ Adefovir  
▪ Tenofovir | |
High Rate of Adefovir Resistance among Patients with Lamivudine Resistance Receiving Adefovir Monotherapy

Fung et al, J Hepatol 2006, Yeon et al, Gut 2006; Lee et al, Hepatology 2006; Chen et al, Antiviral Therapy 2006
High Rate of Entecavir Resistance in Lamivudine-Refractory HBeAg+ Patients

- 72/187 (39%) achieved HBV DNA < 300 cp/mL;
- 3/72 (4%) had subsequent genotypic ETV resistance

Tenney D, Hepatol 2009; 49: 1503
Antiviral-Drug Resistant HBV Remains a Problem in Nucleos(t)ide-experienced Patients

- LAM resistance → ADV monotherapy ~20% ADV resistance after 2 yr
- LAM resistance → ETV 1.0 mg dose ~50% ETV resistance after 5 yr
- ADV resistance → TDF partial virus suppression, persistence of ADV-resistance mutations
- LAM resistance → Switch to ADV monotherapy → ADV resistance → Add LAM → Multi-drug resistance HBV
How to Prevent HBV Antiviral Drug Resistance?

- Judicious use of antiviral treatment
- Use potent drugs that have high genetic barrier to resistance
- Initiate treatment with combination therapy?
- Close monitoring of virologic response and breakthroughs
- Modify treatment in patients with suboptimal viral suppression
- Counseling on medication adherence
### Which Should be the Initial Oral Drug?

<table>
<thead>
<tr>
<th></th>
<th>LAM</th>
<th>ADV</th>
<th>ETV</th>
<th>TBV</th>
<th>TDF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antiviral activity</strong></td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Risk of drug resistance</strong></td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

LAM = lamivudine, ADV = adefovir, ETV = entecavir, TBV= telbivudine, TDF = tenofovir
Can *De Novo* Combination Therapy Prevent Antiviral Resistance?

- **Combination therapy involving antiviral drugs with low genetic barrier to resistance**
  - Reduces but not completely prevent resistance
  - PegIFN + LAM: 1 yr resistance 1-4%
  - LAM + ADV: 2 yr resistance 15%
  - LAM + TBV: 1 yr resistance 10%

- **Combination therapy involving antiviral drugs with high genetic barrier to resistance**
  - Will complete prevention of antiviral resistance be possible?
  - How to prove that combination therapy is superior?
    - To demonstrate decrease in resistance from 1% to 0% will require >1,000 patients in each treatment arm
  - Will this strategy be cost-effective?
Management Roadmap According to Week 24 Virologic Response

Week 24
Assess early predictors of efficacy

Complete response
HBV DNA negative by PCR
Continue therapy

Partial response
HBV DNA 60 to < 2000 IU/mL

Inadequate response
HBV DNA ≥ 2000 IU/mL
Add a more potent drug

Problems with the Proposed Roadmap

• Based on data of drugs with low genetic barrier to resistance

• For patients receiving lamivudine or telbivudine, response at week 24 associated with lower but not 0% drug resistance at week 48

• ~50% of HBeAg+ patients would be considered as having partial or inadequate response at week 24

• For patients receiving entecavir or tenofovir, continued treatment in patients with incomplete response at week 24 associated with very low rate of drug resistance after 3-5 years treatment
Adefovir is More Effective when Added at the First Sign of Lamivudine Breakthrough

Patients with undetectable HBV DNA (%)

<table>
<thead>
<tr>
<th>Months</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>28</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>22</td>
<td>14</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

Patients still at risk

HBV DNA when ADV added to LAM
- 3-6 log HBV-DNA
- 6-8 log HBV-DNA
- >8 log HBV DNA

p<0.0001

Lampertico et al., Hepatology 2005;42:1414
## Rescue Therapy Options for Antiviral Drug-resistance HBV

<table>
<thead>
<tr>
<th>Type of resistance</th>
<th>Preferred rescue therapy</th>
<th>Other options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamivudine or Telbivudine</td>
<td>• Tenofovir – add / switch</td>
<td>• Add adeovir</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Switch to tenofovir + emtricitabine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Switch to entecavir (not preferred)</td>
</tr>
<tr>
<td>Adefovir</td>
<td>• Entecavir – add / switch</td>
<td>• Switch to tenofovir + emtricitabine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Add lamivudine or telbivudine (not preferred if prior lamivudine resistance)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Switch to tenofovir (not preferred, partial cross-resistance)</td>
</tr>
<tr>
<td>Entecavir</td>
<td>• Tenofovir – add / switch</td>
<td>• Add adeovir</td>
</tr>
</tbody>
</table>
Is Antiviral-resistant HBV a Problem of the Past?

- Diminishing problem but not gone
- Nucleos(t)ide-naïve patients – rare if drugs of high genetic barrier used and patient adherent to medications
- Nucleos(t)ide-experienced patients – still a problem particularly if suboptimal rescue therapy used, risk of multi-drug resistance
- More attention to medication adherence is needed
LAM+ETV Resistance

ETV 1.0 mg

HBV DNA (log10 copies/ml)

ALT (IU/L)

Time (months)

0 6 12 16 22 28 34 38 41

HBVDNA
ALT

ETV+LAM-R

L180M+M204V
L180M+M204V+S202G
L180M+M204V+T184I+S202G
L180M+M204V+I169T+T184A
L180M+M204V+I169T+S202G
L180M+M204V+I169T+T184A+S202G
LAM+HBIG, LAM+ADV Resistance

**Graph:**
- **HBV DNA** (log10 copies/ml) over time (months)
- **ALT** (IU/L) over time (months)
- Time (months) 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60
- **HBIG**
- **LAM**
- **ADV 10 mg**
- **HBIG+LAM-R**
- **ADV-R**
- **ADV+LAM-R**
- **OLT**

**Molecular Variants:**
- sG145R+M204I
- sG145R+N236T
- N236T
- V173L+L180M+M204V
- V173L+L180M+M204V+P237H
- L180M+M204V
- V173L+L180M+M204V
- L180M+M204V+P237H
**Tenofovir alone does not adequately suppress adefovir-R HBV**

- **ADV**
- **Tenofovir (TDF)**
- **Truvada (TDF+FTC)**

![Graph showing HBV DNA and ALT levels over time](image)

- **M0**
- **M9**
- **M13**

**HBV DNA Log_{10} copies/mL**

- 0
- 50
- 100
- 150
- 200

**ALT IU/L**

- 0
- 50
- 100
- 150
- 200

**Time (months)**

- -21
- -12
- -3
- 0
- 3
- 6
- 9
- 12
- 15
- 18
- 21
- 24

- Red line: HBV DNA
- Blue line: ALT

**Mutations**

- A181V
- A181V + N236T
- A181V + I233V

*Tan J, J Hepatol 2008; 48: 391*
Monitoring viral response and resistance

- Monitoring of serum HBV DNA
  - Sensitive assay, preferably real-time PCR, lower limit of detection ~30 IU/mL
  - Same assay
  - Baseline, then every 3 months
    - To detect lack of initial response → modify treatment
    - To detect early breakthrough → rescue therapy more effective when serum HBV DNA is low