New (Virological) Tools for Testing and Monitoring Hepatitis B and C

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Outline of Presentation

• Hepatitis C
  – Diagnosis and cure in the era of DAAs
  – Possible Role for Resistance Testing

• Hepatitis B
  – Existing Assays: HBV DNA; qHBeAg and qHBsAg; anti-HBs
  – Assays in Development
    • cccDNA
    • integrated HBV DNA
    • neutralising anti-HBs
WHO Recommendation for Patients with CHC: Treatment Pathway

1. **Screening**
   - HCV antibody screening
     - Screen for other bloodborne viruses
   - RNA test positive
     - Harm reduction
       - Address alcohol use
       - Consider OST
       - Vaccinate for HBV
       - Provide sterile injecting equipment
       - Peer intervention
     - Stage disease
       - Clinical examination exclude decompensation for IFN-containing regimens
       - APRI, FIB4 or TE
   - RNA test negative
     - Harm reduction
       - Address alcohol use
       - Consider OST
       - Vaccinate for HBV
       - Provide sterile injecting equipment
       - Peer intervention
       - Consider retesting (RNA)

2. **Care**
   - If cirrhotic
     - Screen for varices
     - Screen for HCC
     - Consider transplantation
   - Assess for treatment
     - Consider co-morbidities, depression, pregnancy and potential drug–drug interactions
     - Genotype virus

3. **Treatment**
   - Select regimens
   - Monitor for efficacy and toxicity
Hepatitis C

• Cure in the Era of DAAs
  ➢ SVR-12

• Laboratory Tests in DAA Cure Era
  ➢ HCV nucleic acid amplification test (NAAT)
  ➢ In the context of polyvalent platforms [HCV, HBV, HIV]
  ➢ HCV core antigen (cAg) test
NAAT versus cAg in the Era of DAAs

- HCV cAg detectable in blood 1-2 days after HCV RNA becomes positive (window phase)
- 1pg/ml of cAg equivalent 7,900 IU/ml HCV RNA
  
- **Abbott Architect**: 1,000-3,000 IU/ml is equivalent to 3 fmol/L
- sensitivity at 1,000 IU/ml
- thus, 5% of individuals with viremia of 3,000 IU/ml, could be missed on cAg
  
  (Glynn, SA et al 2005. Transfusion;45:994)
- high correlation between HCV RNA and cAg testing at RNA levels >10^3 IU/ml for ALL genotypes
- decline in cAg correlates with decrease in HCV RNA levels
  
- a test for HCV core with a LOD of 1,000 IU/ml should be sufficient for monitoring
Cure in the Era of DAAs

• Latest DAA regimens achieving 90-95% SVR-12
• What about 5% non cure?

Q. Role for resistance associated variants (RAV) testing?
A. Not at Baseline: Probably Not Now!!
   Probably Not Ever
   – Excellent Rescue Regimens for Viral Breakthrough
WHO Recommendations on the Management of persons with CHB
What Would HBV Cure Look Like?

In the blood: HBV DNA/HBsAg negative
anti-HBs positive

In the liver: no HBV cccDNA
no HBV RC/DSL DNA
HBcAg staining negative
± HBsAg (occasional)

[reflecting integrated HBV DNA]

Functional Cure: HBsAg loss/Seroconversion

Absolute or Complete Cure: Maintenance of undetectable serum HBV DNA off-treatment
No cccDNA anywhere
Treatment Challenges: Barriers to Curing Chronic Hepatitis B

1. Reservoir of cccDNA
2. Dysfunctional T-cell Response
3. Insufficient or inadequate B-cell Response

Strategies to overcome these barriers
1. Deplete or Silence cccDNA
2. Improve potency of Pol Inhibitors
3. Broaden Viral Targets: combination DAA Therapy
4. Activate Antiviral Immunity
HBV Lifecycle Showing Novel Approaches for Viral Targets

Tools Needed in 2015 to Demonstrate:

I. Functional Cure
   - PCR for HBV DNA negative in serum
     - Qualitative (and quantitative) HBeAg assay
     - Qualitative (and quantitative) HBsAg assays
     - Neutralizing anti-HBs [epitope mapping]

II. Complete Cure
   - PCR for HBV DNA negative in serum
     - PCR for intrahepatic cccDNA
     - Surrogate for intrahepatic cccDNA [qHBsAg in HBeAg-neg CHB]
     - Surrogate for integrated HBV DNA [serum HBV RNA assay]

Thompson, A et al 2010. Hepatol; 51:1933-1944
**HBV Biology and ARC-520 MOA**

**Untreated**

- HBV Virion → Infection → Hepatocyte → Nucleus → HBV DNA → mRNA → Viral Proteins
- Replication of Virus → Production of Viral Proteins (HBsAg, HBeAg, Core, X)
- Immune Suppression: Liver cancer, Cirrhosis, Death
- Contagion, Reinfection

**ARC-520**

- HBV Virion → Infection → Hepatocyte → Nucleus → HBV DNA → mRNA → Reduced Viral Protein Production
- Reduced Viral Replication
- ARC-520

- Reduced Viral Antigens
- Reversal of Immune Suppression
- HBsAg seroconversion & functional cure
Deep Reduction in HBsAg with ARC-520: HBeAg Pos Chimps are Most Responsive

- Mean knockdown (nadir)
  - HBeAg pos
    99% (2.1 log\(_{10}\))
  - HBeAg neg
    81% (0.7 log\(_{10}\))

- HBsAg trends downward after multiple doses

Wooddell, C & Lanford R 2015. AASLD Hepatology in press
Novel finding: Predominant Liver HBV DNA Differs in HBeAg Neg and HBeAg Pos Chimps

Liver biopsy at initiation of ARC-520 treatment revealed:

- Most HBV DNA in liver of HBeAg pos is cccDNA
- 500-fold less cccDNA in HBeAg neg
  - Only 5% of total HBV DNA in liver in HBeAg neg was cccDNA and total HBV DNA levels were not affected by NUCs
- HBV DNA profile in HBeAg neg chimps is consistent with a high proportion of integrated HBV DNA

Wooddell, C & Lanford R 2015. AASLD Hepatology in press
Chimpanzee ARC-520 Study: Interim Conclusions

• Robust, sustained direct anti-viral effect on HBsAg production observed in all HBeAg pos and neg chimps
  – HBeAg pos chimps displayed highest levels of HBsAg knockdown - up to 2.7 log
  – In HBeAg neg chimps, HBsAg knockdown was also substantial - up to 0.9 log

• ARC-520 was well tolerated after multiple doses up to 4 mg/kg ARC-520 (highest dose tested)

• Evidence indicates integrated HBV DNA is a significant source of total HBsAg, especially in HBeAg neg chimps

Wooddell, C & Lanford R 2015. AASLD Hepatology in press
HBV Replication: Pre ARC-520 Era

1. **Uncoating**
2. **ER**
3. **Mature Nucleocapsid**
4. **Immature Nucleocapsid**
5. **Nuclear Transport**
6. **RC-DNA**
7. **Transcription**
8. **Viral RNA**
9. **Core Polymerase**
10. **Surface**
11. **Precore**
12. **GOLGI**
13. **HBeAg**
14. **Spherical & Filamentous HBsAg**
15. **Mature HBV virion**
16. **Intracellular Conversion Pathway**
17. **Reverse Transcription**
18. **RC-DNA**
19. **Mature Nucleocapsid**
20. **Translation**
New Targets in HBeAg-Neg HBV: ARC-521 + NUC
Summary

Key Virological Findings for ARC-520

• Direct antiviral effect on serum HBsAg, HBeAg, and HBcrAg levels which are substantial

• HBeAg-Pos CHB and HBeAg-Neg CHB have very different viral patho-physiologies

• Importance of integrated HBV DNA as a source of HBsAg

• This has important therapeutic and prognostic significance
Serum Hepatitis B Virus RNA Levels as an Early Predictor of Hepatitis B Envelope Antigen Seroconversion During Treatment With Polymerase Inhibitors

Florian van Bömmel,¹ Anne Bartens,¹,² Alena Mysickova,³ Jörg Hofmann,² Detlev H. Krüger,² Thomas Berg,¹ and Anke Edelmann²

HEPATOLOGY 2015;61:66-76

RACE-based RT-PCR technique used for quantitative analysis
Patient Baseline Characteristics and Mean Levels of HBV fIRNA, HBV trRNA, HBV DNA, and HBsAg During Antiviral Treatment

<table>
<thead>
<tr>
<th></th>
<th>All Patients (n = 62)</th>
<th>(A) HBeAg Positive, Seroconversion (n = 15)</th>
<th>(B) HBeAg Positive, No Seroconversion (n = 35)</th>
<th>P Value (A) vs. (B)</th>
<th>(C) HBeAg Negative (n = 12)</th>
<th>P Value (A) vs. (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>48/14</td>
<td>12/3</td>
<td>25/10</td>
<td>0.73</td>
<td>11/1</td>
<td>0.61</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>44 ± 15 (19-74)</td>
<td>43 ± 13 (19-63)</td>
<td>43 ± 16 (15-74)</td>
<td>0.63</td>
<td>52 ± 17 (24-70)</td>
<td>0.97</td>
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<tr>
<td>Body weight (kg)</td>
<td>73 ± 13 (41-100)</td>
<td>72 ± 14 (47-100)</td>
<td>72 ± 11 (41-89)</td>
<td>0.83</td>
<td>75 ± 10 (65-95)</td>
<td>0.53</td>
</tr>
<tr>
<td>BMI in kg/m²</td>
<td>24.7 ± 3.1 (16.2-34.5)</td>
<td>24.5 ± 4.2 (18.8-34.5)</td>
<td>24.3 ± 2.7 (16.2-30)</td>
<td>0.85</td>
<td>25.3 ± 2.7 (22.2-31)</td>
<td>0.45</td>
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<tr>
<td>HBV genotype</td>
<td></td>
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<tr>
<td>A</td>
<td>18</td>
<td>4</td>
<td>12</td>
<td>0.152</td>
<td></td>
<td>0.121</td>
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<td>B</td>
<td>3</td>
<td>1</td>
<td>1</td>
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<tr>
<td>C</td>
<td>7</td>
<td>1</td>
<td>4</td>
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<tr>
<td>D</td>
<td>33</td>
<td>7</td>
<td>17</td>
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<tr>
<td>E</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Patients receiving TDF</td>
<td>38/24</td>
<td>6/9</td>
<td>30/5</td>
<td>0.003</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>or LMV (n)</td>
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<tr>
<td>ALT (IU/mL)</td>
<td>119 ± 224 (19-1,523)</td>
<td>191 ± 202 (26-816)</td>
<td>55 ± 30 (23-143)</td>
<td>0.02</td>
<td>216 ± 442 (19-1,523)</td>
<td>0.86</td>
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<tr>
<td>Observation time during treatment (months)</td>
<td>30 ± 17 (4-64)</td>
<td>33 ± 18 (4-64)</td>
<td>30 ± 16 (4-64)</td>
<td>0.49</td>
<td>25 ± 10 (7-40)</td>
<td>0.13</td>
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<tr>
<td>HBV fIRNA*</td>
<td></td>
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<tr>
<td>Month 0</td>
<td>5.2 ± 1.6 (2.7-8.0)</td>
<td>4.9 ± 1.3 (2.7-7.2)</td>
<td>5.8 ± 1.5 (2.7-8.0)</td>
<td>0.07</td>
<td>4.3 ± 1.4 (2.7-5.9)</td>
<td>0.11</td>
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<tr>
<td>Month 3</td>
<td>4.8 ± 1.6 (2.7-8.3)</td>
<td>3.8 ± 1.9 (2.7-6.5)</td>
<td>5.6 ± 1.4 (2.7-8.2)</td>
<td>&lt;0.001</td>
<td>3.6 ± 1.0 (2.7-5.0)</td>
<td>0.79</td>
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<tr>
<td>Month 6</td>
<td>4.4 ± 1.6 (2.7-8.0)</td>
<td>3.0 ± 0.7 (2.7-5.1)</td>
<td>5.3 ± 1.4 (2.7-8.0)</td>
<td>&lt;0.001</td>
<td>3.1 ± 0.9 (2.7-5.5)</td>
<td>0.82</td>
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<tr>
<td>HBV trRNA*</td>
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<tr>
<td>Month 0</td>
<td>5.5 ± 1.6 (2.8-8.5)</td>
<td>6.0 ± 1.8 (2.8-8.5)</td>
<td>5.6 ± 1.5 (2.8-8.2)</td>
<td>0.49</td>
<td>4.3 ± 1.2 (2.8-6.0)</td>
<td>0.009</td>
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<tr>
<td>Month 3</td>
<td>4.5 ± 1.4 (2.8-7.6)</td>
<td>3.8 ± 1.1 (2.8-6.1)</td>
<td>5.2 ± 1.2 (2.8-7.7)</td>
<td>&lt;0.001</td>
<td>3.5 ± 0.8 (2.8-4.9)</td>
<td>0.49</td>
</tr>
<tr>
<td>Month 6</td>
<td>4.2 ± 1.4 (2.8-7.5)</td>
<td>2.9 ± 0.3 (2.8-3.9)</td>
<td>4.9 ± 1.2 (2.8-7.5)</td>
<td>&lt;0.001</td>
<td>3.2 ± 0.7 (2.8-4.7)</td>
<td>0.22</td>
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<tr>
<td>HBV DNA*</td>
<td></td>
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</tr>
<tr>
<td>Month 0</td>
<td>6.5 ± 1.9 (1.54-9.0)</td>
<td>7.1 ± 1.8 (3.0-8.5)</td>
<td>6.5 ± 1.9 (1.5-9.0)</td>
<td>0.27</td>
<td>5.5 ± 1.5 (1.5-7.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Month 3</td>
<td>4.3 ± 1.5 (1.5-7.9)</td>
<td>3.9 ± 1.0 (2.5-5.7)</td>
<td>4.6 ± 1.6 (1.5-7.9)</td>
<td>0.096</td>
<td>3.8 ± 1.2 (1.5-6.7)</td>
<td>0.83</td>
</tr>
<tr>
<td>Month 6</td>
<td>3.2 ± 1.4 (1.5-7.3)</td>
<td>2.7 ± 0.7 (1.5-3.7)</td>
<td>3.7 ± 1.6 (1.5-7.3)</td>
<td>0.063</td>
<td>2.6 ± 0.9 (1.5-4.1)</td>
<td>0.76</td>
</tr>
<tr>
<td>HBsAg&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Month 0</td>
<td>3.7 ± 1.0 (0.1-5.6)</td>
<td>3.8 ± 1 (1.2-4.9)</td>
<td>3.9 ± 0.8 (2.4-4.9)</td>
<td>0.89</td>
<td>2.8 ± 1.3 (0.8-3.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>Month 3</td>
<td>3.6 ± 1.0 (0.2-5.6)</td>
<td>3.6 ± 0.9 (1.2-4.8)</td>
<td>3.9 ± 0.7 (1.3-5.6)</td>
<td>0.26</td>
<td>2.7 ± 1.4 (0.8-3.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Month 6</td>
<td>3.5 ± 1.0 (0.0-5.0)</td>
<td>3.4 ± 1 (1.2-4.8)</td>
<td>3.9 ± 0.5 (2.6-5.0)</td>
<td>0.16</td>
<td>2.7 ± 1.4 (0.5-3.8)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

<sup>*</sup>Log<sub>10</sub> IU/mL,
<sup>1</sup>Log<sub>10</sub> copies/mL,
<sup>2</sup>Hypergeometric test; all results in mean values ± standard deviation (range).

van Bommel, F et al 2015. Hepatol;61:66-76
ROC Curves at BL, Month 3 and 6 of Treatment: Predicting HBeAg SC

- BL: HBV fl RNA (AUROC 0.73)
- 3 and 6 months: HBV tr RNA (AUROC 0.9 and 0.85)
- 3 months: > 1.0 log copies/ml
  - [Sensitivity 92%; specificity 82% for eSC]

van Bommel, F et al 2015. Hepatol;61:66-76
Hepatitis B Core-Related Antigen (HBcrAg)

Hepatitis B Virus DNA-negative Dane Particles Lack Core Protein but Contain a 22-kDa Precore Protein without C-terminal Arginine-rich Domain*

Received for publication, February 10, 2005, and in revised form, March 31, 2005
Published, JBC Papers in Press, April 4, 2005, DOI 10.1074/jbc.M501564200

Tatsuji Kimura‡§, Nobuhiko Ohno¶, Nobuo Terada¶, Akinori Rokuhara¶, Akihiro Matsumoto¶, Shintaro Yagi‡, Eiji Tanaka¶, Kendo Kiyosawa¶, Shinichi Ohno¶, and Noboru Maki‡
Electron Microscopy: HBV in Serum
Precore/Core Gene Products and their Processing

- Combined measure of HBcAg, HBeAg and p22cr is known as HB core-related antigen [HBcrAg]

HBcrAg Across Phases of CHB
HBcrAg Concentrations Correlate with the Levels of HBV DNA and HBsAg

Correlation Between HB-crAg, cccDNA and HBV DNA

# Potential Advantages of Applying HBcrAg in Clinical Practice

<table>
<thead>
<tr>
<th>HBcrAg</th>
<th>PROVEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlate strongly with disease activity</td>
<td></td>
</tr>
<tr>
<td>Differentiate ENQ* and ENH* groups in HBeAg-negative disease</td>
<td></td>
</tr>
<tr>
<td>Detect occult HBV infection</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>POTENTIAL (needs further evaluation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predict occurrence of HCC (high levels of HBcrAg)</td>
</tr>
<tr>
<td>Predict HBsAg seroclearance (low levels of HBcrAg)</td>
</tr>
<tr>
<td>Stop Therapy (very low levels of HBcrAg)</td>
</tr>
</tbody>
</table>

*ENH: HBeAg-negative CHB
*ENQ: HBeAg-negative quiescent

HBsAg Neutralisation Domain

The major anti-HBs binding domain or ‘a’ determinant (aa99-169), contains the immunogenic epitope 139-147

The ‘a’ determinant is highly conformational, with a raft of cysteine & proline residues forming discrete loops


HBsAg ‘a’ determinant topology alterations directly influence the HBV neutralisation phenotype

[Adapted from Torresi et al., Virology. 2002. 293:305-313]
BioPlex Multiplex HBsAg Epitope Mapping Assay

Multiplex assay to identify/predict HBs variants & VEM’s by mapping the HBsAg fingerprint

Developed a 19plex panel of anti-HBs mAbs covering HBsAg ‘a’ determinant and C-terminal domain (residues 99-226)

[Adapted from Bio-Rad; www.bio-rad.com]

Anti-HBs antibodies were sourced from industry & academic collaborators

Genotype/serotype or mutations alter the HBsAg ‘a’ determinant structure, & consequently effect the epitope binding profile & phenotype
Aberrant sG145R VEM phenotype detected across 9/19 mAbs

VEM predictive mAb = 17 (loop2), increased epitope binding for sG145R VEM

VEM k/o phenotype across 3 domains: loop1 (6), loop2 (8,12,16), Conformational (9)

Loop 2 sG145R VEM alters HBsAg to confer an aberrant phenotype across multiple epitopes or ‘a’ determinant domains
**Hypothesis**

HBsAg epitope profiles are sensitive to therapeutic and immune (i.e. recovery of the B cell / Ab & innate immune responses on-treatment), and can be predictive of HBsAg response (loss or seroconversion) on-treatment

**HBsAg clearance profile (CP)**

HBsAg epitope pressure (reduced recognition) at *both* loop 1 **AND** loop 2 epitopes

- associated with HBsAg response/decline (>1log) and potentially HBsAg loss/seroconversion

**HBsAg non-clearance (or escape) profile (NCP)**

No change in HBsAg epitope profile, OR reduced epitope binding at *only* one loop

- associated with no HBsAg response/decline (<1log)
Mapping HBsAg epitope profiles to predict HBsAg loss/seroconversion in a treatment naïve cohort of genotype A chronic hepatitis B (CHB) patients receiving tenofovir disoproxil fumarate (TDF) therapy

GS-US-174-0103 (G103) study cohort: HBsAg epitope profile analysis of HBeAg+ patients on NA therapy:

- Treatment naïve CHB patients in the immune clearance phase (HBeAg positive)
- Treated with tenofovir (TDF) for 48 weeks, & continued/followed out to > 4 years
- 96% achieved viral suppression & 15% HBsAg loss (22/142) at 4 years

We studied a subset of 25 HBV genotype A patients:

- 14 achieved HBsAg loss/seroconversion
- 11 had no change in HBsAg (<1log decline to 4 years)

HBsAg clearance profile (CP) v’s non-clearance profile (NCP) conclusions:

Significant association ($p < 0.02$) between the development of a HBsAg CP and HBsAg Loss/Seroconversion [PPV 83%] by 48 weeks of treatment

Walsh, R et al 2015. AASLD Hepatology in press
Future Perspectives and Developments

- The goalposts are shifting in hepatitis B and C
- In hepatitis B, the medium-term aim for the field is to achieve “cure”
  - HBsAg loss ideally with HBsAg seroconversion
  - An immunomodulator may be required
- Many new DAAs for CHB are starting to emerge
  - Improved delivery to the liver for molecular therapeutics
  - New HBV biomarkers such as serum HBV RNA and HBsAg epitope profile (CP) promising
  - New concepts for HBeAg-pos (cccDNA) versus HBeAg-neg (integrated DNA) CHB

PALPABLE OPTIMISM and EXCITEMENT

- Hepatitis C: “done and dusted”
What Might a HBV Curative Regimen Look Like?

Potent NA

agent to prevent viral spread and cccDNA re-amplification

cccDNA Inhibitor

safe and selective agent to reduce or silence cccDNA

Immune Activator

agent(s) to activate specific antiviral immune responses or relieve repression/exhaustion of the system

HBV Antigen Inhibitor

agent(s) to block/inhibit the HBV life-cycle [entry, cell-spread, capsid assembly, HBx, HBeAg, HBsAg]