Understanding HBV Testing: HBsAg, HBV RNA, cccDNA, HBeAg and HBcrAg in Context of Antiviral Drug Development

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## Disclosure

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<tr>
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<th>Gilead Sciences Inc</th>
<th>Arrowhead Research Corp</th>
<th>Spring Bank Pharmaceuticals, Inc.</th>
<th>Roche Molecular</th>
<th>AusBio Ltd</th>
<th>Janssen (J&amp;J)</th>
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<td>Consulting Fees (eg. Advisory Boards)</td>
<td>yes</td>
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<td>Contract Research (grant)</td>
<td>yes</td>
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# Viral Biomarker Scenarios: DAAs and Cytokines

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<th>Possible Interpretation*</th>
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                     | • Pol-5´-ε binding/encapsidation |
| HBV RNA:           | • Pol-5´ε binding  
                    | • priming RT  
                    | • encapsidation inhibition  
                    | • nucleocapsid assembly inhibition  
                    | • cccDNA dependent |
| • pgRNA [full length] |                           |
| Other [Truncated]  | • ? splice HBV RNAs  
                    | • ? chimeric HBV RNAs |
| HBeAg              | • precore mRNA [cccDNA dependent] |
| HBcrAg [HBcAg; HBeAg; p22cr] | • pregenomic RNA [cccDNA dependent]  
                              | • precore mRNA [cccDNA dependent]  
                              | • cccDNA “activity” |
| HBsAg              | • phase of CHB [set-points]  
                     | • episomal HBV (ccc)DNA [HBeAg-POS]  
                     | • integrated HBV DNA [HBeAg-NEG] |

* Substantial Overlap
Biomarkers and MOA of DAAs

Old Concept

- HBeAg-Pos and HBeAg-Neg Replication Same
- Very low level of HBV Integration
Novel Findings ARC-520 Studies: 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

Changes in Serum HBsAg are Correlated with Changes in cccDNA Titer: HBeAg-pos vs neg

HBeAg-NEG different transcriptome than HBeAg-POS

Hepatic HBV cccDNA Levels in Different Patient Populations

- cccDNA persists through all phases of the natural history of chronic hepatitis B
- PCR Measures Level of cccDNA NOT Activity
- **Copy number 0.1-10 cccDNA/hepatocyte**

Partial Reduction of cccDNA by NUCs

Role of Intracellular Conversion Pathway

- cccDNA = 21 Topoisomers
  [NOT a single entity]
- Difference in Transcriptional Activities

cccDNA Transcriptional Activity

- **Virion Productivity**: “the number of intrahepatic (IH) replicating HBV DNA molecules per cccDNA molecule” (Volz T, et al 2007. Gastroenterol;133:843-52)

  \[
  \text{Total IH HBV DNA} - \frac{\text{cccDNA}}{\text{cccDNA}}
  \]


- **Epigenetic State**: cccDNA acetylation assay (Pollicino, T et al 2006. Gastroenterol;130:823))
  - CHIP assay - HBV replication parallels the acetylation status of HBV cccDNA-bound H3 and H4 histones
HBV cccDNA Replicative Activity (pgRNA transcripts produced per cccDNA molecule) in HBeAg(+) (open squares) and HBeAg(-) (closed circles) patients

Serum HBV RNA

- **Mimic what’s happening in the liver with cccDNA levels**
- **RNA in serum may reflect the presence and active transcription of cccDNA in the liver** *(Wang J et al. J Hepatol 2016)*
- Typically lower than HBV DNA levels (but abundant)
- Serum RNA levels vary significantly from other viral markers during AV therapy
  - eg. in HBeAg pos pts there is a stronger decline in HBV DNA levels cf with RNA levels
  - highlighting potential as an independent marker in the evaluation of pts with CHB *(Jansen L et al. JID 2015)*
- Persistence of serum HBV RNA associated with risk of viral rebound following discontinuation of NUC therapy (reflect level of intrahepatic cccDNA?) *(Wang et al 2016. J Hepatol;65:700-710)*
Model of the Production of Enveloped pgRNA Virions and Their Infectious Potential: Entry and Re-entry

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
HBV Full Length RNA

Phase 1b Clinical Trial: CpAM NVR 3-778 Reduces Serum HBV DNA and RNA

Pre-clinical evaluation in hepatocyte culture and chimeric mouse models

- Serum HBV DNA: mean 1.7 log reduction (600 mg BID)
- Serum HBV RNA: mean 0.86 log reduction (600 mg BID)

Lam A, et al. AASLD 2015, San Francisco. #33
HBV RNA from Hepatitis B Patient Sera Contains Significant Amounts of Encapsidated Spliced HBV RNA Variants (2)

- HBV RNA is secreted within virus-like particles consist of envelope and capsid
- Extracellular HBV RNA particles contain pgRNA and spliced RNA variants
- HBV CAM blocks production of pgRNA and spliced RNA containing particles
- 3 new spliced variants identified

Patient sera contains encapsidated **spliced** HBV RNA variants (known and novel) and may be potential treatment response biomarkers depending on the DIA

Espiritu C, et al. AASLD 2016, Boston. #17
Antiviral Activity of SB9200 (Inarigivir)
HBV RNA and HBcrAg Profile of ACHIEVE Trial

In HBeAg-NEG group: 3 log rapid decline HBV RNA whilst 1 log gradual decline HBV DNA

See AASLD Abstract #39 and Late Breaker Poster
HBV Encapsidation: POL-5′-ε pgRNA Binding

STOICHEOMETRIC IMBALANCE: One pgRNA; One Polymerase; 240 Core submits

ABSOLUTE REQUIREMENT: 5′-ε pgRNA

SMALL CAPSID SYNDROME

Proposed Model for Direct Antiviral Effect of SB 9200

HBV Encapsidation: The Packaging Reaction

- RT1
- CHAPERONE ACTIVATION
- RT2 - Hsp90
- ε RNA BINDING (Pregenome)
- SB 9200
- Core Protein
- INITIATION OF PROTEIN PRIMING
- RT4 - dGAA- ε
- TRANSLOCATION AND (-) DNA SYNTHESIS

Quantitative HBeAg Testing

- Role for **quantitative HBeAg titre** in predicting treatment outcome has been proposed: *(Fried, Hepatology, 2008)*

Pegylated-interferon therapy:
- HBeAg seroconversion
  - Baseline HBeAg titre < 31 PE IU/ml – PPV for seroconversion = 51%
  - 24 week HBeAg titre > 100 PE IU/ml – NPV for seroconversion = 96%
  
Vs 24 week HBV DNA > 9 log copies/ml – NPV = 86%

Population Distribution of HBeAg Titre

In 40% the HBeAg titre <1000 PE IU/ml (n=85)  
Most patients have a titre <100 PE IU/ml

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*Thompson A, et al. AASLD 2008*
HBV Replication: HBeAg (Secretory) Pathway

- pre-core mRNA Derived [full length HBV DNA]
- made from cccDNA templates only
HEPATITIS B CORE-RELATED ANTIGEN (HBcrAg) PRECORE/CORE GENE PRODUCTS AND THEIR PROCESSING


HBcrAg = combined measure of HBcAg, HBeAg and p22cr
Electron Microscopy: HBV in Serum

Empty particles: p22cr Capsids
Correlation Between HBcrAg, cccDNA and HBV DNA

In HBeAg-neg CHB, what is being measured??

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See AASLD Abstract #39 and Late Breaker Poster
Biomarkers and MOA of DAAs

**OLD**
- HBV Virions (DNA)
- HBV Virions (RNA)
- HBeAg
- HBcrAg
- HBsAg

**NEW**
- Minichromosome
- cccDNA
- pgRNA
- subgenomic mRNA
- HBx RNA
- Fusion transcripts

**NUCLEUS**
- RC DNA
- PF-RC DNA
- Minichromosome cccDNA

**CYTOPLASM**
- DSL DNA
- pgRNA
- HBx RNA

**EXTRACELLULAR / SERUM**
- DSL DNA
- HBeAg
- HBcrAg
- HBsAg


Integrated DSL HBV DNA
HBcrAg Across Phases of CHB
Hepatic HBV cccDNA Levels in Different Patient Populations

- cccDNA persists through all phases of the natural history of chronic hepatitis B
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Natural History: HBsAg Levels are Lowest in the Immune Control Phase

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<th>HBeAg-negative</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>Immune tolerance</td>
<td>Immune clearance</td>
<td>Immune clearance/ Reactivated</td>
</tr>
<tr>
<td>ASIA</td>
<td>4.53</td>
<td>4.03</td>
<td>3.35</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>55</td>
<td>83</td>
</tr>
<tr>
<td>EUROPE</td>
<td>4.96</td>
<td>4.37</td>
<td>3.89</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>48</td>
<td>68</td>
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Reliable identification of inactive carriers through a combination of HBV DNA <2000 IU/mL and HBsAg <1000 IU/mL

Nguyen T, et al. J Hepatol 2010
Jaroszewicz J, et al. J Hepatol 2010
Serum qHBsAg Predict HBsAg Loss in HBeAg Seroconverters

- 390 patients who spontaneously underwent HBeAg SC (genotype B/C)
- Low serum levels of HBsAg (alone or in association with HBV DNA levels*) 1.0 year after HBeAg SC can predict HBsAg loss:

  **PPV HBsAg Loss Within 6 years**

  - HBsAg < 100 IU/ml  46%
  - HBsAg 100-999 IU/ml  29%
  - HBsAg > 1000 IU/ml  < 10%

* HBV DNA < 200 IU/ml

CONCEPT: VIROLOGICAL SET POINTS

Tseng, T-C et al 2011. Gastroenterology;141:577
Response Guided Therapy for Peg-Interferon in the Treatment of Hepatitis B

Week 12 and 24 are the Key

HBeAg - Positive

Week 12 – Define Possible Non-Responders

Criteria:
1) Absence of HBsAg decline OR
2) HBsAg > 20,000 IU/ml

HBeAg - Negative

Week 24 – Define the Level of Treatment Response

Criteria:
1) Absence of HBsAg decline AND
2) HBV DNA reduction < 2 log

High:
HBsAg < 1,500 IU/ml
Mid: HBsAg 1,500 to 20,000 IU/ml
Low: HBsAg > 20,000 IU/ml

High: HBsAg decline >10%
Low: HBsAg decline < 10%


INTEGRATE WITH HBV RNA, HBcrAg, and qHBeAg TO IMPROVE PPV
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Conclusion: Key Serum Biomarkers

• Phase of CHB
  * HBV DNA
  * qHBsAg
  * qual HBeAg/anti-HBe
    [transition vs flip-flop]
  * HBV RNA
  * HBcrAg

• Interpret ALL available serum markers in context of CHB Natural History in order to define both known and new viral targets [packaging vs core assembly inhibitors]

• View HBV Lifecycle in full context for insight into mechanism(s) of action of DAA and cytokines [identification of regulatory pathways: virus replication eg: cccDNA ↔ envelope protein]