What's on the Horizon for Treatment for CHB? New Targets for HBV?

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In many regions the cost of anti-CHB therapy poses significant financial burden to patients and to the resource-constrained national healthcare systems. Financial burden of treatment remains unaffordable for most patients because of lack of full or adequate reimbursement for treatment.

### Guidelines HBV Treatment

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<tr>
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<th>AASLD 2009</th>
<th>EASL 2012</th>
<th>APASL 2012</th>
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<td>Lamivudine</td>
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<td>Adefovir</td>
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<td>Entecavir</td>
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<td>Telbivudine</td>
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<td>Tenofovir</td>
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<td>PEG-IFN</td>
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*Lok and McMahon 2009, Liaw et al., 2012, EASL 2012*
Benefits of HBsAg Clearance

- ↓ Hepatic decompensation
- ↓ HCC
- ↑ Survival
- ↓ Levels of cccDNA
- **As close to cure as we can expect to achieve in chronic hepatitis B**

What’s New?

• **Refining current treatments**
  – Combination therapy
    • PEG-IFN + NA

• **New treatment approaches**
  – NA - TAF
  – Novel antivirals
  – Novel immune stimulants
PEG-IFN Add-On (ARES Study)

- HBeAg positive study
- Multicenter, open-label, randomized controlled trial

**Response?**

- **Entecavir 0.5 mg daily**
- **PEG-IFN alfa-2a 180μg**

**Follow-up**

Response: combined presence of HBeAg loss and HBV DNA level < 200 IU/ml at week 48

Sonneveld et al. AASLD 2012
ETV and PEG-IFN (ARES Study): HBV DNA, HBeAg, HBsAg During Therapy

Sonneveld et al. AASLD 2012
PEG-IFN Switch (OSST Study)

- Randomized, multicentre, open-label study
- Primary endpoint: HBeAg seroconversion at end of treatment (week 48)
- Secondary endpoint: HBsAg loss at week 48

- HBV DNA ≤ 10^3 copies/ml

ETV 0.5 mg QD (N=200)

~ 9-36 months

Switch to PegIFNa-2a 180 μg/week for 48 weeks (n=100)

ETV 0.5 mg QD for 8 weeks

ETV 0.5 mg QD for 48 weeks (n=100)

QD = once daily; PEIU = validated with in-house reference standards obtained from Paul Ehrlich

Ning et al., AASLD, 2012
OSST Study: Response Rates at Week 48 of Treatment (ITT)

* Fisher Exact test, other p-values are using Chi-Squared Test
† Updated data from time of abstract submission
ITT = intention-to-treat

Ning et al., AASLD, 2012
OSST Study: HBsAg Decline Greater in PEG-IFN Responders

Ning et al., AASLD, 2012
### New Treatment Approaches

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Target</th>
<th>Agents</th>
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<tr>
<td><strong>HBV life cycle</strong></td>
<td><strong>HBV Pol</strong></td>
<td><strong>TAF</strong></td>
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<tr>
<td>Viral entry</td>
<td>Myrcludex-B</td>
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<tr>
<td>cccDNA</td>
<td>Zinc finger nucleases</td>
<td>cccDNA conversion inhibitors</td>
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<td>mRNA transcription/ stability</td>
<td>Zinc finger proteins</td>
<td>Epigenetic silencers</td>
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<td>RNA silencing</td>
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<td>- Antisense OGNs</td>
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<td>- Ribozymes</td>
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<td>- RNAi</td>
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<td>Viral assembly</td>
<td>HAPs</td>
<td>Phenylpropenamides</td>
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<td>HBV antigen secretion</td>
<td>REP 9AC’</td>
<td>Small molecule inhibitors of HBsAg secretion</td>
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<td>e.g. glucovirs</td>
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<td>e.g. triazolo-pyrimidines</td>
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<td><strong>Immunotherapeutic</strong></td>
<td>PegIFN-λ1a (IL29)</td>
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<td>Cytokines</td>
<td>rIL-7</td>
<td>rIL-21</td>
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<td>TLR agonists</td>
<td>TLR7 (GS-9620)</td>
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<td>Therapeutic vaccines</td>
<td>Adeno-virus approaches (TG1050)</td>
<td>Tarmogen</td>
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<td>(GI-13020)</td>
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<td>Blocking T cell inhibitory receptors</td>
<td>Anti-PD-1 moAB (BMS936558)</td>
<td>Anti-PD-L1 moAb (BMS936559)</td>
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<td>Intrahepatic blocking of suppressive cytokines / regulatory T cells</td>
<td>TGF-β inhibitors</td>
<td>T reg depletion (e.g. α-CD25, daclizumab)</td>
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Inhibition of HBV Nucleos(t)ide Analogues

- Attachment and Penetration
- DNA repair
- Transport to cell nucleus
- Nucleus
- Pregenomic RNA
- HBV RNA transcripts
- HBV polymerase protein
- Envelope proteins S, M, L
- Core proteins
- Golgi complex
- Release
- TRANSLATION
- Translocation
- \( 5' \) Cap

Inhibition of priming

Inhibition of chain elongation
Tenofovir Alafenamide (TAF)

- TAF = orally bioavailable phosphonoamidate prodrug of tenofovir (TDF)
- In comparison with tenofovir, TAF enables enhanced delivery of the parent nucleotide and its active diphosphate metabolite into lymphoid cells and hepatocytes.
- This is attributed to an improved plasma stability and differential intracellular activation mechanism for TAF relative to TDF

<table>
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<tr>
<th>EC$_{50}$ HIV-1 (PBMCs)</th>
<th>Tenofovir</th>
<th>Tenofovir Disoproxil</th>
<th>TAF</th>
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<tr>
<td>1.2 µM</td>
<td>0.015 µM</td>
<td>0.003 µM</td>
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Prevention of Infection: Entry Inhibitors

Acylated Pre-S1 Peptides

Petersen J, Urban S. et al 2008. Nature (Biotechnology);26:335
Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus

Huan Yan¹,²†, Guocai Zhong²†, Guangwei Xu², Wenhui He²,³, Zhiyi Jing², Zhenchao Gao¹,², Yi Huang²,³, Yonghe Qi², Bo Peng², Haimin Wang², Liran Fu²,³, Mei Song²,³, Pan Chen²,³, Wenqing Gao², Bijie Ren², Yinyan Sun², Tao Cai², Xiaofeng Feng², Jianhua Sui², Wenhui Li²*¹

¹Graduate program in School of Life Sciences, Peking University, Beijing, China; ²National Institute of Biological Sciences, Beijing, China; ³Graduate program in Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

NTCP = a receptor for HBV

*Yan et al, eLife, November 2012
A Synthetic Peptide Derived from the Large Envelope Protein of HBV Blocks HBV Infection in Susceptible Cells….

Incubation o/n at 37°C

Collection of supernatant of days 8-12p.i.

Measurement of secreted HBsAg/HBeAg etc.

Infection of HepaRG cells or PHH

Incubation o/n at 37°C

Gripon et al., PNAS, 99 (24) 2002
Urban et al., J. Virol, 79 (3), 2005
Glebe et al., Gastroenterology, 129, 2005
Engelke et al., Hepatology, 43, 2006
Schulze et al., Hepatology, 46, 2007
Status of Myrcludex B the First in Class Entry Inhibitor of HBV and Hepatitis Delta Virus (HDV).

- The GMP synthesis of 100 g Myrcludex B (API) is finished.
- A formulation for s.c. application has been developed.
- Vials for clinical studies have been filled.
- Myrcludex B has been characterized for purity, stability etc.
Mechanism of RNA Interference (RNAi)

**Natural Process of RNAi**

- **dsRNA**
- **dicer**
- **cleavage**
- **RISC**
- **complementary pairing**
- **mRNA degradation**
- **cleaved mRNA**

**Therapeutic Gene Silencing**

- **Synthetic siRNAs**
- **strand separation**
- **siRNAs**
- **(A)_n**
RNAi treatment for chronic Hepatitis B

siRNA design and in vitro screening

• Designed 140 siRNAs targeting conserved regions of HBV genotypes A-D
• Confirmed conservation in genotypes E-H as well.

• Screened candidate siRNAs in a cell culture system
• 4 highly potent siRNAs chosen for further testing in animal models
• siHBV-74 and siHBV-77 chosen as leads

Roche-Kulmbach (Axolabs GmbH)
Dynamic Polyconjugate (DPC) technology for siRNA delivery in vivo

- **DPC polymer composition and physical characteristics**
  - Amphipathic peptide
  - peptide amines reversibly “masked” with CDM
  - Slightly negatively charged

- **Cellular uptake of peptide** is ligand-driven (N-acetyl galactosamine (NAG)) for hepatocytes

- **siRNA** is made liver tropic by attachment of lipophilic ligand (e.g. cholesterol)

- **↓ pH in endosomes** drives peptide unmasking

- Unmasked peptide disrupts endosomal membrane

- **siRNA** released to cytoplasm
Reduction in HBV after administration of ARC-520 in a chronically infected chimp

- Log$_{10}$ reduction in HBV DNA (95%), HBeAg (90%) and HBsAg (90%)
- First demonstration of RNAi efficacy in the chimp HBV model
- KD comparable to that achieved in mouse HBV models at similar dose level
- Further reduction after a subsequent dose

REVIVAL OF IMMUNE RESPONSES AND FUNCTIONAL CURE

Dr. Robert Lanford, Texas Biomedical Research Institute
Immune-Based Therapies for Chronic Hepatitis B
HBV Life Cycle and Innate Immunity

Potential intracellular PAMPS:
- nucleocapsid
- viral DNA
- viral RNA (ss and ds)
- viral proteins

Potential PAMPS for virus and sub-viral particles:
- glycoproteins (HBsAg)
- nucleocapsid (HBcAg)
- rcDNA

Other potential PAMPS:
- secreted HBsAg
- secreted HBeAg
- secreted non enveloped nucleocapsids
- free viral nucleic acids

TLR2 Stimulation Inhibits HBV Replication: WT (HBeAg-Positive) HBV (*in vitro*)

Thompson, A et al 2009. Antiviral Therapy; 14:797–808
GS-9620: Oral TLR-7 Agonist

- TLR-7
  - Intracellular pathogen sensor
    - endolysosomal RNA
  - Agonism induces anti-viral response via innate immune activation
- GS-9620
  - Oral
  - Nanomolar potency
  - Selective (TLR-7 >>> TLR-8)
  - Pharmacodynamic effects in mouse, woodchuck, cyno, chimp, human
GS-9620: Reduction in HBV DNA, and Serum HBsAg and HBeAg in Chimpanzee

Lanford et al. EASL 2011
Reversal of Immune Exhaustion

i. Role of Immune Regulatory Receptors
   • in CHB, immune regulatory receptors (IRR) are the key drivers of T-cell dysfunction [eg: PD-1]
     (Fisicaro, P et al 2010. Gasto; 138:682-693.,
   • blocking these inhibitory IRRs has the potential to restore T-cell function [eg: anti-PD-1/PD-L1]

ii. Follicular Helper T-Cells (Tfh)
   • Tfh (CXCR5⁺ CD4⁺) under influence of IL-21 provide help to B-cells
   • IL-21 levels associated with HBeAg seroconversion
     (Ma, S-W et al 2012. J Hepatol; 56:775-781)
Reverse T cell exhaustion by PD-1/PD-L1 pathway blockade

Exhausted T cell:
- Decreased proliferation
- Decreased cytokine secretion
- Decreased cytotoxicity

Functional T cell:
- Increased proliferation
- Increased IFN-γ, TNF-α, IL-2
- Increased cytotoxicity
*In vivo* PD-L1 blockade synergizes with therapeutic vaccination to enhance WHcAg-specific T cell immunity

Jia Liu et al, PLOS Pathogens under revision
*In vivo* PD-L1 blockade synergizes with therapeutic vaccination to control WHV replication.
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]
Future Perspectives

• Futility rules for PEG-IFN therapy identified
  – RGT needs to be explored

• The goalposts are shifting

• The long-term aim for the field is to achieve “cure”
  – HBsAg seroconversion
  – An immunomodulator is likely to be required

• Emerging strategies explore the use of combination, or “add-on” PEG-IFN plus NA therapy

• New agents for CHB are starting to emerge
  – Identification of the HBV-R (NTCP) may be paradigm shifting
  – Improved delivery to the liver for molecular therapeutics

PALPABLE OPTIMISM