New HBV Treatment Options on the Horizon

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Key Considerations for Current Treatment Options

- All HBV nucleos(t)ides are generally well tolerated but with low rates of successful discontinuation.

- The long-term safety of nucleos(t)ide-analogues remains to be determined.

- PEG-IFN monotherapy is finite but only effective in subgroup of patients and its use is limited due to toxicity.
Why is Finite Therapy a Goal for HBV Treatment?

- Younger patients may find lifelong treatment hard to accept
- Women who want to become pregnant
- Patients reluctant to start treatment
- Working days lost to hospital visits
- Cost savings to healthcare system
- Long-term adherence issues
Is HBV Treatment Paradigm changing?

<table>
<thead>
<tr>
<th>Current PARADIGM</th>
<th>New PARADIGM</th>
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<tbody>
<tr>
<td>• Indefinite Treatment</td>
<td>• Finite treatment duration</td>
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<td>• Poor off-Rx response</td>
<td>• Sustained off-Rx response shift towards endpoint of true immune control &amp;HBsAg seroconversion</td>
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<td>• Reduces overall mortality</td>
<td>• No increased risk of mortality and HCC</td>
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<td>• Reduce but does not eliminate the risk of HCC</td>
<td>• New HBV treatments with increased chance of curing disease</td>
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<td>• Potent NAs : suppresses viral replication but cannot cure the disease</td>
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</table>
New Strategies for Finite HBV Treatment

Strategies with Licensed Medication

Strategies with Unlicensed Medication
New Strategies for Finite HBV Treatment

Strategies with Licensed Medication

Strategies with Unlicensed Medication
How to enhance response rate through combination therapy? What’s the best combination therapy?

• The discussion about the combination of IFN and nucleos(t)ide analogues has never ceased.

\[ \text{Nucleos(t)ide analogues} + \text{Peg IFN} = ? \]

• The clinical trials on IFN and other nucleos(t)ide analogues combination thus far did not provide sufficient evidence, proving that combination therapy can bring more benefit.
Current Initiatives PEG-IFN Treatment

- PEG IFN in immune tolerant patients (NIH)
- PEG-IFN in combination with TDF (NIH, Gilead)
- PEG-IFN add-on in virally suppressed patients on NA (PEGON, Fudan)
- PEG-IFN in combination with ETV (ARES)
- PEG-IFN RGT study (EXCEL)
- PEG-IFN alfa 2b registration study in Asia (Merck)
Pre-Treatment with Nucleos(t)ide Analogues?

- Partial immune restoration (NK cells, dendritic cells and T cells) after viral load reduction with almost all NA’s

- In clinical practice hardly sustained off-treatment immune control

- However could be used as priming therapy for PEG-IFN

ETV and PEG-IFN (ARES Study)

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

Response? *

Entecavir 0.5 mg daily
Pegi-IFN alfa-2a 180µg

Response: combined presence of HBeAg loss and HBV DNA level < 200 IU/ml at week 48
Week 96 results: PEG-IFN addition leads to increased response rates (ARES Study)

**PEG-IFN add-on therapy:**
- Significantly associated with response (OR 3.1, p=0.007)
- Significantly more decline in HBsAg, HBeAg and HBV DNA during treatment
- More sustained response after ETV discontinuation at wk72

Brouwer et al. Hepatology 2014
New Strategies for Finite HBV Treatment

Strategies with Licensed Medication

Strategies with Unlicensed Medication
HBV Life cycle
Towards New HBV Treatment Targets

- Myrcludex-B
- Cyclosporin
- HAP
- Phenypropenamide
- Isothiawlude
- NVR-1221
- RNAi
- ASO
- ISIS-HBVRx
- ALN-HBV
- Interferon-α
- LTβR
- ZFNs
- Pegylated-Lambda
- TLR-7 agonist
- RNA Interference Gene Silencer
- Translation
- Encapsidation priming RNA
- Assembly Effectors
- Nucleotide Analogues
- TAF
- AGX-1009
- Besitovir
- Lagociclovir valactate
- NAP(Rep 9AC)
- Nitazoxanide
- Hepatitis B virus life cycle components
- Entry inhibitor
- cccDNA Degradation
- HBsAg release inhibitor
- NTCP
- Small HBs
- Medium HBs
- Large HBs
- Core
- Recombinant DNA
- POL
- Pre-genomic RNA (3.5kb)
- DNA(-) to DNA(+)
- Receptor Ligand
- Lipid Envelope
- Partially dsDNA
- DNA Polymerase
- Inner protein core (HBcAg)
- Spheres & filaments containing HBsAg
New HBV Treatments

**Virology**
- New Nucleos(t)ide Analogues
- HBsAg release inhibitors
- Antisense oligonucleotides/si-RNA’s
- Entry inhibitors

**Immunology**
- PEG-IFN Lambda
- TLR agonists
- Therapeutic vaccination
- PD-1, PDL-1 Blocking
New HBV Treatments

**Virology**

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Tenofovir Alafenamide Fumarate (TAF)
Nucleotide Analogue Reverse Transcriptase Inhibitor (NRTIs)

- Improved stability in plasma:
  - Enhanced delivery of active form (TFV-DP) to hepatocytes
  - Lower doses are used; systemic exposures of TFV reduced

 CES1 = carboxylesterase 1; DP = di-phosphate; MP = mono-phosphate.

Agarwal K et al. AASLD 2013
Murakami E et al. HepDART 2013
**Tenofovir Alafenamide Fumarate (TAF)**
Nucleotide Analogue Reverse Transcriptase Inhibitor (NRTIs)

- "Super" Tenofovir
- Once daily oral single regimen use in HIV
- 25 mg TAF similar antiviral activity but reduces systemic exposure to < 8% of exposures generated by 300 mg tenofovir
- Probably less nefrotoxic than tenofovir
- Also beneficial for HBV+HIV co-infected

Agarwal et al. AASLD 2013
• 2 phase 3, randomized, double-blind studies
• Primary endpoint (non inferiority margin of 10%)
  – HBV DNA <29 IU/mL at Week 48
• Secondary endpoints
  – Bone mineral density
  – Renal parameters
Besifovir (LB80380)

- Acyclic Nucleotide Phosphonate
- Effective for Naïve and LAM resistant
- Multicenter phase II trial in Asia: Besifovir 90mg,150 mg for 48 weeks was found to be non-inferior to 0.5 mg Entecavir
- No resistant mutations or renal toxicity
- Besifovir group needed oral L-carnitine supplementation

Lai CL et al. Gut 2013
Scientists from National Institute of Biological Sciences (NIBS), Beijing discovered a functional receptor for Hepatitis B virus (HBV) infection

- It opens doors for future high throughput drug screen
- as well as revealed an important new target for treating HBV infection and related diseases

Myrcludex B: Acylated HBV preS1-derived peptides block HBV infection in vitro – entry inhibitor

Chemically synthesized lipopeptides derived from the envelope of HBV block virus infection in cell culture (HepaRG & PTH, PHH)

Gripen 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
MyrB blocks NTCP-mediated Bile Salt Transport & Inhibits HBV infection

- Normal function of NTCP is to import bile salts
- Whether HBV exploits this property for entry into hepatocytes?

Myristoylated preS1 peptide

Inhibits transport of taurocholate into hNTCP transfected HepG2 cells

Several bile salts inhibit binding of the preS1 peptide

Inhibit HBV infection of hNTCP transfected Huh7 cells


Taurocholate, taurodeoxycholate & taurochenodeoxycholate, in the presence of MyrB atto (500 µm), all substrates profoundly interfered with preS- binding
Myrcludex B prevents the establishment of de novo HBV infection in vivo

- Strongly limited HBV dissemination and ccc DNA accumulation in mice
- Phase 2 study currently conducted for HBV and HDV in Russia (Heptera)
- Results expected late 2014

RNA Interference

- HBV is susceptible to RNAi because it replicates via an RNA intermediate

- Small interfering RNA (siRNA) therapeutics have the capacity to knockdown production of all hepatitis B genes and thereby significantly decrease the number of infectious viral particles and viral antigens

- This reduction in viral and antigen load is designed to permit the immune system to mount an effective response to CHB

- Phase II study of ARC 520 (Arrowhead) ongoing

- Delivery is possible through different platforms, Nanoparticles (Tekmira)
RNA Interference

Antisense Oligonucleotides

• Investigational drug designed to target viral mRNA -> reduce production of viral protein (HBsAg) associated with HBV infection and replication

• Dose dependent reduction in liver and serum viral DNA and proteins in animal models of HBV infection

• Phase I randomized, placebo-controlled dose escalation study in healthy volunteers (ISIS)

• Enhances viral + immune system activity against HBV virus
What are the new Strategies for Finite HBV Treatment?

**Virus Targeting**

**Entry Inhibitors**
- Targets NTCP receptor to inhibit viral infection

**Assembly Effectors**
- Inhibits HBV replication by causing destabilization of viral nucleocapsid

**RNA Interference**
- RNA molecules inhibiting gene expression and release of new virions

**New Nucleos(t)ide Analogues**
- DNA polymerase inhibitor

**HBsAg Release Inhibitor**
- Inhibits the release of HBsAg SVPs and boosts restoration of the immune response

**cccDNA Degradation**
- Up-regulation of APOBEC3A and APOBEC3B causing cccDNA degradation
New HBV Treatments

Virology
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- HBsAg release inhibitors
- Antisense oligonucleotides/si-RNA’s
- Entry inhibitors

Immunology
- PEG-IFN Lambda
- TLR agonists
- Therapeutic vaccination
- PD-1, PDL-1 Blocking
Week 48

**Peg-IFN Lambda vs Alfa One Year of Therapy**

**HBeAg Seroconversion**

- **Baseline**
  - Peg-IFN Lambda 180 µg
    - *N = 80*
    - 14/80 (18%)

- **Week 48**
  - Peg-IFN Lambda 180 µg
    - 14/80 (18%)
  - Peg-IFN Alfa 180 µg
    - *N = 83*
    - 14/83 (17%)

- **Week 24 post-dosing**
  - Peg-IFN Lambda 180 µg
    - 11/80 (14%)
  - Peg-IFN Alfa 180 µg
    - 25/83 (30%)

*Chan HL, et al. EASL 2014*
Toll Like Receptor (TLR) 7 Agonist

- TLR-7 is a pattern recognition receptor in endolysosomol compartment of plasmacytoid dendritic cells (pDC) and B cells
- Agonism induces anti-viral response via innate immune activation

- GS-9620
  - Potent oral TLR-7 agonist tested in several animal models
  - Decline in HBVDNA and HBsAg during GS-9620 therapy in HBV-infected chimpanzees
  - Safe and well tolerated in 84 patients, significant dose dependant ISG-15 mRNA induction was observed in peripheral blood

Lanford R et al. Gastroenterology 2013; Gane E et al. AASLD 2013
Tarmogens are made from genetically modified yeast that express one or more disease-associated antigens.

- Activate T cells to specifically target and eliminate diseased cells with the same target antigen.
- Elicited an immune response to recombinant antigens and peptides in healthy volunteers (independent of host HLA alleles); well-tolerated.
- Further evaluation of GS-4774 in virally suppressed chronic HBV patients is ongoing.

Gaggar et al. AASLD 2013
Therapeutic Vaccination
TG1050

- Based on a non-replicative E1 and E3 deleted human adenovirus fusion protein serotype 5
- HBV Core (truncated) fused to a deleted and mutated HBV polymerase (full length) and 2 selected HBsAg domains (genotype D sequence)
- Capable of inducing a potent, multispecific, sustained and cross reactive T cell responses in mice
- First in human phase 1 trial in 2014

<table>
<thead>
<tr>
<th>Core t</th>
<th>Pol1</th>
<th>Env1</th>
<th>Pol2</th>
<th>Env2</th>
<th>Pol3*</th>
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<tr>
<td>148</td>
<td>(37 aa)</td>
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<td>(29 aa)</td>
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# Therapeutic vaccines for CHB

| **GS-4774** [Gilead] USA | **Preclinical**  
Tarmogen product candidate expressing HBV antigens (HBV X, S and Core antigens) for the treatment of chronic HBV infection.  
Commercialisation strategy will be to combine GI-13020 with Viread to determine if the combination can reduce or eliminate HBV infection. | • Vaccine is not delivering a polymerase antigen.  
• Vaccine is delivering the surface antigen known to be subject to strong immune tolerance.  
• X antigen is poorly immunogenic  
• Likely issue related to anti-vector neutralising immunity  
• The technology demonstrated low levels of immunogenicity in chronic viral infection in a HCV vaccine trial. |
| **TG-1050** Transgene, France | **Preclinical proof of concept**  
Recombinant non-replicative human adenovirus expressing multiple specific HBV antigens (Core, Envelope and Polymerase) from genotype D. | • Adenoviruses have a limited use due to pre-existing immunity  
• Viral vectors induce anti-vector immunity reducing vaccine immunogenicity upon repeat injection  
• Likely to be restricted in terms of genotype coverage |
Reversing the exhausted Phenotype of HBV-specific T cells

• Inability to eliminate virus in CHB has been attributed to high levels of expression of programmed death 1 (PD-1) and its ligand (PD-L1/B7-H1) on viral antigen-specific T-cells and APC’s

• Blocking the PD-1/PD-L1 interaction in vitro reversed exhausted cytokine production and proliferation of these HBV specific T cells
<table>
<thead>
<tr>
<th>Therapeutic Targets</th>
<th>Example</th>
<th>Mechanism of Action</th>
<th>Phase of Clinical Trial</th>
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<tbody>
<tr>
<td>New Nucleos(t)ide Analogues</td>
<td>Tenofovir Alafenamide</td>
<td>DNA polymerase inhibitor</td>
<td>III</td>
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<tr>
<td></td>
<td>AGX-1009</td>
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<td>II</td>
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<td></td>
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<td>Lagociclovir valactate</td>
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<td>Immune Modulators</td>
<td>Pegylated-Lambda</td>
<td>Cytokines with immunomodulating and antiviral effects</td>
<td>II</td>
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<td></td>
<td>TLR-7 agonist</td>
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<td>Targets NTCP receptor to inhibit viral infection</td>
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<td>Cyclosporins</td>
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<td></td>
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<td></td>
<td>NVR-1221</td>
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<td>HBsAg Release Inhibitor</td>
<td>REP 9AC</td>
<td>Inhibits the release of HBsAg SVPs and boosts restoration of the immune response</td>
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<td>RNA interference/ RNAi Gene silencer</td>
<td>ARC-520</td>
<td>RNA molecules inhibiting gene expression and release of new virions</td>
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<td></td>
<td>Antisense Oligonucleotide</td>
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<td>ISIS- HBVRX</td>
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<td>ALN-HBV</td>
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<td>Preclinical</td>
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<td>TKM-HBV</td>
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<td>NUC B 1000</td>
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<td>Preclinical</td>
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<td>Therapeutic Vaccines</td>
<td>Tarmogen</td>
<td>Induce and stimulate CD4 + and CD8 + T-cell response</td>
<td>III</td>
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<td></td>
<td>Transgene</td>
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<td>Lymphotixin-β receptor</td>
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<tr>
<td>Inhibitory T lymphocytes</td>
<td>PD-L1 Blockade</td>
<td>Blockade of the inhibitory signal and/or activation of costimulatory signal</td>
<td>Ila</td>
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HBV Curative Regimen?

**Antiviral**
- Agent to prevent viral spread, cccDNA re-amplification

**Immune activator**
- Agents to activate antiviral immunity or relieve repression of the system

**cccDNA inhibitor**
- Selective agent to deplete or perturb cccDNA

**HBV antigen inhibition**
- Agents to inhibit other components in the HBV life cycle [entry or cell-spread, capsid, HBX, HBsAg]
Conclusions

• Shift towards endpoint of true immune control and HBsAg seroconversion
• Combination of the most potent nucleos(t)ide: Peg-IFN add on therapy in different regimens
• HBV entry inhibitors, antisense therapy promising but early in development
• Direct ccc-DNA inhibition may be needed but is difficult to reach
• Peg-IFN lambda disappointing thus far
• Different immune modifying agents with hopefully limited systemic effects potentially to be combined with antiviral agents