Rationale for Different Virological Targets in HBV

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# Disclosure

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<th>Clear-B Therapeutics</th>
<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>
<th>AbbVie</th>
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<td>Consulting Fees (eg. Advisory Boards)</td>
<td>YES</td>
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<td>Contract Research (grant)</td>
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Outline of Presentation

1. Overview of Current Virological Targets

2. Possible New Directions

3. Opportunities for Integration into Existing and New Therapies

“Combine or Perish”
Current Efforts

1. Entry (Myrcludex B)

2. Viral Transcriptome (RNAi)

3. Capsid Assembly – Disassembly (CpAM)

Q. How many targets need to be blocked to achieve cure?
HBV Lifecycle Showing Novel Approaches for Viral Targets

HBV Entry into Hepatocytes

- Internalization via Clathrin-Mediated Endocytosis
- IMPORTANT CONCEPT EMERGING: PROTECT CELLS FROM MULTIPLE ROUNDS OF VIRAL RE-INFECTION

(Urban, S)

Entry & Re-Entry and HBV

- HBV WT and HBV DSL DNA
- HBV SPLICE VARIANTS
- HBV FL RNA
HBx RNA Was Detected Very Early After HBV Infection

*Mock infection. LHB, large HBV surface protein; d, day; h, hour; MHB, middle HBV surface protein; ORF, open reading frame; SHB, small HBV surface protein.

Beran, R et al 2017. EASL
HBV DNA levels decline during Myrcludex B treatment in 4/8 patients (consistent with HBV trial).

More pronounced decline of HBV DNA in the Myrcludex B/PEG-IFNα group (5/8 patients).

No significant changes (except patient 1027) in HBsAg levels.
HBV Genome and Proteome

- 3.2kb DNA virus
- *Hepadnaviridae, Orthohepadnavirus*
- 10 well described genotypes (A-J)
- rcDNA (+strand incomplete)
- 4 overlapping genes (P, S, X, C)
- HBV proteins (HBsAg, HBeAg, HBcAg, HBx, Pol)
- Multiple splice variants

**Molecular Variants Generated:**
- G → A hypermutation (RT)
- APOBEC-3
- Recombination
- Splice Variants

Viral Transcriptome: Mechanism of RNA Interference (RNAi)

Natural Process of RNAi

Therapeutic Gene Silencing

- dsRNA
  - dicer
  - cleavage
  - strand separation
  - RISC
  - complementary pairing
  - cleavage

- siRNAs
- mRNA degradation
- (A)n

- Synthetic siRNAs
- mRNA (A)n
- cleaved mRNA
Mean Decline in HBsAg by Cohort with HBV-ARO

Will this result in restoration of immune responses?

Gane, E et al 2018. AASLD Late Breaker [LB-25]
Phase 1b Clinical Trial: CpAM NVR 3-778 Reduces Serum HBV DNA and RNA

Efficacy shown in hepatocyte culture and chimeric mouse models of HBV.

Clinical studies:
Serum HBV DNA: mean 1.7 log reduction (600 mg BID)
Serum HBV RNA: mean 0.86 log reduction (600 mg BID)
New Understanding of HBV Life-Cycles and Potential Antiviral Opportunities

1. HBeAg-Pos vs HBeAg –Neg HBV
2. Chromatinisation (HBx)/Epigenetic Regulation Minichromosome (cccDNA)
3. HBV DNA Integration
4. RNA and RNA Splicing
5. Packaging pgRNA and Reverse Transcription (Protein Priming/Translocation)
6. Entry and Re-Entry (HBx)
HBV Replication: Pre ARC-520 Era (HBeAg-Positive HBV)

- cccDNA/minichromosome based replication
- “minimal” integration
HBsAg derived predominately from integrated HBV DNA
The HB Core Protein is a Component of the Minichromosome

- **Low Replication Phenotype**
  - Quiescent or active
  - Medium to Low or No Viraemia

- **High Replication Phenotype**
  - Transcriptionally Active
  - High Viraemia

- **HBcAg binds to CpG Island II**
  (Guo, YH et al 2011. Epigenetics;6:720)

- **cccDNA = 21 Topoisomers** [NOT a single entity]

Chromatinization mediated by Smc5/6 Complex:
- involved in DNA repair, chromosome topology and organization
- Fully chromatinized MC are transcriptionally SILENT

- Reflects Differences in Transcriptional Activities
HBx Promotes Degradation of the Smc5/6 Complex to Prevent Silencing of HBV cccDNA

HBx-minus or HBx variant HBV strains essentially transcriptionally SILENT

Addressing the HBV DNA Integration Issue

- Integration occurs early when infection is first established (Tu, T et al 2018. J Virol;92(11):e02007-17)
- integrated HBV DNA is an important source of HBsAg
- in the context of natural history, 1-2% of patients do lose HBsAg
- mechanism involved double-stranded linear (DSL) HBV DNA as major precursor
- role in clonal expansion of “resistant” hepatocytes (Mason, WS et al 2016. Gastroenterol;151(5):986-998)
- PCR Assay available for HBV DSL DNA in serum (Zhao, X-L et al 2016. GUT;65:502-511)
- can be successfully targeted with molecular based therapeutics
  - RNAi: ARO-HBV
HBV Splicing and Natural History of CHB

- Splicing of HBV is a common event during chronic infection, occurring in over 80% of patients
  - HBV splicing levels change dramatically over time, suggesting a highly dynamic process
  - Higher HBV viral load results in increased HBV splicing
  - Splicing increases each year prior to the development of HCC
    (Bayliss, J et al 2013. J Hepatol;59:1022)
  - Asian HBV genotypes (B and C) have significantly greater levels of HBV splicing than European genotypes (A and D)
- Up to 10% of the virion DNA populations contain spliced genomes
- splice mutants are replication defective due to intron removal generating truncated viral proteins (Sommer et al Virology, 1997;239:402)
- three neoproteins translated from spRNA: HBSP, HBDSP, P-S FP
- secreted splice RNA recently identified in patient serum (Espiritu..Lam, AASLD, 2016)

- Clinical, virological and pathological significance now emerging
Regulation of HBV Pre-S/S mRNA and HBsAg Production by Spliced Pre-S/S mRNA


Lessons Learned: HBV Splicing and OBI

- From a reactivation case of OBI, G458A mutation identified (affected the 5' splice site by disrupting RNA secondary structure/stem loop formation) which inhibited Pre-S2/S splicing, resulting in a marked decrease in unspliced Pre-S2/S transcript and HBsAg (Hass, M et al 2005. Hepatol;42:93)

- Candotti, D et al 2012. GUT;61:1744 reported on mutations in the 5' splice-site 458 vicinity:
  - 25/55 (45%) OBI_B vs 5/47 (11%) non-OBI_B
  - 14/33 (42%) OIB_C vs 5/48 (10%) non-OBI_C

- Amongst OBI Variants: 44% OIB_B and 36% OBI_C splice donor region mutations disrupted stem loop structure as described with the G458A mutation

- None of the (few) substitutions found in non-OBI variants predictive of affecting the RNA stem loop structure

IMPLICATIONS FOR HBsAg LOSS VIA Pre-S2/S 5' SS MUTATIONS
**Key Step 1: Conversion of RC DNA into cccDNA**

1. Removal of RT
2. Removal of r
3. Ligation of (-) DNA
4. Completion of (+) DNA
5. Removal of capped RNA
6. Ligation of (+) DNA

**Host Enzymes**

**Key Step 2: Reverse Transcription**

**CORE-CAPSID PROTEIN DEPENDENT**
Assessment of Antiviral Effect of SB9200 Compared to Other HBV Inhibitors

- Antiviral effect involves inhibition of HBV replication at the level of reverse transcription without affecting nucleocapsid assembly [novel MOA]
- Blocking priming or primer translocation within the capsid

Colledge, D et al. 2018 AASLD Poster # 383
IN ALL COHORTS: BASELINE HBsAg PREDICTS RESPONSE OF BOTH DNA AND RNA TO INARIGIVIR

HBV DNA

Log$_{10}$ Decline HBV DNA

Subjects with Baseline HBsAg <4 log

Subjects with Baseline HBsAg >4 log

p<0.001

Patients: HBsAg <4 log: 16 HBeAg –ve, 10 HBeAg +ve

HBV RNA

Log$_{10}$ Decline HBV RNA

Subjects with Baseline HBsAg <4 log

Subjects with Baseline HBsAg >4 log

p<0.007

Patients: HBsAg >4 log: 1 HBeAg –ve, 19 HBeAg +ve
HBsAg Targeting Strategies

- HBsAg clearance an **endpoint of therapy**
- Decline in HBsAg levels may **restore the antiviral activity of exhausted T/B/NK cells**
- **Several strategies** in evaluation
  - RNA interference (siRNA): « gene silencing »
  - Anti-HBs antibodies
Immune Regulation by HBsAg

- HBsAg secreted in vast excess over virions (>10^3 fold)
- Circulate in blood 100-400 µg/ml (1% of total serum protein)
- Unique conformational structure (8 cysteines ● and 8 prolines ○)
- Associated with increased risk of HCC (Yuen, MF. et al 2008. Gastro; 135:1192–1199)
- Plays a key role in HBV persistence
- Suppress both innate (TLR-2, TLR-9 and IFN-α) as well as adaptive (mDC) responses to infection

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

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

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

- The medium-term aim for the field is to achieve “functional cure”
  - HBsAg seroconversion off treatment

**How Many Targets to be Blocked to Achieve Functional Cure?**