Treatment of Hepatitis B: From Virology to Strategies for a Cure

T. Jake Liang, M.D.
Liver Diseases Branch, NIDDK, NIH
Why New Therapies?

**Ultimate goals of HBV treatment**
- Eradicate HBV
- Reverse liver damage
- Prevent cirrhosis and HCC

**Efficacy of existing treatment**
- Suppress but not eradicate HBV
- Low rate of HBsAg loss
- Partial reversal of inflammation and fibrosis
- Decrease but not eliminate risk of HCC
- Prolonged therapy, side effect & cost
HBV Life Cycle

- Viral Entry
- Receptor
- Uncoating
- Nuclear Import
- Vesicular Transport
- Budding
- Assembly
- ER
- Positive Strand Synthesis
- Removal of Pregenome
- Negative Strand Synthesis
- Encapsulation

Key:
- NTCP
- HBx
- cccDNA
- HBsAg
- 3.5 kb RNA
- 2.4/2.1 kb RNA
- Repair
- Transcription
- Translation
HBV cccDNA Inhibitor
**HBV cccDNA**

- Requires viral replication: recycling of rcDNA-containing nucleocapsid for amplification
- Conversion of rc to cccDNA mediated by host proteins (removal of pol by TDP2)
- Negatively regulated by LHBsAg
- ~10 copies/cell
- Minichromosomes: histones, nonhistone proteins and viral proteins (core, HBx)

*Levrero et al, J Hepatol 2009; Koniger et al, PNAS 2014*
HBV cccDNA

- Transcriptional activity regulated by hepatocyte-specific factors (HNF1, 3 & 4)
- Epigenetic regulation: histone (H3 and H4) acetylation (↑) and methylation (↓)
- IFN-α recruits histone deacetylase 1 and suppresses transcription
- Correlation of cccDNA with viremic levels; HBeAg+ > HBeAg- patients

Levrero et al, J Hepatol 2009
**HBV cccDNA**

- Long half-life, stable in quiescent cells
- Turnover controlled by
  - Cell death: immune cytopathic mechanism
  - Dilution by cell proliferation: liver regeneration
  - Cell cure: immune noncytopathic mechanism, IFNs & other cytokines
- Persistence of low-level cccDNA in hepatocytes, even in long-term treated patients

HBV cccDNA Inhibitor

Viral Entry → Vesicular Transport → Assembly → Budding → ER → Positive Strand Synthesis → Removal of Pregenome → Negative Strand Synthesis → Encapsidation

Nucleus
- Transcription
  - 3.5 kb RNA
  - 2.4/2.1 kb RNA
  - Translation

cccDNA
- Repair
- HBx
- NTCP
HBV cccDNA Inhibitor

- Topoisomerase inhibitor (Pommier et al, ACS Chem Biol 2013)
- Disubstituted sulfonamide (Cai et al, AAC 2012)
HBV cccDNA Inhibitor

Viral Entry
NTCP
Receptor
Uncoating
Nuclear Import
Assembly
Vesicular Transport
Budding
Glycosylation Myristoylation
ER
Positive Strand Synthesis
Remove of Pregenome
Negative Strand Synthesis
Encapsidation
Translation
HBsAg
2.4/2.1 kb RNA
3' 5' 3'
Transcription
3.5 kb RNA
5' HBx
Repair
Degradation
Translation
Interferons & cytokines
Effect of IFN-α on HBV Replication

Lucifora et al, Science 2014
Effect of LTβR Activation on HBV Replication

Lucifora et al, Science 2014
Induction of APOBEC by IFN-α and LTβR Activation

*Lucifora et al, Science 2014*
DNA Cytidine Deaminase

- Activation-induced deaminase (AID): antibody diversification
  - A3G & F on retroviruses (HIV)
  - A3A on paroviruses (AAV)
  - All A3’s on foreign DNA
  - Not active on self-DNA

Stenglein et al, Nat Struct Mol Biol 2010
Deamination of HBV cccDNA by IFN-α and LTβR Activation

Differential DNA Denaturation PCR

Lucifora et al, Science 2014
Degradation of cccDNA by IFN-α or LTβR activation

Lucifora et al, Science 2014
Immune Modulation of cccDNA

- Innate mechanism of cccDNA turn-over
- Induction of degradative pathway by immune modulators or other means
- Other biochemical mechanisms controlling this degradative pathway?
- Enhanced degradation by pharmacologic agents?
- Combination with potent nucleoside analogs
HBV Entry Inhibitor

Urban et al, Gastroenterol 2014
HBV Entry Inhibitor

- Myrcludex B is a HBV preS-derived lipopeptide that binds to NTCP
- Myrcludex B inhibits HBV receptor function of NTCP in vitro and in animal models
- Myrcludex B inhibits NTCP-mediated bile salt uptake
- Myrcludex B specifically targets the liver
- Myrcludex B showed good safety profile in phase 1a trial in humans
- In phase 1b trial, Myrcludex B inhibited HBV DNA levels and improved ALT

Meier et al, Hepatol 2013; Volz et al, J Hepatol 2013
HBV Transcription Inhibitor

Viral Entry → Vesicular Transport → Assembly → Budding → ER → Positive Strand Synthesis → Removal of Pregenome → Negative Strand Synthesis → Encapsidation

Nucleus

NTCP

Receptor → Uncoating → Nuclear Import → Transcription → Repair → cccDNA → Transcription → Translation → HBsAg

3.5 kb RNA → 2.4/2.1 kb RNA → Translation

HBx
SiRNA-mediated Inhibition of HBV

• DPC polymer composition and physical characteristics
  – amphipathic peptide
  – peptide amines reversibly “masked” with CDM
  – slightly negatively charged

• Liver-targeting by attachment of lipophilic ligand (e.g. cholesterol)

• Cellular uptake via ligand-driven (N-acetyl galactosamine) endocytosis

• Unmasking of peptide by ↓ pH in endosomes

• Disruption endosomal membrane by unmasked peptide

• Release of siRNA into cytoplasm
SiRNA-Mediated Inhibition of HBV

Wooddell et al, Mol THr 2013; Robert Lanford, unpublished data
HBV Encapsidation Inhibitor

Viral Entry → Receptor → Uncoating → Nuclear Import → Nucleus

Nucleus → Transcription → 3.5 kb RNA → Translation

cccDNA → HBx → Repair → Transcription

Positive Strand Synthesis → Glycosylation Myristoylation → HBsAg → Translation

Encapsidation

Removal of Pregenome → Negative Strand Synthesis

Assembly → Budding → ER → Vesicular Transport

NTCP

HBx
HBV Encapsidation Inhibitor

- Heteroaryldihydropyrimidines (HAP-1, BAY 41-109 series) [Bayer/AiCuris]
- Phenylpropenamide Derivatives (AT-61, AT-130) [Gilead]
- Sulfamoylbenzamide Derivatives (NVR-1221) [Novira]
- BCM-599 [2-amino-N-(2, 6-dichloropyridin-3-yl) acetamide family]
- Isothiafludine (pgRNA packaging)

HBV Encapsidation Inhibitor

**Heteroaryldihydropyrimidine (HAP)**

- HAP binds to core protein
- HAP inhibits nucleocapsid formation
- HAP destabilizes nucleocapsid formation
- HAP inhibits HBV replication in HBV transgenic mouse model with a similar efficacy as nucleoside analogs

*Deres et al, Science 2003; Stray et al, PNAS 2005*
HBx Inhibitor

• HBx-deficient HBV as attenuated virus in vivo
• Promiscuous transcriptional co-activator (episomal form)
• Interacts with the ubiquitin proteasome system
• Present on cccDNA mini-chromosome
• Specifically activates transcription from cccDNA via epigenetic modifications

HBV Assembly Inhibitor

Glucosidase inhibitor (Block et al, Nat Med 1998)
"HBV Cure"

Liang, Chang, Lok, et al, Hepatology 2015