Understanding mechanisms and effects of oligonucleotide-based drugs in chronic HBV infection

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Introduction

The understanding of mechanisms underlying HBsAg declines during antisense (ASO) of synthetic interfering RNA (siRNA) treatment is confounded by limitations in rodent *in vivo* models, the effect of different delivery strategies on the accumulation of oligonucleotides in parenchymal and non-parenchymal cells, immunostimulatory properties of oligonucleotides and how these properties are impacted by oligonucleotide modification. A global analysis of HBsAg response with these agents to date considering these variables is presented.

Results

Pharmacodynamic response to GalNAc-ASO and GalNAc-siRNA and dose saturation for ASO / siRNA effects are indistinguishable in humans. Single point mutations similarly abolish ASO and siRNA activity. As such, HBsAg response to ASOs informs on mechanisms of HBsAg response to siRNA and vice versa. HBV infection is genetically highly plastic (Figure 1) allowing rapid evolution of ASO and siRNA escape mutants. This feature of HBV infection is only well modeled in ducks and woodchucks (Table 1). Rebound of viremia during LNP-siRNA treatment of WHV-infected woodchucks demonstrated rapid evolution of escape mutants with multiple triggers (Figure 2). This was confirmed in NUC-suppressed chronic HBV with a combination of three LNP-siRNA triggers in S and X (ARB-1467) (Figure 3).

Transition of siRNA from LNP formulation to GalNAc conjugation drives substantial exposure of double stranded RNA (dsRNA) into non-parechymal cells (sinusoidal endothelial cells [LSECs] and Kupffer cells [KCs]), triggering stronger TLR3 activation (Figure 4 and 5). A highly conserved 90% decline in liver target proteins 15 days after the first dose of GalNAc-conjugated siRNA or GalNAc-conjugated ASOs is well established clinically for numerous liver targets but is absent with HBsAg for both siRNA and ASOs (Figure 6). HBsAg response to all GalNAc RNAi currently in development (AB-729, JNJ-3938, VIR-2218, RG 6346) is similarly hypervariable and universally delayed, consistent with delayed HBsAg response to dsRNA mediated TLR3 stimulation in mice. Universally rapid HBV RNA and HBcrAg response with GalNAc-siRNA is absent with LNP-siRNA (Figure 3 and 7) and is consistent with TLR3 mediated inactivation of cccDNA. HBsAg isoform response shows no selective decline in S-HBsAg (Figure 8) demonstrating that HBV subviral particles (and therefore mRNA degradation) are not impacted by siRNA.

Bepirovirsen is an unconjugated ASO containing a class II CpG motif (TLR9 stimulatory, Figure 9) with 70% of liver dose accumulating in non-parenchymal (and more highly immunoreactive) liver cells (sinusoidal endothelial cells and Kupffer cells, Figure 4). Immune activation consistent with TLR9 stimulation occurs with bepirovirsen but a strong HBsAg decline is restricted to patients with baseline HBsAg < 1000 IU/mL. These effects are absent when this ASO is shifted to hepatocyte accumulation by GalNAc conjugation (GSK 3389404).

Conclusions

- ***In vivo* and clinical data indicate HBsAg response to siRNA or ASOs is driven by stimulation of innate immunity.**
- **Saturated HBsAg decline with siRNA reflects persistence of SVP derived from integrated HBV DNA (Figure 10).**
- **The immunoreactive properties of ASOs and siRNA must be considered in the evaluation of these agents.**

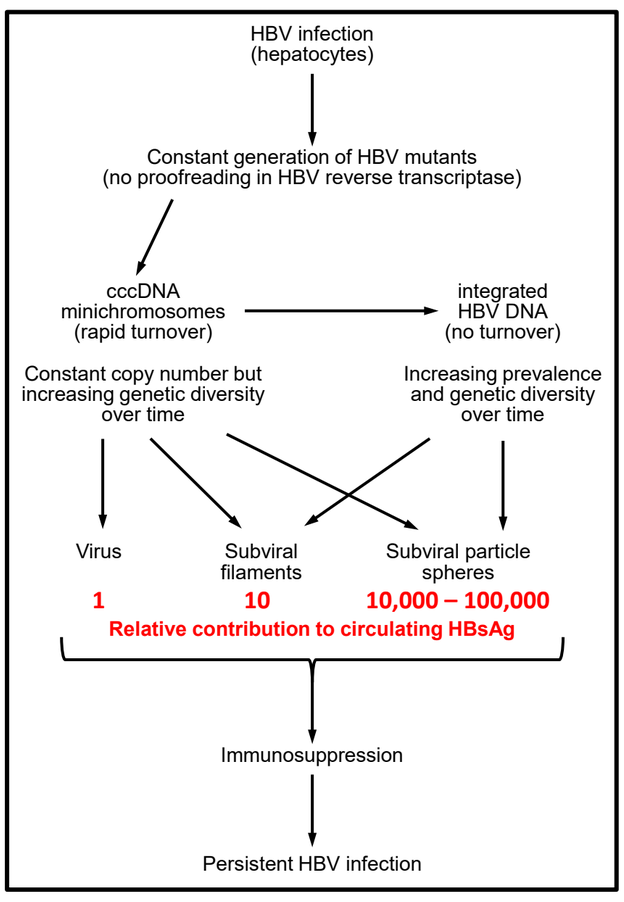

Figure 1. Genetic plasticity of chronic HBV infection

Table 1. Limitations of animal models in assessing oligonucleotide-based drugs for HBV

Model	SVP production	Genetic diversity (pre-existing ASO/siRNA escape mutants)	Rapid turnover of cccDNA (evolution of ASO/RNAi escape mutants)	TLR9 activity (CpG DNA)	TLR3 reactivity (dsRNA / RNAi)
Human	LDL-based (SVP are spherical)	Present	Present	Present (KCs)	Present (LSECs, KCs)
Transgenic mice	LDL metabolism opposite to humans (SVP are octahedral)	None	Present but turnover unknown	Yes but human and rodent CpG sequences differ	Stronger vs primate
AAV / HDI-mice	LDL metabolism opposite to humans (SVP are octahedral)	None	Present but turnover unknown	Yes but human and rodent CpG sequences differ	Stronger vs primate
Scid-Hu mice	SVP production is attenuated (altered lipid metabolism)	Yes (limited due to short term infection)	Yes	Yes but human and rodent CpG sequences differ	Stronger vs primate
Ducks	LDL metabolism similar to humans	Yes (limited due to short term infection)	Yes	Yes but via altered reactivity by TLR15	Similar to primate
Woodchucks	LDL metabolism opposite to humans	Yes (chronic infection)	Yes	Yes but human and rodent CpG sequences differ	Similar to primate

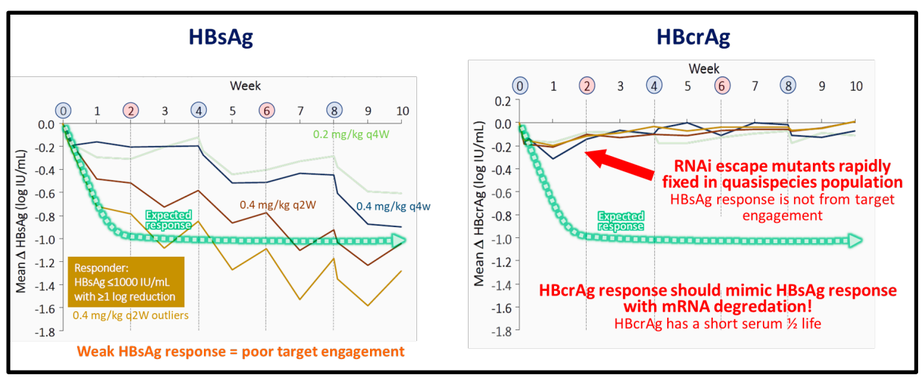

Figure 3. Rebound of viremia in NUC suppressed chronic HBV with TKM-HBV (ARB-1467) (AASLD 2017).

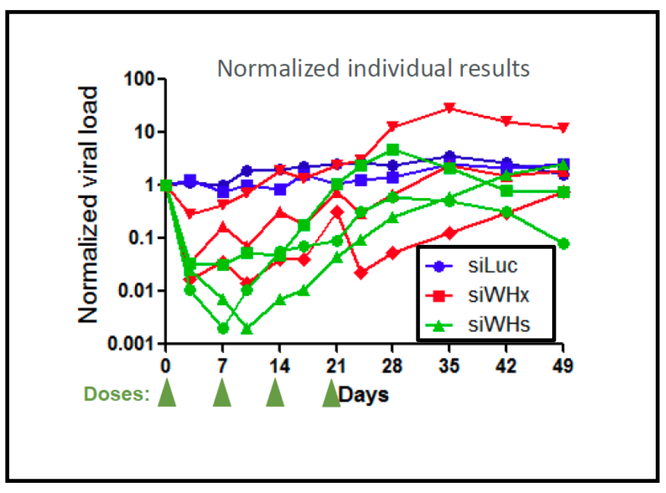

Figure 2. Rebound of viremia during LNP-siRNA treatment of WHBV infected woodchucks. (Tekmira, DIA 2015)

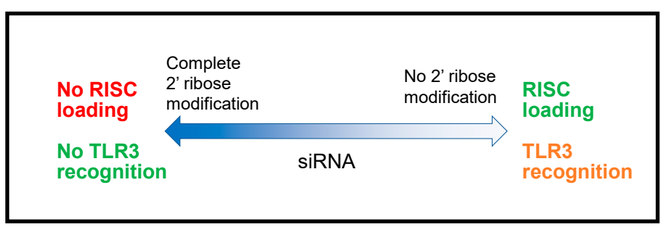

Figure 4. TLR3 stimulation by siRNA is sequence independent. Oligonucleotide modification reducing TLR reactivity also blocks RISC loading and RNAi effect.

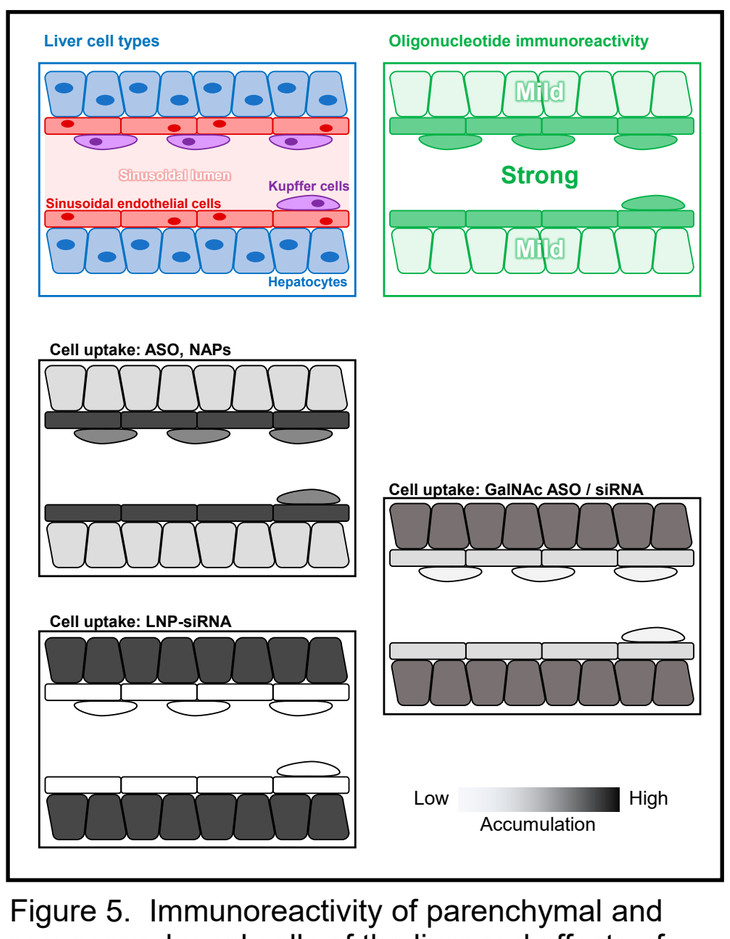

Figure 5. Immunoreactivity of parenchymal and non-parenchymal cells of the liver and effects of various delivery strategies on accumulation of oligonucleotides in different cell populations.

Figure 7. Virologic response to siRNA (JNJ-3839) showing rapid inactivation of cccDNA (with no response to HBsAg).

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Response to this GalNAc-siRNA stands in stark contrast to LNP-siRNA (TKM-HBV) where, despite more efficient hepatocyte targeting and three siRNA triggers, rapid HBcrAg and HBV RNA rebound were observed.

Figure 8. HBsAg isoform response to Gal-NAc siRNA (AB-729). No selective S-HBsAg isoform declines consistent with targeting of SVP were observed. Selective declines in M- and L-HBsAg suggest inactivation of cccDNA and or enhanced autophagy (EASL 2021).

Selective S-HBsAg declines are observed with NAPs in clinical studies using identical assay platforms.

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Figure 6. Summary of HBsAg responses to oligonucleotide-based therapies observed in clinical studies.

Figure 9. The curious case of bepirovirsen.

This fully phosphorothioated oligonucleotide is blocked from TLR7/8 stimulation by 2'MOE modification at the ends and contains a TLR9 stimulatory motif within its unprotected DNA core.

Accumulation of this oligonucleotide in non-parenchymal cells (bepirovirsen) results in strong HBsAg declines with host mediated transaminase flares restricted to patients with low baseline HBsAg.

These effects are absent when this oligonucleotide is targeted to hepatocytes via GalNAc conjugation (GSK 3389404).

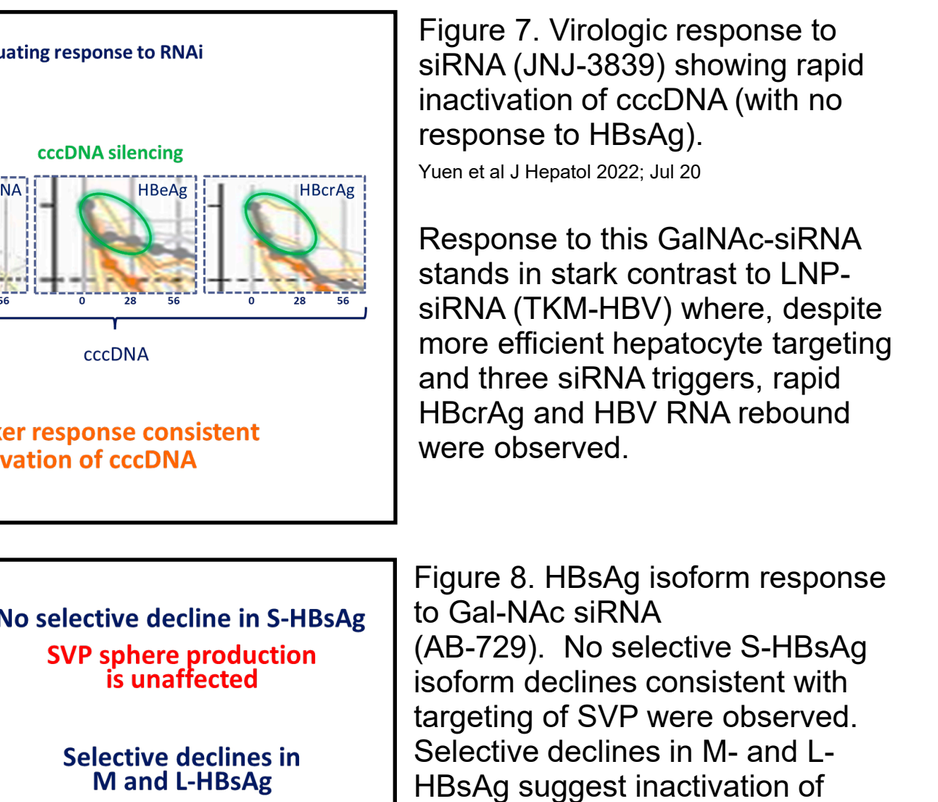

Figure 10. Impact of oligonucleotide-based therapies on circulating HBsAg