Andrew Vaillant

Replicor Inc.

6100 Royalmount Avenue

Montreal, Quebec Canada H4P 2R2

+1 514 733-1998

availlant@replicor.com

Word count: 824

Figures: 0

Tables: 0

AV is an employee and shareholder of Replicor Inc.

This manuscript was conceived and written by AV.

The preparation of this manuscript was supported by Replicor Inc. Replicor Inc had no input into its preparation.

Data availability: not applicable.
Yuen et al.[1] suggest that HBsAg reductions observed with the GalNAc conjugated antisense oligonucleotide (ASO) GSK 3389404 reflect ASO-mediated HBV mRNA degradation. This interpretation is inconsistent with in vivo and clinical data accumulated over 15 years establishing the highly conserved pharmacodynamic protein response signatures and sensitivity to single point mutations shared by GalNAc-ASOs and GalNAc-siRNA.

Individual HBV infection is comprised of thousands of viral quasispecies (the HBV RT has no proofreading functionality) which can fluctuate rapidly with rapid cccDNA turnover[2]. Both ASO and siRNA functionality is abolished by single nucleotide mismatch in the mRNA target sequence[3] so the persistence of any ASO or siRNA effect in HBV infection is extremely unlikely. This was verified with the rapid rebounds in viremia observed with siRNA triggers in HBsAg and HBx in WHV infected woodchucks[4] and with simultaneous treatment of three siRNA triggers (in HBsAg and HBx) in humans[5] with high efficiency (LNP-mediated) delivery to hepatocytes. These pivotal data demonstrate that ASO and siRNA effects in HBV are driven by off-target effects.

Degradation of mRNA with either GalNAc conjugated ASOs or siRNA achieves a highly uniform 90% reduction in liver target protein 15 days following the first dose, an effect saturated at 40mg qW[6, 7]. The rapid turnover of HBsAg [8] and the absence of HBsAg decline at doses saturating for ASO effects with GalNAc conjugation (30mg) and the absence of an ASO-diagnostic HBsAg response at all doses of GSK3389404 (up to 120mg) indicate rapid emergence of ASO/siRNA escape mutants, consistent with virologic rebound during ARB-1467 therapy in humans[5]. HBsAg responses diagnostic for an ASO/siRNA effect are also absent with the ASO RO7062931 / RG 6004 and the GalNAc conjugated siRNAs JNJ-73763989 (ARO-HBV), AB-729, VIR-2218 (ALN-HBV02) and RG6346 (DCR-HBVS).

Bepirovirsen and GSK3389404 share an identically modified oligonucleotide sequence containing a class II CpG motif (5-GCAGAGGTGAGCGAAGTCG-3’). This CpG motif is not shielded by 2’ribose modification, allowing stimulation of the innate immune response via activation of TLR9. Liver accumulation of this sequence is ~70% in liver sinusoidal epithelial cells (LSECs) and Kupffer cells (KC)
with the unconjugated bepirovirsen[9] and ~70% in hepatocytes with the GalNAc-conjugated GSK3389404[10]. HBsAg responses with bepirovirsen in treatment naïve patients were absent in 4/6 patients at doses (150mg) which normally elicit uniform ASO effect with unconjugated ASOs[11]. At high dose (300mg), multilog IU/mL HBsAg decline from baseline was restricted to treatment naïve patients with low (~ 1000 IU/mL) baseline HBsAg[12] and in patients with baseline HBsAg > 1000 IU/mL were gradual and < 1log_{10} IU/mL from baseline after 4 weeks of therapy (again inconsistent with the ASO effect). Even though GalNAc conjugation of ASOs improves their potency 10-fold[10], The HBsAg reductions with GSK3389404 were much weaker than with bepirovirsen [1]. These HBsAg response patterns with both bepirovirsen and GSK3389404 exclude mRNA degradation as they do not confirm to the well-established protein response patterns for all ASO or siRNA (described above); HBsAg responses are absent at doses normally producing protein response with all other ASO / siRNA and do not confirm to the conserved pattern of universal 1 log_{10} protein decline within 15 weeks with ASO and siRNA. These HBsAg response patterns are however entirely consistent with TLR9-mediated stimulation of innate immunity, they are stronger with accumulation of this oligonucleotide in non-parenchymal cells in the liver, are correlated with TLR-mediated immunoreactivity[13] and much stronger with reduced HBsAg load at the start of therapy.

Recent data has now shown that HBsAg response to bepirovirsen is correlated with the immunostimulatory properties of this oligonucleotide and it has been suggested that bepirovirsen acts via stimulating TLR8[13]. While the immunostimulatory properties of bepirovirsen are expected via TLR stimulation, assigning TLR8 as the reactive host sensor is inconsistent with the chemical modifications and sequence present in this oligonucleotide. TLR8 recognizes unmodified RNA which in bepirovirsen is efficiently blocked by 2’methoxyethyl ribose modification and an exposed class II CpG motif can stimulate TLR9. This disconnect between the possible TLR-reactivity of bepirovirsen and the assignment of TLR8 may be due to the use of mouse systems, which do not accurately model TLR reactivity in humans and the different CpG motif reactivity in mice and humans.
HBsAg response to bepirovirsen and GSK3389404 is consistent with the TLR reactive properties of their shared oligonucleotide sequence and distribution in immunoreactive cells in the liver: bepirovirsen accumulates primarily in LSEC and KCs and drives mild HBsAg reduction via TLR stimulation in all patients but much stronger HBsAg reduction when low levels of circulating HBsAg are present (a condition where reduced inhibition of innate signalling mechanisms is occurring). GSK 3398404 results in substantially lower accumulation in LSECs and KCs resulting in much weaker TLR-mediated HBsAg response, even in patients with lower baseline HBsAg.

Correct clinical assessment of HBsAg responses to ASO and siRNA must consider their well-established biochemistry and pharmacology, including the impact of different delivery strategies on accumulation in different liver cell populations and the ability of oligonucleotides to stimulate innate immunity via TLR recognition.

References


