Editorial

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Antisense oligonucleotides (ASOs) in chronic hepatitis B infection: Opportunities and challenging the orthodoxy

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Chronic hepatitis B (CHB) is a global health problem with an estimated 296 million people affected worldwide.[1] Nucleos(t)ide analogues (NAs) are currently the mainstay of treatment, suppressing viral replication, improving hepatic fibrosis, and reducing the risk of oncogenesis.[2] However, these agents have no direct effect on covalently closed circular DNA (cccDNA) and so life-long therapy is usually required. This leads to high treatment costs, possibility of non-adherence or virological breakthrough, and cumulative toxicity. Moreover, a residual risk of hepatocellular carcinoma (HCC) remains.[3]

Thus, finite duration therapies that can achieve durable off-treatment clearance of circulating HBsAg and HBV DNA, so-called functional cure, remain an unmet need. To achieve these aims, it is likely that a combination of therapies will be required that inhibit viral replication, reduce antigenic burden, and restore HBV-specific immune control. A plethora of new antiviral and immuno-modulatory agents have entered clinical development, but no clear roadmap to functional cure has been established, and the optimal combination of treatments remains uncertain. Whilst this is an exciting arena, the complexities of HBV and pace of drug development have generated more questions than answers.

Translation inhibitors, such as small-interfering RNA (siRNA) and antisense oligonucleotides (ASOs), have the potential to treat a wide range of inherited and infectious diseases by silencing the production of proteins and enzymes at the transcriptional level. Licensed treatments now exist for several disease conditions, including hypercholesterolaemia (inclisiran),[4] transthyretin-mediated amyloidosis (vutrisiran),[5] and primary hyperoxaluria type 1 (lumasiran).[6] Chronic HBV infection is an ideal target for translational inhibitors for 2 reasons. Firstly, the organisation of the compact HBV genome with overlapping reading frames enables a single targeted sequence (trigger) to silence multiple transcripts, potentially blocking the production of the core, polymerase, surface, and X protein simultaneously. Secondly, the reduced expression of tolerogenic antigens (HBsAg, HBeAg) should indirectly restore HBV-specific immunity.

ASOs are single-stranded nucleic acid polymers, typically 16-30 nucleotides in length, that cause RNaseH-mediated degradation of mRNA transcripts or steric hindrance of spliceosomes.[7] Unlike siRNAs, naked ASOs can freely enter cells and the high doses needed to attain sufficient hepatocyte concentrations may result in extrahepatic toxicities such as thrombocytopenia and kidney injury. In order to reduce systemic exposure, ASOs can be conjugated with N-acetylgalactosamine (GalNAc), thereby enhancing hepatocyte uptake via the asialoglycoprotein receptor (ASGPR). This is a high-capacity system (~500,000 copies per cell) with rapid receptor turnover (~15 min is required for recycling to the plasma membrane).[8] GalNAc conjugation has been shown to increase hepatocyte uptake of ASO molecules by more than 10-fold compared to the naked molecule.[9] It is now the preferred hepatocyte-targeted delivery system for many therapeutics, facilitating subcutaneous administration and longer dosing intervals. To date, 4 different ASOs have entered clinical trials for the treatment of CHB infection: **GSK3389404** (GSK), **GSK3228836/Bepirovirsen** (GSK), **RO7062931** (Roche) and **ALG-020572** (Aligos).

In this edition of the *Journal of Hepatology*, Yuen *et al.* assessed the safety, tolerability, and antiviral activity of GSK3389404 in patients with CHB on stable NA therapy.[10] GSK3389404 is a GalNAc-conjugated version of the prodrug GSK3228836/bepirovirsen and contains three GalNAc moieties covalently linked to the oligonucleotide backbone. In this phase IIa double-blinded, placebo-controlled study, patients (n = 66) were randomised in a 11:2 ratio to receive GSK3389404 (30, 60, 120 mg weekly or 120 mg bi-weekly) or placebo for a 12-week treatment period. The study was conducted across 22 sites in the Asia Pacific region, and the mean HBsAg titre at baseline was 2.94 $\log_{10}$ IU/ml. A dose-dependent reduction in HBsAg was noted and the magnitude of change was similar in HBeAg-positive and HBeAg-negative individuals. Only modest reductions in HBsAg levels were observed – three patients achieved ≥1.5 $\log_{10}$ IU/ml reductions from baseline and no patients lost HBsAg.[10] Reductions in HBsAg levels were transient and rebounded to baseline within 2 weeks.

Conversely, bepirovirsen (the "naked" unconjugated ASO GSK3228836) achieves more profound suppression of HBsAg titres, compared with the GalNAc-conjugated GSK3389404. In a phase IIa study (NCT02981602), the administration of 6 doses of 300 mg bepirovirsen over a 4-week period resulted in a mean reduction of 1.99 log IU/ml from baseline in NA-treated individuals (n = 5) at day 29.[11] Similar findings have been shown in

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the interim results of the phase IIb study (B-CLEAR, NCT04449029). Four hundred and fifty-seven patients with CHB infection (n = 227 stable on NA treatment, n = 230 not currently on NA treatment) were randomised in 3:3:3:1 ratio to receive different regimens of bepirovirsen (150 mg or 300 mg) or placebo for 12 or 24 weeks. In the group of NA-suppressed patients (n = 68) who received weekly injections of bepirovirsen 300 mg for 24 weeks, 28% achieved HBsAg and HBV DNA below the lower limit of quantification at the end of treatment, and more than 60% achieved HBsAg titres <100 IU/ml.[12] Similar results were observed in the group of patients not suppressed on NAs.[13] Off-treatment follow-up will be needed to determine whether functional cure can be achieved with bepirovirsen. Two other GalNAc-conjugated ASOs have been explored in CHB infection, namely RO7062931 (Roche) and ALG-020572 (Aligos). RO7062931 achieved modest reductions in HBsAg levels at the highest doses (0.50 $\log_{10}$ IU/ml), similar to GSK3389404.[14] ALG-020572 development was discontinued prematurely due to multiple cases of severe alanine aminotransferase flares in the phase I study.[15]

The reasons for the superior effect of the unconjugated bepirovirsen over GSK3389404 is unclear. It has been proposed that the difference in efficacy may be due to the administration of higher doses of bepirovirsen. However, conjugation with GalNAc is proposed to significantly increase uptake of the oligonucleotide into hepatocytes by binding to ASGPR.[8] Given the dose-dependent reductions in HBsAg levels achieved by GSK3389404 and RO7062931, it is unlikely that saturation of the ASGPR was the primary cause of the limited treatment response of these compounds.[10,14]

The superior efficacy of bepirovirsen may (partly) be explained by its immunostimulatory effects on the non-parenchymal cells within the liver. In a mouse model, Prakash *et al.* (2014) showed that unconjugated ASOs are predominantly taken up by the non-parenchymal population within the liver (>70%), whereas GalNAc-conjugated ASOs concentrate in the parenchymal tissue (>80%).[16] Oligonucleotide drugs have the potential to stimulate immune reactions via pattern recognition receptors. For example, Toll-like receptor (TLR)7 and TLR8 recognise single-stranded RNA motifs, whilst TLR3 and RIG-I recognises double-stranded RNA structures.[17] A *post hoc* analysis of the NCT02981602 study found that bepirovirsen activates the TLR8 receptor in humans, which was confirmed in transfection studies and transgenic mice. Within 6-24 hours of bepirovirsen dosing, there was noticeable induction of IL12B, IFNγ and IL-1Ra. Principal component analysis of longitudinal serum analysis showed that the elevation of these TLR8 signature cytokines was correlated with a reduction in HBsAg. A similar cytokine response was much weaker with GSK3389404, indicating specificity of TLR8 activation for the non-GalNAc compound.[18]

Bepirovirsen enters phase III clinical studies in 2023 and GSK have terminated further development of GSK3389404 because of inferior efficacy. However, many questions remain unanswered about the mechanism of action of bepirovirsen, and that of other ASOs. The potential immunostimulatory properties of bepirovirsen are intriguing, however, and raise the possibility of synergism when combined with therapeutic vaccines, checkpoint inhibitors and/or TLR agonists. Studies exploring the effect of these drugs on the intra-hepatic immune milieu will be important and the B-Fine study, in which patients receiving bepirovirsen undergo longitudinal fine needle aspirations, may be informative. There should also be greater efforts to directly measure on-treatment intrahepatic viral transcriptional activity, perhaps using *in situ* hybridisation technologies. Clearly we will need to improve our understanding of the mechanism of action of new therapeutic agents, and the potential for synergistic or inhibitory effects when prescribed in combination, if we are to develop therapies that can regularly achieve functional CHB cure.

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