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Spotlight

Making safe sense of an anti-sense!

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Bepiroversen has been developed and trialed for the cure of HBV. Yuen et al.[1] report on the safety and antiviral efficacy of this agent. We "spotlight" key findings of this study and its impact for future clinical trial design.

Several novel agents have entered clinical trials for the functional cure of chronic hepatitis B (CHB), which target steps of hepatitis B virus (HBV) replication and/or restoring immune responses.[2] Antisense oligonucleotides (ASOs) are one of the agents being tested in the HBV-cure program. ASOs inhibit the replication of several viruses, with targeted delivery to hepatocytes by receptor binding and internalization via receptor-mediated endocytosis. Pre-clinical models using HBV transgenic and hydrodynamic transfection mice along with cell culture models have tested ASOs *in vivo* and *in vitro*, where on-treatment reductions in HBsAg were noted but rebounded post ASO termination. ASO treatment with nucleos(t)ide analogs (NAs) showed superior HBsAg decline compared with NAs alone, thus in a pre-clinical setting, demonstrable virological responses were noted leading to this technology being utilized in HBV-infected patients.[3]

In the October issue of *Nature Medicine*, Yuen et al. assessed the safety, tolerability, and antiviral activity of the ASO, bepirovirsen, in a phase 2 randomized double-blinded, placebo-controlled, dose escalation trial.[1] The binding site of bepirovirsen is present in all HBV mRNA, and is thus anticipated to attenuate all HBV mRNA's including pre-genomic RNA.[3] This study included treatment-naive and experienced, non-cirrhotic patients, where 2 subcutaneous injection doses of bepirovirsen, 150 mg and 300 mg, were tested. Treatment-naive patients were randomized to placebo (n = 6), or bepirovirsen 150 mg (n = 6) or 300 mg (n = 12) with NA-experienced patients randomized to placebo (n = 3) or bepirovirsen 300 mg (n = 5). Treatment-naive patients were a mixed cohort of HBeAg positive (n = 13) and negative (n = 11), whereas all on-NA patients were HBeAg negative. Bepirovirsen was administered twice weekly for the first 2 weeks, then weekly during week 3 and 4, resulting in patients receiving 6 injections and followed for 26 weeks. Here, the overall study population was small, with further limitations of patient groups once these are divided into subgroups; despite this, dose-dependent reductions in HBsAg and HBV DNA levels were seen in the active ASO groups compared with placebo ($p = 0.001$). Higher-dose bepirovirsen (300 mg) was required to achieve statistically significant reductions in HBsAg, especially in the treatment-naive group. The absolute reduction of HBsAg levels in the on-NA group was seen at the higher dose, but this was not statistically significant. Transient HBsAg loss was noted in 4 patients (13%) during high-dose bepirovirsen administration, with demonstration of its return following therapy cessation. Reductions in HBsAg, however, were detected in both HBeAg positive and negative groups, thus the target sequence for this ASO may be present even if HBsAg is derived from integrated HBV DNA/genome, thus having the potential for limiting HCC risk.[4] Interestingly, there was an observed transient appearance of anti-HBs antibody in patients with HBsAg loss, consistent with the potential synthesis of antigen-antibody complex formations; the reduction in HBsAg may lead to free HBs-antibody presence, but this requires further work to decipher the mechanisms underlying this process.[4] Overall sustained reductions in HBV DNA levels (<20 IU/mL) were noted in all participants as NA-treatment was administered following bepirovirsen therapy. Interestingly, overall absolute reductions in HBV DNA and HBsAg along with HBcrAg and HBV RNA were greater in HBeAg negative patients, suggesting higher baseline antigenic load may be a poor prognostic indicator, thus, reducing HBV DNA loads may be an important pre-requisite for HBV cure. It is not certain whether this translates into improved immune responses and other host factors, such as patient age and timing of treatment initiation, require due consideration.[5]

Biochemical activity was assessed with ALT flares noted in approximately half of the patients. Notably, flares were transient and temporally linked to HBsAg reductions and not evident in those on placebo or without HBsAg decline. HBsAg decline either preceded or coincided with the ALT flare, and it is well reported that biochemical activity may be important in generating an immune response leading to HBV clearance.[6] The timing of ALT flares is, of course, critical; intriguingly, with bepirovirsen, it may be that this reduces HBsAg first, and the subsequent (or concomitant) increase in ALT is because of an immune response secondary to infected hepatocyte clearance. A mild-moderate ALT flare may augment immune responses for HBV clearance, evident both in studies of the natural history of HBV and flares prior to commencing therapy and following treatment discontinuation.[7] The key question

is determining the timing and reasons for the ALT flare, whether it is directly due to ASO therapy and/or host-related factors, and this will require important mechanistic studies, which are currently underway with bepirovirsen, investigating peripheral and hepatic compartmentalized viral and immune responses with the B-Fine study.

Central to this study, the safety and tolerability of bepirovirsen was evaluated, demonstrating an acceptable profile, with the main adverse events (AEs) being fever and injection site reactions recorded as grade 1 and not leading to dose interruptions. The validation of limited AEs is required in a larger study; these are undergoing with the B-Clear clinical trial.

Bepirovirsen is potentially a novel agent added to the arsenal for HBV-cure. It is, however, difficult to determine its true efficacy given this small study size, thus the results observed require further investigation. Indeed, the rebound of HBsAg following ASO cessation warrants increased therapy duration, but at the expense of remaining finite, one injection monthly and longer-term may be acceptable. Response rates of current therapies also need to be considered; in certain cases, pegylated interferon alpha (Peg-IFNα) remains efficacious, which may be augmented with NAs in the correct populations.[8] Moreover, the study of immune responses in sequential and add-on therapies may still provide further information about future clinical trial design.[9] The B-Together clinical trial will assess the sequential use of Peg-IFNα with bepirovirsen where HBsAg loss may be greater. The importance of studying on-treatment viral and immune responses will provide key data on why agents are efficacious or, on the contrary when they are not and what adaptations are required. The B-Fine study with bepirovirsen will provide such data where participants are undergoing longitudinal fine needle aspiration of the liver to detect changes in intrahepatic responses along with those in the periphery, which we have shown are different[10] and thus require study in tandem.

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