Potent and sustainable HBsAg clearance and HBV DNA negative by a liver-targeting PD-L1 siRNA in mice

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Introduction

- Chronic hepatitis B (CHB) is a global public health burden affecting approximately 257.5 million people worldwide [1]. Although tremendous progress has be made for CHB drug development, the low functional cure rate and the high ratio of viral rebound after the discontinuation of medication are still two major challenges need to be addressed.
- PD-1/PD-L1 checkpoint antibodies has been proven to be effective in CHB clinical studies [2]. CHB patients with low HBsAg baseline showed HBsAg loss and seroconversion, suggesting that immunomodulatory treatments can help CHB functional cure in human.
- SA012, a N-acetylgalactosamine (GalNAc)-conjugated siRNA which specifically targets the mRNA of PD-L1 gene in hepatocytes, has been developed for the treatment of CHB.

Aim

We want to explore the efficacy and safety profiles of SA012 for the functional cure of CHB. Here we report the efficacy results for SA012 in mono- and combo-therapies in mice, and safety profiles in rats and monkeys.

Method

hPD-1/PD-L1 AAV-HBV mice have been used in the efficacy evaluation studies below.

- SA012 alone was injected subcutaneously at 7.5 mg/kg QW for 8 doses (monotherapy study).
- VIR-2218 (siRNA) 1 mg/kg Q4W was injected subcutaneously for 2 doses and SA012 was injected at 7.5 mg/kg QW for 8 doses. Bepirovirsen (ASO) 10 mg/kg QW with loading dose (Day0, 4, 7) was injected subcutaneously for 6 doses and SA012 was injected at 7.5 mg/kg QW for 5 doses (combination studies).
- Serum HBsAg, HBV DNA and/or HBsAb were detected at different time points during each study. The change of liver immune cells after SA012 treatments were also studied.

In the Tox studies of SA012, biochemistry, routine blood test and histopathology were assessed in rat and cynomolgus monkeys at 100 mg/kg dosage, Q2W for 3 doses.

Results

SA012 reduced HBsAg and HBV DNA in hPD-1/PD-L1 mice (monotherapy study)

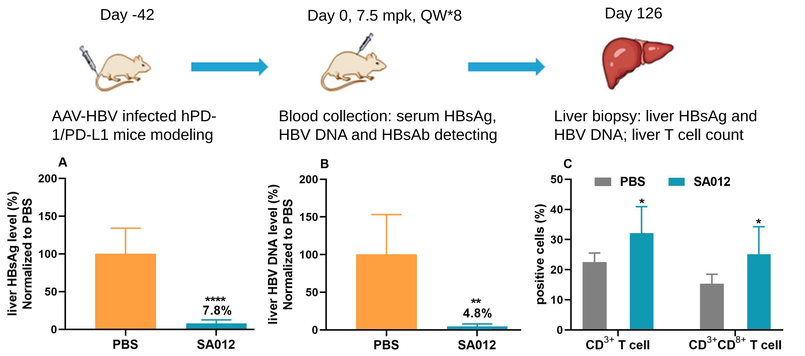
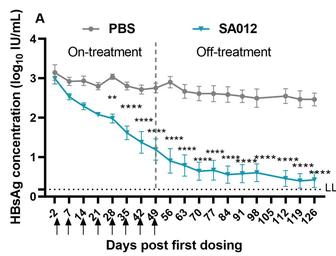
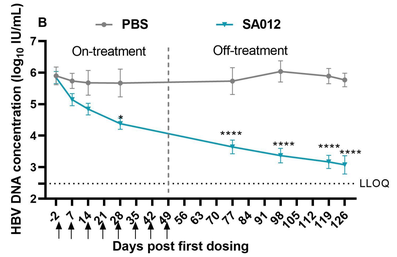


Figure 1. **Liver HBsAg, HBV DNA and T cell change in AAV-HBV infected hPD-1/PD-L1 mice at Day 126**

A: Liver HBsAg level change. B: Liver HBV DNA level change. C: Liver CD^{3+} T cell and $CD^{8+}CD^{3+}$ T cell change.

- Liver HBsAg and HBV DNA levels were dramatically reduced after SA012 treatment (*Fig 1A* and *Fig 1B*). The number and percentage of CD^{3+} and $CD^{3+}CD^{8+}$ T cells increased significantly in liver, which indicates the restoration of acquired immunity towards HBV infection.





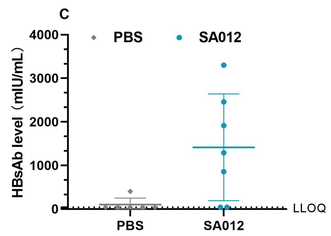
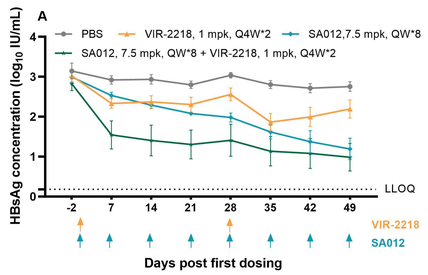


Figure 2. **Serum HBsAg, HBV DNA and HBsAb change in AAV-HBV infected hPD-1/PD-L1 mice**

A: Serum HBsAg change after treatment; B: Serum HBV DNA change after treatment; C: Serum HBsAb change at the end of study (Day 126).

- Serum HBsAg and HBV DNA were significantly reduced after SA012 treatment. 5/7 and 4/7 mice achieved the low limit of quantification (LLOQ) for HBsAg and HBV DNA, respectively. No obvious viral rebound was observed 11 weeks after SA012 discontinuation. (*Fig 2A* and *Fig 2B*)
- SA012 induced high proportion and high levels of HBsAb (seroconversion) after treatment. At the end of the experiments, 5/7 mice reached seroconversion. (*Fig 2C*).

SA012 had synergistic effect of HBsAg when combined with VIR-2218/Bepirovirsen (combination study)



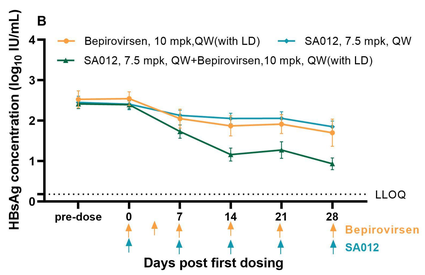


Figure 3. **Serum HBsAg change after SA012 combo treatments in AAV-HBV infected mice**

VIR-2218 is an anti-HBV siRNA, Bepirovirsen is an anti-HBV ASO. They had shown good results in HBV clinical trials. Figure 3A: Serum HBsAg change after SA012+VIR-2218 treatments; Figure 3B: Serum HBsAg change after SA012+Bepirovisen treatments. Combo treatment studies are still ongoing to obtain further results.

- When SA012 was combined with VIR-2218 or Bepirovirsen, synergistic effect in HBsAg reduction was observed (*Fig 3A* and *Fig 3B*).

Excellent preclinical safety profile of SA012

A. In a 4-Week Subcutaneous Repeat-Dose Toxicity Study, the NOAEL was at least 100 mg/kg in Sprague Dawley Rats. 300 mg/kg experiments are ongoing.

- ✓ No averse effect was observed in Tox studies, including weight, clinical hematology and biochemistry;
- ✓ Pathology dissection revealed mild changes in liver (vacuolar in some animals, which is common for GalNAc-conjugated siRNA). No abnormalities was observed in the kidneys or injection sites.

B. In a 4-Week Subcutaneous Repeat-Dose Toxicity Study, the NOAEL was at least 100 mg/kg in Cynomolgus Monkeys. 300 mg/kg experiments are ongoing.

- ✓ No test sample-related adverse effect was observed, including food intake, body weight, hematology and coagulation, serum biochemistry, immune functions and histopathological examinations.

- SA012 showed excellent preclinical safety profiles in cynomolgus monkeys and in rats.

Conclusions

- SA012 monotherapy showed potent and sustainable clearance of HBsAg and HBV DNA in AAV-HBV infected mice. High ratio of seroconversion was achieved at the end of experiments.
- Significant synergistic effects were observed when SA012 was injected in combination with other anti-HBV drugs, such as VIR-2218 or Bepirovirsen. No viral rebound was observed during the entire studies.
- SA012 showed excellent safety profiles in rats and cynomolgus monkeys, which supports its further clinical development.
- SA012 may help to restore HBV-specific acquired immunity, which maybe the pivotal point to prevent viral rebound. Because of its unique mechanism of action, SA012 has great potentials for the functional cure of CHB as monotherapy and in combination therapies with many other types of antiviral drugs.

References

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Acknowledgements

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