nature portfolio

Peer Review File

An RNA interference therapeutic potentially achieves functional cure of chronic hepatitis B virus infection

Corresponding Author: Professor Geng-Shen Song

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This study reported a pan-genotypic siRNA targeting the HBs region of HBV, KC13-M2G2, which demonstrated high efficacy in both AAV-HBV and HBVTG mouse models. The clinical-stage drugs elebsiran and bepirovirsen were evaluated in parallel, and KC13-M2G2 showed superior antiviral effects compared to both. Safety profiles were also confirmed in rats and monkeys. Given the multiple siRNAs currently under development for HBV, the novelty of this work should lie in demonstrating a significant breakthrough in efficacy and/or mechanism of action. While the reported efficacy appears to differ from existing siRNAs in development, the underlying mechanisms remain unexplained. Several issues should be addressed and clarified:
1. The authors stated that two rounds of screening were conducted and that chemical modifications were applied. However, these screening and modification processes should be presented in the main manuscript and described in detail to demonstrate how KC13-M2G2 was selected and why it is superior to other sequences. A dose-dependent reduction of viral antigens and DNA was evaluated in HepG2.2.15 cells, yet IC50 values were neither shown nor discussed in the manuscript.
2.According to the authors, KC13-M2G2 is not conserved in HBV genotypes F and H. However, further experiments showed that KC13-M2G2 suppressed viral antigens across all HBV genotypes, including F and H. The observed mismatches in the target sequence appeared to have no effect on siRNA susceptibility. How can this be explained? Could the observed efficacy be attributable to the immunogenicity of the siRNA? Is there any experimental evidence that supports or rules out this possibility?
3.The anti-HBV activity of KC13-M2G2 was evaluated in AAV-HBV mice at a dose of 3 mg/kg and compared to elebsiran. The efficacy of a single-dose regimen should be shown to illustrate both the potency and kinetics of the siRNA response. Given that elebsiran targets conserved regions of all HBV-derived RNAs (including HBx), a more appropriate positive control targeting the HBs region should be included—such as xalnesiran (RG6346), BW-20507, or HT-101.
4.Approximately half of the mice treated with KC13-M2G2 exhibited seroconversion, but the mechanism underlying this outcome remains unclear. Since even siRNAs targeting the same region can exhibit markedly different effects depending on chemical modifications and delivery strategies, and these aspects are not clearly described in the manuscript, it is difficult to assess whether the observed efficacy is attributable to the target sequence itself or to other factors, including potential artifacts related to the disease model used.

Reviewer #2

(Remarks to the Author)
This is a preclinical study on KC13-M2G2, which is a novel GalNAc-conjugated siRNA targeting the S region. The authors studied the compound in HepG2.2.15 cells transfected with HBV plasmid & HBV-infected primary human hepatocytes, mice and cynomolgus monkeys. The cells demonstrate no cytotoxicity, lower IC50 values than elebsiran, and pan-genotypic potency in reducing HBsAg, HBV DNA, HBeAg, HBV S mRNA and total RNA. Sustained viral suppression without rebound was also demonstrated in mice. Variable doses, dosing frequency, high HBsAg expressing-mice were investigated. Positive control with elebsiran and bepirovirsen was used.
The following are my comments:
- P.1 the abstract: there should be “levels” following high circulating hepatitis B surface (HBsAg).
- P.2 first paragraph: HBV rcDNA is “converted” not “repaired” by endogenous enzymes….
- P.2 first paragraph: cccDNA does not only encode the 4 antigens mentioned, but also the polymerase, and the pre-genomic RNA.

- P.2 second paragraph: omit “tend” after “likely”
- P.3 first paragraph: “…but it is limited by poor tolerability and patient’s stage” – The meaning of ‘patient’s stage’ is unclear.
- P.3 second paragraph: JNJ-3989 now is named as Daplusiran/Tomligisiran
https://adisinsight.springer.com › drugs
- P.2 second paragraph: “…indicating that immune system had taken over the duty of HBV suppression” the meaning is unclear.
- P.3 first paragraph: “NAs can suppress HBV replication but generally requires lifelong therapy and cannot cause HBsAg loss.” The rate of HBsAg seroclearance on NA is about 1-2% per year, which is similar to untreated patients. The authors should replace ‘cause’ by ‘improve the rate of’.
- p.19 second paragraph: AB-729 is now named Imdusiran
- P.4 third paragraph: please use the full term of AGO2 i.e. Argonaute 2
- How were the levels of viral antigens and RNA measured in mice?
- What is the dosing regimen for elebsiran?
- In figure 4, it seemed that elebsiran had a greater reduction of HBeAg levels compared to KC13-M2G2. Do the authors have any postulations on this finding?
- Lack of significant ALT elevation and the ‘silent cellular immune response’ in mice experiment needs to be interpreted with caution. The transfected mice model cannot directly be related to human HBV infection.
- Efficacy and safety evaluation in monkeys: more data in the monkey experiments would be preferred, as they more closely simulate the HBV infection in humans. The currently presented data only showed ALT, AST, bilirubin, urea, ALP and body weight in rats as shown in supplementary figure 9. There was no clinical, biochemical or microscopy data for monkeys presented as mentioned in the results on P.16 of the manuscript. There was also no efficacy data as the experiments did not include HBV-infected or transfected monkeys.
- It has recently been reported that siRNA-induced HBsAg suppression is associated with ameliorated T cell exhaustion in humans (EASL 2025 oral abstract #071). The findings from the current study were opposite to what was reported in humans.
- P.29-30: antiviral activity evaluation in rAAV-HBV mice and HBV-transgenic mice: ‘KC13-M2G1’ and ‘KC13-M1G1’ were given instead of ‘KC13-M2G2’.

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The revised manuscript shows considerable improvement, with added details on siRNA screening, efficacy data, and comparative analyses that enhance the study’s completeness. However, several issues remain to be clarified.
While the authors describe the chemical modification types, the exact modification sites and whether systematic comparisons of different modification patterns were performed remain unclear.
The efficacy evaluation relies mainly on serum biomarkers, lacking intrahepatic indicators such as HBV RNA, cccDNA or vector DNA residues to evaluate the pattern of viral suppression.
The immunogenicity assessment is still limited — only IL-2, IL-6, IL-10, TNF-α, and IFN-γ were measured, without inclusion of type I interferons (IFN-α/β), which are critical for assessing innate immune activation by exogenous nucleic acids. Additional clarification or expanded discussion would strengthen the work.
Overall, the revision represents substantial progress and clearer data presentation, demonstrating strong HBsAg reduction. Nonetheless, mechanistic insight remains limited, and the current evidence cannot yet distinguish whether the superior efficacy derives from target selection, chemical modification, or delivery optimization, or potentially other contributing factors.

Reviewer #2

(Remarks to the Author)
The authors had revised satisfactorily to the suggestions and comments made previously. I have no further requests.

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Point-by-point Response to Reviewers' Comments:

We sincerely thank all reviewers for their insightful comments, which have improved our manuscript. Our replies to the comments are provided in blue below. Manuscript text and figures have also been revised (highlighted in blue) according to the reviewers' comments.

Reviewer #1 (Remarks to the Author):

This study reported a pan-genotypic siRNA targeting the HBs region of HBV, KC13-M2G2, which demonstrated high efficacy in both AAV-HBV and HBV TG mouse models. The clinical-stage drugs elebsiran and bepirovirsen were evaluated in parallel, and KC13-M2G2 showed superior antiviral effects compared to both. Safety profiles were also confirmed in rats and monkeys. Given the multiple siRNAs currently under development for HBV, the novelty of this work should lie in demonstrating a significant breakthrough in efficacy and/or mechanism of action. While the reported efficacy appears to differ from existing siRNAs in development, the underlying mechanisms remain unexplained. Several issues should be addressed and clarified:

1. The authors stated that two rounds of screening were conducted and that chemical modifications were applied. However, these screening and modification processes should be presented in the main manuscript and described in detail to demonstrate how KC13-M2G2 was selected and why it is superior to other sequences. A dose-dependent reduction of viral antigens and DNA was evaluated in HepG2.2.15 cells, yet IC_{50} values were neither shown nor discussed in the manuscript.

Response: We thank the reviewer for raising these important points. We have added the screening processes and IC_{50} values to our revised manuscript.

- In Results (Screening of siRNAs targeting HBV): "Briefly, siRNA candidates targeting HBV were designed using publicly available algorithms, including siDirect, DSIR, DsiRNA, and sFold, based on the reference sequence NC_003977.2. A total of 26 siRNAs were manually selected according to the criterion of high target sequence conservation. The region downstream of nucleotide position 1600 was excluded from siRNA design due to its possible loss during HBV DNA integration into the host genome. The initial screening round aimed to identify sensitive target sites. All 26 siRNAs were uniformly chemically modified using the same pattern, which included the incorporation of 2'-O-methyl, 2'-fluoro and phosphorothioate modifications at specific sites to enhance siRNA stability and specificity. In addition, (E)-vinylphosphonate was introduced at the 5' end of the antisense strands to promote efficient loading into Argonaute 2. Among the 26 siRNAs, five exhibited superior anti-HBV activity compared to the others (Supplementary Fig. 1a). Based on these five lead candidates, a total of 95 siRNAs were designed by optimizing chemical modification patterns, and were subsequently evaluated in a second screening round (Supplementary Fig. 1b). Among these candidates, a total of 9 siRNAs were selected for further evaluation

in rAAV-HBV mice based on a comprehensive consideration of anti-HBV efficacy and patent conflict avoidance. The selected siRNAs were conjugated with a proprietary GalNAc ligand (G1) and administered subcutaneously at a dose of 3 mg/kg once weekly for a total of three injections. The siRNAs KC13-M1G1 and KC13-M2G1, which share the same nucleotide sequence (KC13), but differ in their modification patterns (M1 vs. M2), demonstrated superior efficacy compared to the other candidates (Fig. R1). In a subsequent lower-dose study (0.5 mg/kg), KC13-M2G1 exhibited more potent anti-HBV activity than KC13-M1G1 (Fig. R2). Furthermore, conjugation of KC13-M2 with an alternative GalNAc ligand (G2) led to a further enhancement of antiviral efficacy (Fig. R3). These results established KC13-M2G2 as the most promising siRNA candidate for the treatment of HBV infection."

The Figs. R1-R3 were added in the revised Supplementary Information (Supplementary Figs. 2-4).

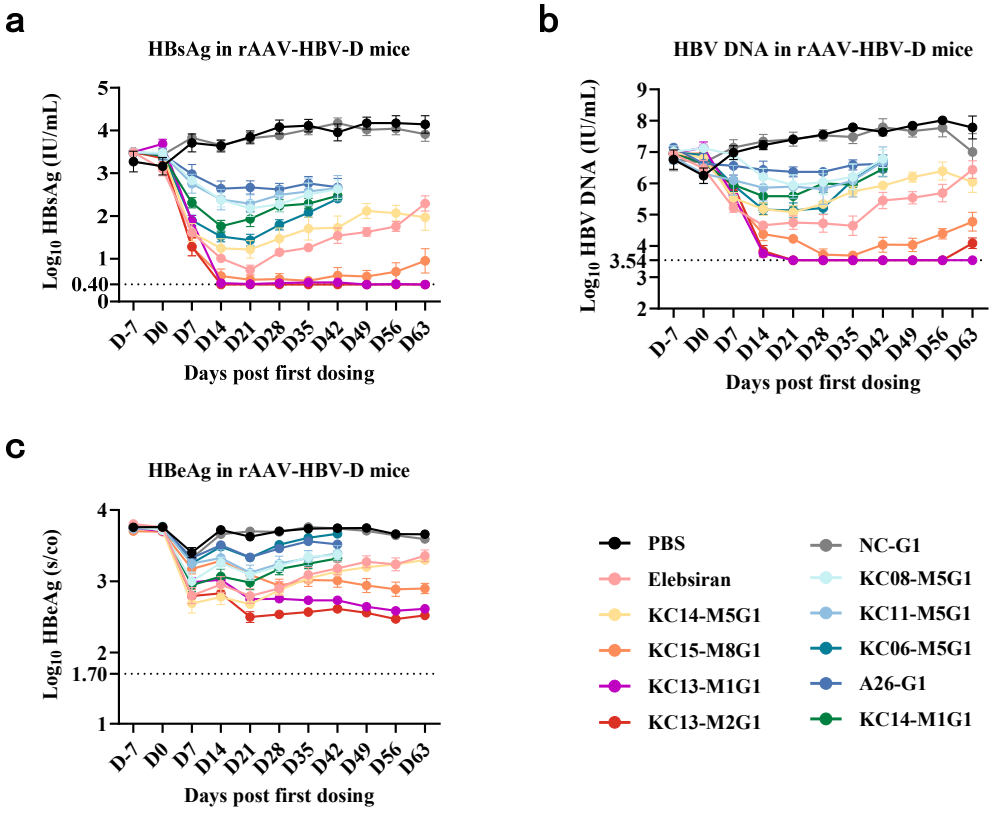

Fig. R1. ***In vivo*** **screening of GalNAc-conjugated siRNA candidates in rAAV-HBV-D mice.** Nine selected siRNAs conjugated with the proprietary GalNAc ligand G1 were administered subcutaneously at a dose of 3 mg/kg once weekly for three weeks. Plasma levels of HBsAg (**a**) and HBeAg (**c**) were quantified by chemiluminescence immunoassay (CLIA) assays on commercial analytical systems, HBV DNA (**b**) by PCR-fluorescence probing assay at certain timepoints, with the first dosing day defined

as D0. Data are showed as mean ± SEM (n=6). Dashed lines indicate LLOQs.

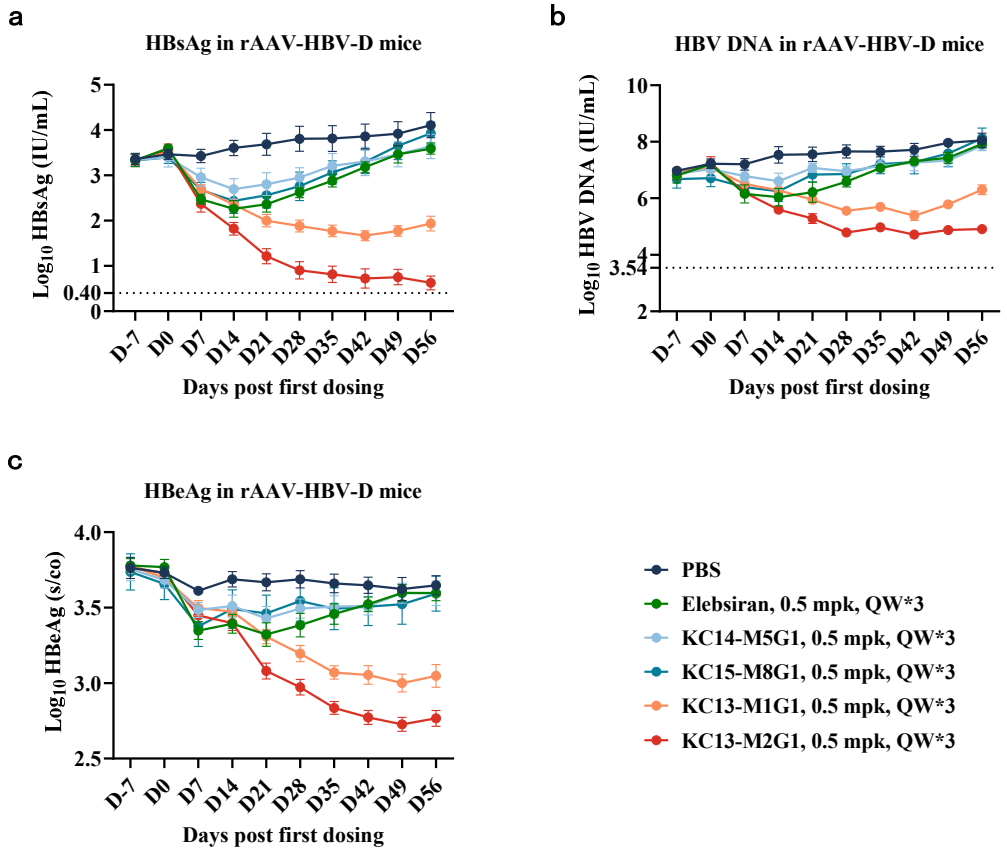

Fig. R2: **Lower-dose study (0.5 mg/kg, once weekly for three weeks) for siRNA screening in rAAV-HBV-D mice.** Four selected siRNAs, conjugated with the proprietary GalNAc ligand (G1), were evaluated. Plasma levels of HBsAg (**a**) and HBeAg (**c**) were quantified by chemiluminescence immunoassay (CLIA) assays on commercial analytical systems, HBV DNA (**b**) by PCR-fluorescence probing assay at certain timepoints. The first dosing day was defined as D0. Data are shown as mean ± SEM (n=6). Dashed lines indicate LLOQs.

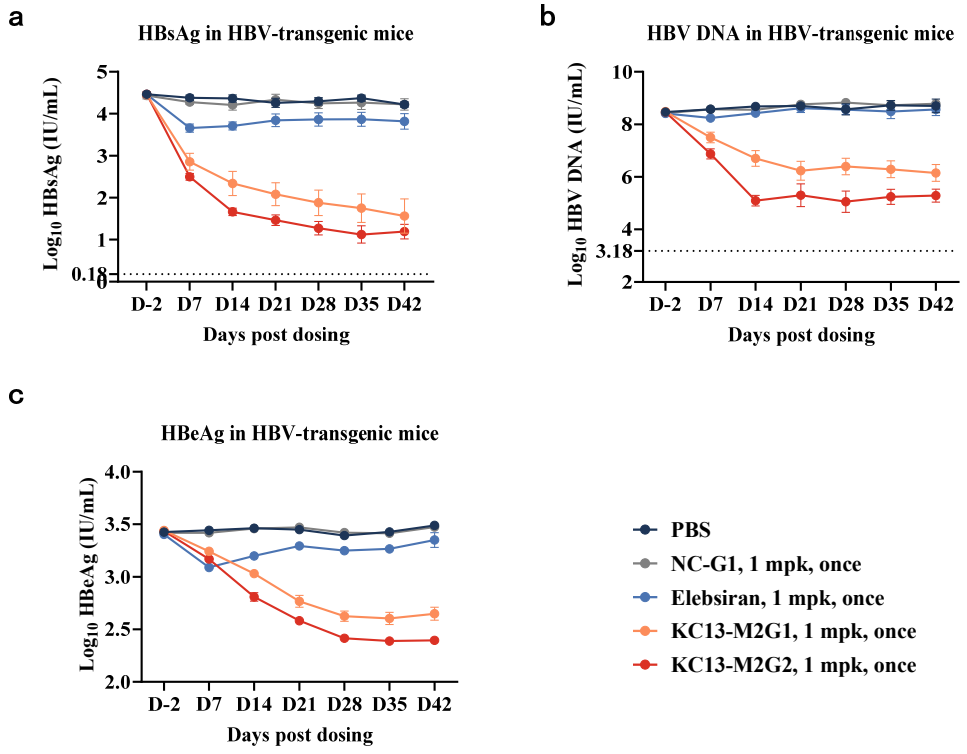

Fig. R3: **GalNAc screening for KC13-M2 in HBV-transgenic mice.** KC13-M2G2 conjugated with either proprietary GalNAc ligand G1 or G2 was administered subcutaneously at a single dose of 1 mg/kg. Plasma levels of HBsAg (**a**) and HBeAg (**c**) were quantified by chemiluminescence immunoassay (CLIA) assays on commercial analytical systems, HBV DNA (**b**) by PCR-fluorescence probing assay at certain timepoints. The dosing day was defined as D0. Data are shown as mean ± SEM (n=6). Dashed lines indicate LLOQs.

- We have revised the text in Results (*In vitro* efficacy of KC13-M2G2): "All IC_{50} values of KC13-M2G2 were lower than those of elebsiran." to "KC13-M2G2 showed superior antiviral efficacy than elebsiran, with IC_{50} values of 0.03 vs. 0.07 nM for HBsAg, 0.08 vs. 0.11 nM for HBV DNA, 0.47 vs. 0.51 nM for HBeAg, 0.20 vs. 0.44 nM for HBV S mRNA, and 0.25 vs. 0.50 nM for HBV total RNA."

2.According to the authors, KC13-M2G2 is not conserved in HBV genotypes F and H. However, further experiments showed that KC13-M2G2 suppressed viral antigens across all HBV genotypes, including F and H. The observed mismatches in the target sequence appeared to have no effect on siRNA susceptibility. How can this be explained? Could the observed efficacy be attributable to the immunogenicity of the siRNA? Is there any experimental evidence that supports or rules out this possibility?

Response: We thank the reviewer for raising this insightful point.

- Our sequence conservation analysis of KC13-M2G2 across different HBV

genotypes was conducted based on full-length sequences. Notably, its nucleotide sequence in the seed region showed 93.9% identity with all HBV genotypes (Table 1-R). As is well established, the seed region of siRNAs plays a critical role in determining pharmacodynamic efficacy. Thus, although KC13-M2G2 is not conserved across HBV genotypes F and H when evaluated by full-length sequence alignment, it exhibited effective activity against these two genotypes.

- We have added the text in Results (Conservation analysis and pan-genotypic efficacy of KC13-M2G2): "However, the seed region (position 2-8 of antisense strand) was conserved in 93.9% of all HBV genotypes from A to H." Table 1 has also been revised accordingly.

Table 1-R: **Conservation of KC13-M2G2 across HBV genotypes A to H.**

genotype	A	B	C	D	E	F	G	H	All
% global infection[28]	16.9	13.5	26.1	22.1	17.6	0.86	0.04	0.07	97.2
Number of sequences analyzed[a]	1038	1922	2954	1701	353	296	41	31	8336
% core region conservation[b]	96.4	79.7	91.5	95.9	94.6	0.7	95.1	0	86.9
% seed region conservation[c]	98.4	80.9	97.0	99.4	98.9	91.6	100	96.8	93.9

[a] 8336 HBV genotype A−H sequences downloaded on May 28, 2025.

[b] Complete complementarity to position 2−19 of antisense strand.

[c] Complete complementarity to position 2−8 of antisense strand.

- Moreover, the mismatches between KC13-M2G2 and HBV genotypes F and H occur at antisense strand positions 15 and/or 18. It has been reported that siRNA antisense strand could be divided into four functional domains: the seed region (position 2-8), the central region (position 9-12), the supplementary region (position 13-17), and the tail region (position 18-21)[1]. The recognition and cleavage of target RNAs depend critically on perfect complementarity with the seed and central regions. The mismatches between KC13-M2G2 and HBV genotypes F and H are located outside these critical regions and involve only one or two bases, offering little opportunity to significantly impair siRNA susceptibility. Furthermore, mismatches within the tail region have been reported to promote the release of either the sense strand or the target mRNA from the RISC complex by reducing structural strain in the RNA duplex, thereby enhancing cleavage efficiency[2].

- We have discussed these in the manuscript (Discussion): "In this study, we found that even mismatches exist, KC13-M2G2 still obviously inhibited HBsAg

of HBV F and H genotypes at picomolar levels. As reported, siRNA antisense strand could be divided into four functional domains: the seed (position 2-8), central (position 9-12), supplementary (position 13-17), and tail (position 18-21) regions[35]. Our results revealed that some mismatches in the supplementary and tail regions will not affect siRNAs' efficacy. That means perfect complementarity with position 2-12, other than 2-19, could be a more suitable criterion when designing siRNA for highly variable viruses."

- Furthermore, it seems the anti-HBV oligonucleotide drugs, such as ASO drug bepirovirsen or AHB-137, harbors intrinsic immune activity, imparting an additional immunomodulatory property. However, the siRNA KC13-M2G2 was heavily modified with 2'-O-methyl, which were reported to help reduce the immunogenicity of siRNA[3]. In fact, in the non-clinical toxicity study of KC13-M2G2, we systematically examined the immunogenicity of KC13-M2G2 in SD rats and cynomolgus monkeys. There were no KC13-M2G2-related changes in the serum levels of IL-2, IL-6, IL-10, TNF-α and IFN-γ were observed (Fig. R4a-e and Fig. R5a-e). The absolute number and percentages of lymphocytes and the subpopulations (total T cells, cytotoxic T cells and helper T cells) also did not show significant differences between the saline and KC13-M2G2 groups (Fig. R4f-l and Fig. R5f-l). Although some fluctuations were noted at individual time points in some animals, these fluctuations exhibited no time- or dose-dependent trends and were thus considered to be caused by individual differences rather than KC13-M2G2 treatment. We have added these data to Results (Immunogenicity of KC13-M2G2 in SD rats and cynomolgus monkeys). The Figs. R4 and R5 were added in the revised manuscript (Supplementary Fig. 13 and Fig. 10).

- Accordingly, we have added the text to Methods (Immunogenicity study of KC13-M2G2 in SD rats and cynomolgus monkeys): "KC13-M2G2 was administered repeatedly via subcutaneous injection to SD rats at 25, 50 or 100 mg/kg once every two weeks for 4 consecutive weeks (on day 0, 14, and 28) with an 8-week recovery period. Serum cytokine levels (including IL-2, IL-6, IL-10, TNF-α and IFN-γ) were assayed 4 h after the first dose and the final dose (day 0-4 h and day 28-4 h), 24 h after the first dose and the final dose (day 1 and day 29) and at the study terminus (day 85), using Rat Luminex® Discovery Assay (R&D) according to the instructions. Serum lymphocyte profiles were detected on day 1, day 29 and day 85, employing an immunophenotyping panel to assess the percentages and absolute numbers of lymphocyte subpopulations of total T cells ($CD45^+$ $CD3^+$), helper T cells ($CD45^+$ $CD3^+$ $CD4^+$ $CD8a^-$) and cytotoxic T cells ($CD45^+$ $CD3^+$ $CD4^-$ $CD8a^+$) with flow cytometry (FACS Canto II, BD). Briefly, the blood samples were washed with DPBS and subsequently incubated with pre-mixed antibody for 30 min at room temperature on an orbital shaker. FACS Lysing Solution (diluted 1:10 using deionized water) was added to each sample and incubated for additional 10 min. The samples were subsequently washed with DPBS and resuspended in DPBS for analysis on flow cytometer. The antibodies

were listed in Supplementary Table 6.

Similar parameters were investigated in cynomolgus monkeys after three doses of 30, 100, or 200 mg/kg administered on day 0, 14, and 28, with an 8-week recovery period. Serum cytokine levels (including IL-2, IL-6, IL-10, TNF-α and IFN-γ) in monkeys were assessed at the same timepoint as in SD rats, using NHP XL Cytokine Luminex® Performance Premixed Kit (R&D) according to the manufacturer's protocols. Serum lymphocyte profiles were detected as described above. The antibodies were listed in Supplementary Table 6."

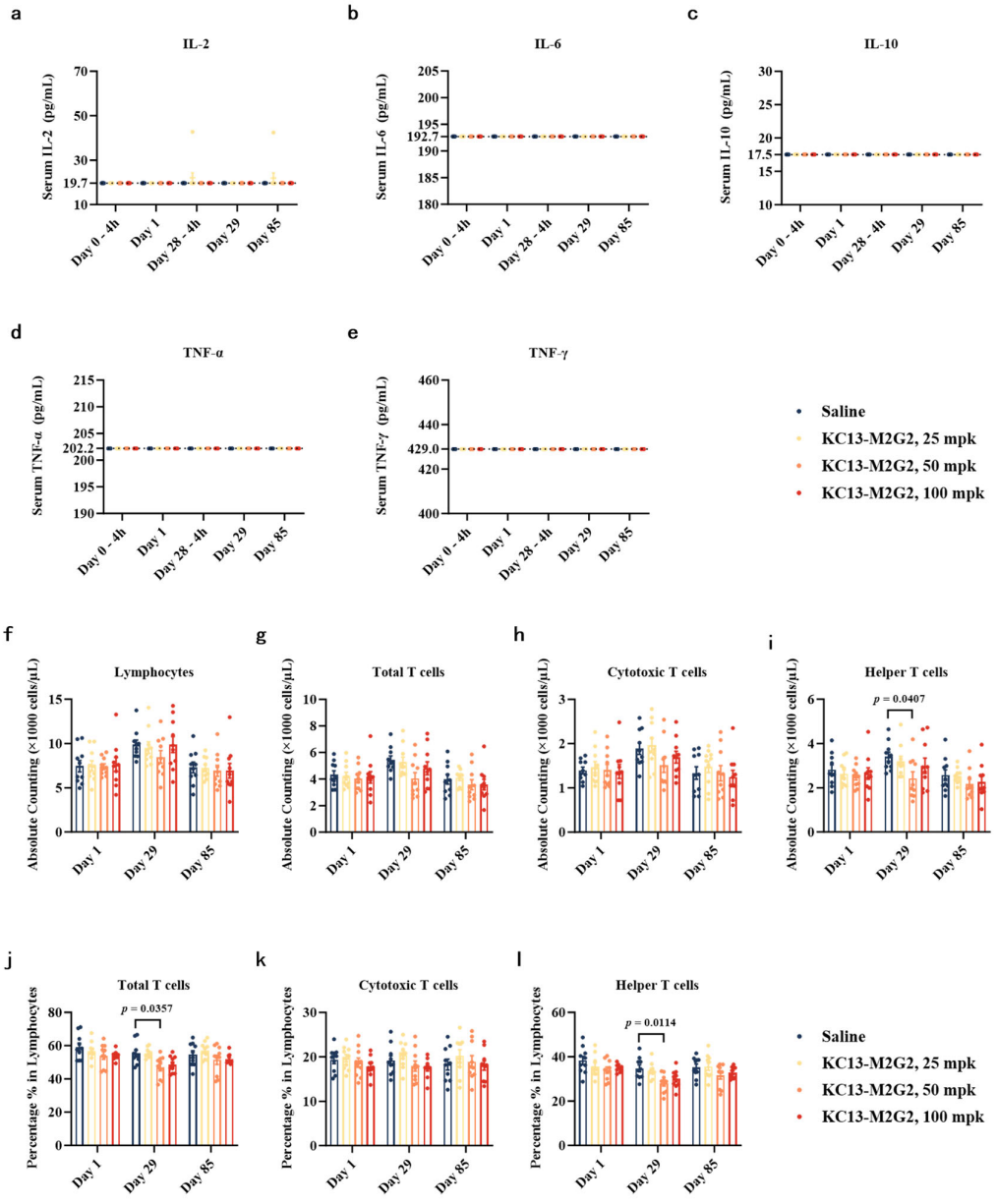

Fig. R4: **Immunogenicity of KC13-M2G2 in SD rats.** KC13-M2G2 was administered repeatedly via subcutaneous injection to SD rats at 25, 50 or 100 mg/kg on day 0, 14,

and 28 with an 8-week recovery period. **a-e** Serum cytokine levels (including IL-2, IL-6, IL-10, TNF-α and IFN-γ) were assayed 4 h after the first dose and the final dose (Day 0-4 h and Day 28-4 h), 24 h after the first dose and the final dose (Day 1 and Day 29) and at the recovery period terminus (Day 85), using Rat Luminex® Discovery Assay. **f-l** Serum absolute numbers and percentages of lymphocyte subpopulations of total T cells ($CD45^+$ $CD3^+$), cytotoxic T cells ($CD45^+$ $CD3^+$ $CD4^-$ $CD8a^+$), and helper T cells ($CD45^+$ $CD3^+$ $CD4^+$ $CD8a^-$) were detected on day 1, day 29 and day 85 by flow cytometry, employing an immunophenotyping panel. The first dosing day was defined as Day 0. Data are shown as mean ± SEM (n=10). Dashed lines indicate LLOQs. Only statistically significant differences are indicated (one-way ANOVA test or Kruskal-Wallis test with Dunn's post-hoc test for multiple groups).

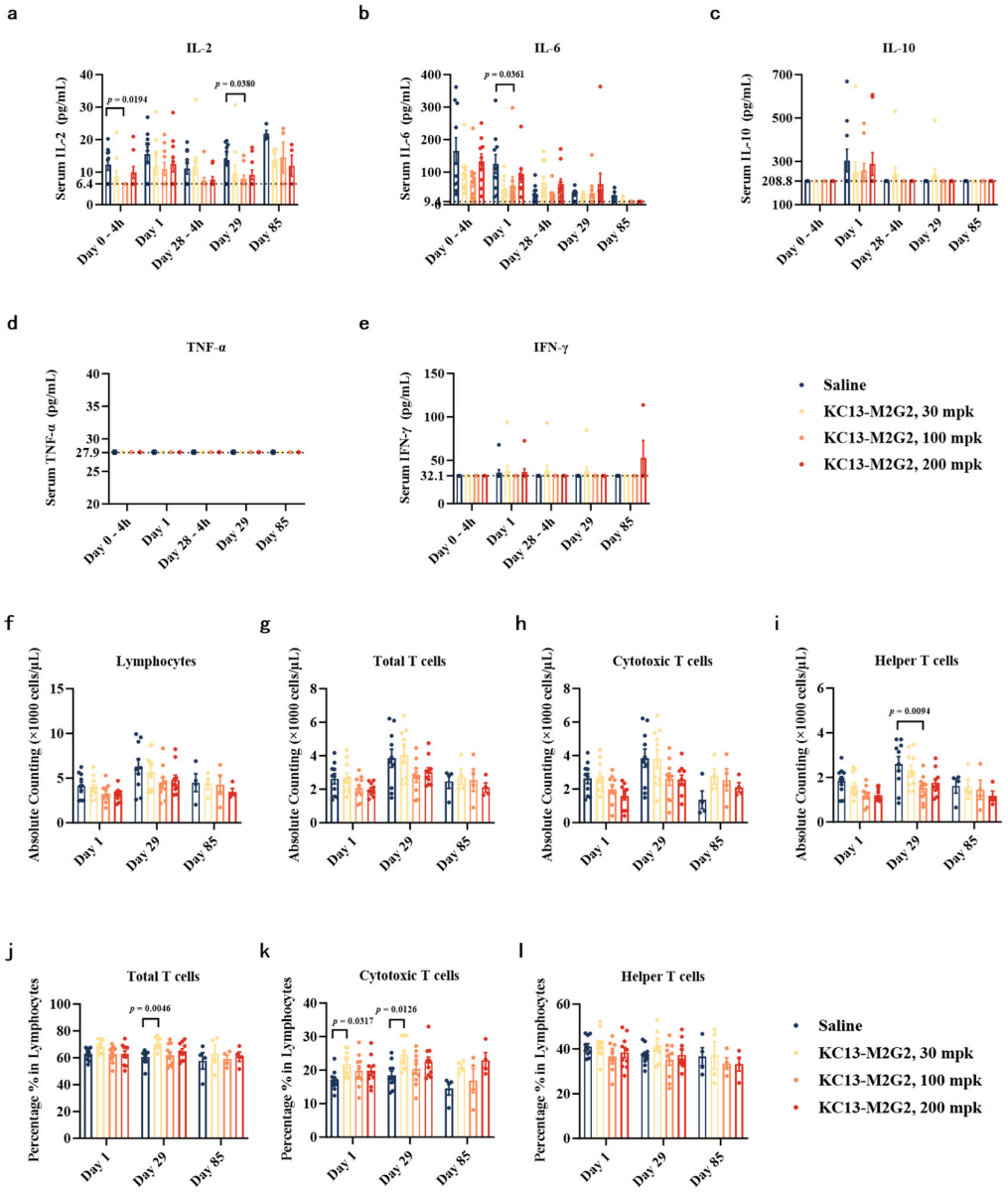

Fig. R5: **Immunogenicity of KC13-M2G2 in cynomolgus monkeys.** KC13-M2G2 was administered repeatedly via subcutaneous injection to cynomolgus monkeys at 30, 100 or 200 mg/kg on day 0, 14, and 28 with an 8-week recovery period. **a-e** Serum cytokine levels (including IL-2, IL-6, IL-10, TNF-α and IFN-γ) were assayed 4 h after the first dose and the final dose (Day 0-4 h and Day 28-4 h), 24 h after the first dose and the final dose (Day 1 and Day 29) and at the recovery period terminus (Day 85), using NHP XL Cytokine Luminex® Performance Premixed Kit. **f-l** Serum absolute numbers and percentages of lymphocyte subpopulations of total T cells ($CD45^+$ $CD3^+$), cytotoxic T cells ($CD45^+$ $CD3^+$ $CD4^-$ $CD8a^+$), and helper T cells ($CD45^+$ $CD3^+$ $CD4^+$ $CD8a^-$) were detected on day 1, day 29 and day 85 by flow cytometry, employing an immunophenotyping panel. The first dosing day was defined as Day 0. Data are shown as mean ± SEM (n=4 or 10). Dashed lines indicate LLOQs. Only statistically significant differences are indicated (one-way ANOVA test or Kruskal-Wallis test with Dunn's post-hoc test for multiple groups).

3.The anti-HBV activity of KC13-M2G2 was evaluated in AAV-HBV mice at a dose of 3 mg/kg and compared to elebsiran. The efficacy of a single-dose regimen should be shown to illustrate both the potency and kinetics of the siRNA response. Given that elebsiran targets conserved regions of all HBV-derived RNAs (including HBx), a more appropriate positive control targeting the HBs region should be included—such as xalnesiran (RG6346), BW-20507, or HT-101.

Response: We appreciate these valuable points.

- We have evaluated the efficacy of a single-dose regimen of KC13-M2G2 in both rAAV-HBV-D mice and HBV-transgenic mice. In rAAV-HBV-D mice, KC13-M2G2 was administrated at single dose of 0.3, 1, 3, and 9 mg/kg. Data showed even at a lower dose of 1 mg/kg, KC13-M2G2 exhibited significant inhibitions on HBsAg and HBV DNA levels without obvious rebound throughout the study, as shown in Supplementary Fig. 8 (labeled as Supplementary Fig. 5 in the initial manuscript). In HBV-transgenic mice, KC13-M2G2 was administrated at a single dose of 3 mg/kg. The results showed that a maximum 3 $\log_{10}$ IU/mL reduction in HBsAg was triggered and up to 6/12 mice achieved HBsAg loss at the nadir, as shown in Supplementary Fig. 10 (labeled as Supplementary Fig. 7 in the initial manuscript).

- As suggested by the reviewer, we obtained commercial xalnesiran from MedChemExpress (MCE) and verified its identity using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/substance/472422861). The efficacy differences between xalnesiran and KC13-M2G2 were assessed *in vitro*. In HepG2.2.15 cells, KC13-M2G2 demonstrated more potent inhibition of HBsAg than xalnesiran, while exhibiting comparable efficacy against HBeAg (Fig. R6).

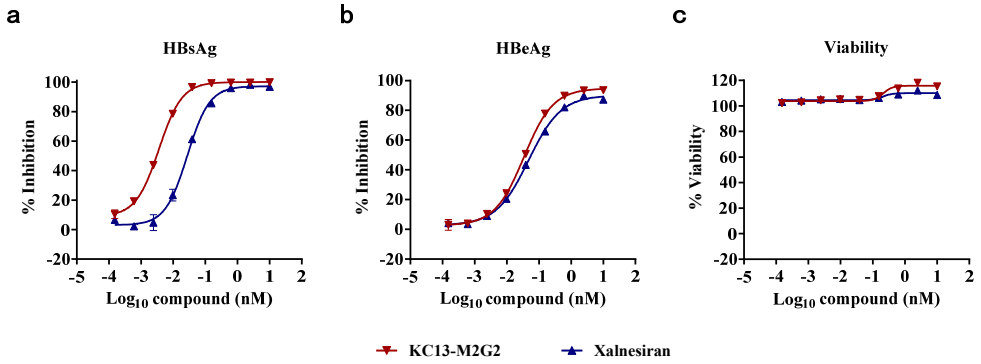

Fig. R6: **Comparison of the efficacy of KC13-M2G2 and xalnesiran in HepG2.2.15 cells.** KC13-M2G2 and xalnesiran were transfected into cells at concentrations of 10 to 0.00015 nM. The medium was replaced with fresh medium on day 3 post-transfection. On day 6 post-transfection, HBsAg (**a**), and HBeAg (**b**) in supernatants were quantified using chemiluminescence immunoassay (CLIA) commercial Kits. Cell viabilities (**c**) were determined using CellTiter-Glo (CTG) according to the manuals. Data are shown as mean ± SEM (n=3).

- Although the chemical structures of BW-20507 and HT-101 could not be ascertained from the public resources, we noticed the preclinical study of HT-101 and discussed in this study (Discussion): "The preclinical study on HT-101 showed a maximum HBsAg reduction of 1.97 $\log_{10}$ IU/mL in rAAV-HBV mice at a single dose of 3 mg/kg[42], much lower than that of KC13-M2G2 (3.21 $\log_{10}$ IU/mL) at the same dosing regime. Meanwhile, the article noted that HT-101 induced long-lasting suppression of HBsAg below the LLOQ. We noticed that the LLOQ mentioned here is 1.78 $\log_{10}$ IU/mL, equal to 60.26 IU/mL, much higher than that in our study (2.5 IU/mL). High LLOQ value may overestimate the anti-HBV activity."

4.Approximately half of the mice treated with KC13-M2G2 exhibited seroconversion, but the mechanism underlying this outcome remains unclear. Since even siRNAs targeting the same region can exhibit markedly different effects depending on chemical modifications and delivery strategies, and these aspects are not clearly described in the manuscript, it is difficult to assess whether the observed efficacy is attributable to the target sequence itself or to other factors, including potential artifacts related to the disease model used.

Response: We appreciate this valuable point.

- We have evaluated efficacy of siRNAs targeting other HBV sites in rAAV-HBV mice as described in the response to the first comment. Variable antiviral efficacy was observed across different siRNA-treatment groups (Fig. R1, above). For instance, although KC13-M1G1 and KC14-M1G1 share the same chemical modification pattern and GalNAc ligand, they exhibited markedly different

antiviral outcomes. KC13-M1G1 suppressed HBsAg to levels below LLOQ without rebound throughout the experimental period, whereas KC14-M1G1 achieved a maximum HBsAg reduction of less than 1 $\log_{10}$ IU/mL, followed by rapid viral rebound (Fig. R1, above). Neither the PBS group nor the negative control group (scrambled sequence of elebsiran) showed any inhibition on viral biomarkers. These results confirmed that the reduction in viral antigen expression was specifically attributable to siRNA-mediated silencing rather than non-specific effects.

- Furthermore, we also evaluated efficacy of the siRNAs with the same nucleotide sequence but different chemical modifications and delivery ligands. As described in the response to the first comment, KC13-M1G1, KC13-M2G1, and KC13-M2G2 share the same nucleotide sequence (KC13), but differ in their modification patterns (M1 vs. M2) or GalNAc ligands (G1 vs. G2). The *in vivo* study showed that KC13-M2G1 exhibited more potent antiviral activity than KC13-M1G1 (Fig. R2, above), and KC13-M2G2 led to a further enhancement of antiviral efficacy than KC13-M2G1 (Fig. R3, above). These results suggest that the functional properties of siRNA are influenced by its chemical modification pattern and GalNAc ligand, consistent with the reviewer's comment.

- The rAAV-HBV mouse model is widely used in studies of chronic HBV infection and has been extensively applied in the development of oligonucleotide-based therapies, neutralizing antibodies, and therapeutic vaccines[4,5]. This model has been validated to show a characteristic immunotolerant status, marked by high frequencies of hepatic $CD4^+CD25^+$ $Foxp3^+$ T cells, upregulation of inhibitory immune checkpoint molecules (including PD-1, LAG-3, and TIM-3), as well as impaired cytokine production in both $CD4^+$ T and $CD8^+$ T cells[6]. These features closely mimic the immunotolerant environment observed in human chronic HBV carriers. Furthermore, in our study, rAAV-HBV mice exhibited persistent HBV viremia lasting for at least 189 days, with no observed HBsAb (Fig. 6-R, PBS group). Collectively, these results support the suitability of the rAAV-HBV mouse model for studying chronic HBV infection.
  Fig. 6 has been updated to include data from day 70 to day 189, shown as Fig. 6-R.

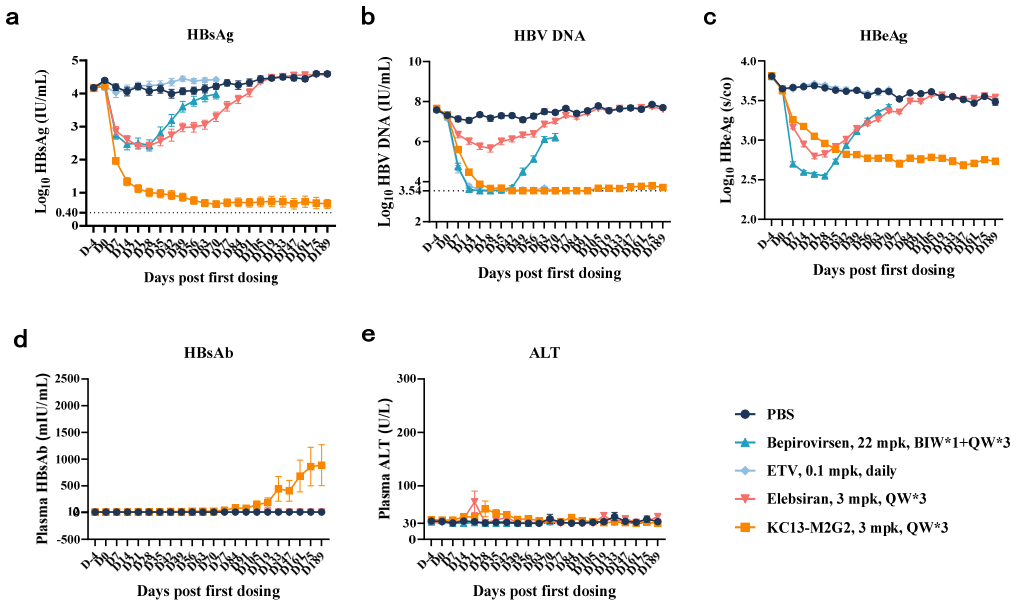

Fig. 6-R: Efficacy of KC13-M2G2 in rAAV-HBV-D mice with high HBsAg baseline at three weekly doses of 3 mg/kg. Elebsiran, bepirovirsen and ETV served as positive controls. The first dosing day was defined as D0. Plasma HBsAg, HBV DNA, HBeAg, HBsAb and ALT were detected at certain timepoints. Data are shown as mean ± SEM (n=10). Dashed lines indicate LLOQs.

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4. Iwakawa, H. O., & Tomari, Y. Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. *Mol. Cell* **82**, 30–43 (2022).
5. Wee, L, M., Flores-Jasso, C. F., Salomon, W. E. Zamore, P. D. Argonaute divides its RNA guide into domains with distinct functions and RNA-binding properties. *Cell* **151**, 1055-1067 (2012).
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Reviewer #2 (Remarks to the Author):
This is a preclinical study on KC13-M2G2, which is a novel GalNAc-conjugated siRNA targeting the S region. The authors studied the compound in HepG2.2.15 cells transfected with HBV plasmid & HBV-infected primary human hepatocytes, mice and cynomolgus monkeys. The cells demonstrate no cytotoxicity, lower IC50 values than elebsiran, and pan-genotypic potency in reducing HBsAg, HBV DNA, HBeAg, HBV S mRNA and total RNA. Sustained viral suppression without rebound was also demonstrated in mice. Variable doses, dosing frequency, high HBsAg expressing-mice were investigated. Positive control with elebsiran and bepirovirsen was used.
The following are my comments:

- P.1 the abstract: there should be "levels" following high circulating hepatitis B surface (HBsAg).

Response: Thanks. We have revised this sentence as suggested.

- P.2 first paragraph: HBV rcDNA is "converted" not "repaired" by endogenous enzymes….

Response: Thanks for this precise suggestion. We have corrected it as suggested.

- P.2 first paragraph: cccDNA does not only encode the 4 antigens mentioned, but also the polymerase, and the pre-genomic RNA.

Response: Thanks for raising this point. We have revised the sentence from "… which encodes four viral antigens..." to "…which encodes polymerase, pre-genomic RNA, and four viral antigens…"

- P.2 second paragraph: omit "tend" after "likely"

Response: Thanks for raising this point. We have corrected it accordingly.

- P.3 first paragraph: "…but it is limited by poor tolerability and patient's stage" – The meaning of 'patient's stage' is unclear.

Response: We have revised this sentence to "…but it is limited by poor tolerability. Furthermore, young children with CHB may derive greater benefit from current IFN-α and NAs treatments than those adult patients[18,19]."

- P.3 second paragraph: JNJ-3989 now is named as Daplusiran/Tomligisiran
https://adisinsight.springer.com › drugs

Response: Thanks for raising this point. We have corrected it.

- P.2 second paragraph: "…indicating that immune system had taken over the duty of HBV suppression" the meaning is unclear.

Response: Thanks for raising this point. We have revised this sentence to "…indicating successful restoration of HBV-specific immune responses".

- P.3 first paragraph: "NAs can suppress HBV replication but generally requires lifelong therapy and cannot cause HBsAg loss." The rate of HBsAg seroclearance on NA is about 1-2% per year, which is similar to untreated patients. The authors should replace 'cause' by 'improve the rate of'.

Response: Thanks for raising this point. We have corrected it as suggested.

- p.19 second paragraph: AB-729 is now named Imdusiran

Response: Thanks for raising this point. We have replaced "AB-729" by "Imdusiran" throughout the manuscript.

- P.4 third paragraph: please use the full term of AGO2 i.e. Argonaute 2

Response: Thanks for raising this point. We have corrected it accordingly in P.5 of the revised manuscript.

- How were the levels of viral antigens and RNA measured in mice?

Response: We measured the plasma levels of HBsAg and HBeAg in mice were using chemiluminescence immunoassay (CLIA) kits on commercial analytical systems (Abbott or Maccura), the plasma levels of HBV DNA using PCR-fluorescence probing assay kit (Sansure Biotech) according to the manufacturer's instructions. We have added the methods to figure legends correspondingly. The detailed description of measurement method has been added to Methods (Antiviral activity evaluation in rAAV-HBV mice and HBV-transgenic mice): "The plasma levels of HBsAg, HBeAg, and HBsAb were quantified using CLIA assay kits on commercial analytical systems (Abbott for rAAV-HBV mice and Maccura for HBV-transgenic mice). The plasma levels of HBV DNA were quantified using PCR-fluorescence probing assay kit (Sansure Biotech) according to the manufacturer's instructions. The plasma levels of ALT were quantified by an enzymatic method with alanine aminotransferase detection kits (Zybio)."

- What is the dosing regimen for elebsiran?

Response: In rAAV-HBV-B and rAAV-HBV-C mice, elebsiran was administered at 3 mg/kg once weekly for three doses. In rAAV-HBV-D mice, elebsiran was administered

as a single dose, three weekly doses, or three biweekly doses of 3 mg/kg. In HBV-transgenic mice, elebsiran was administered either as a single dose or as three weekly doses of 3 mg/kg. Thanks for highlighting this issue. We have clarified the dosing regimens in revised manuscript.

- In figure 4, it seemed that elebsiran had a greater reduction of HBeAg levels compared to KC13-M2G2. Do the authors have any postulations on this finding?

Response: Thanks for raising this point. As known, elebsiran targets all HBV-derived RNA transcripts, including preC RNA, pgRNA, preS1 RNA, preS2/S RNA, and X RNA, whereas KC13-M2G2 targets all HBV-derived RNAs, except the X RNA. It has been reported that HBx protein plays an important role in HBV' life and could activate HBV RNA transcription[1], enhance HBV replication[1], and induce degradation of host Smc5/6 complex[2]. We hypothesize that elebsiran's inhibition of X RNA contributes to its greater HBeAg reduction in these models. Furthermore, differences in the secondary structures and relative abundance of various HBV RNA transcripts may influence the binding affinities and silencing efficiencies of elebsiran and KC13-M2G2. All these factors could account for the divergent functional outcomes.

- Lack of significant ALT elevation and the 'silent cellular immune response' in mice experiment needs to be interpreted with caution. The transfected mice model cannot directly be related to human HBV infection.

Response: Thanks for raising this important point. We have deleted the sentence "The silent cellular immune response could also be inferred from the low ALT levels (Supplementary Fig. 6c)." in P.15 of the revised manuscript.

- Efficacy and safety evaluation in monkeys: more data in the monkey experiments would be preferred, as they more closely simulate the HBV infection in humans. The currently presented data only showed ALT, AST, bilirubin, urea, ALP and body weight in rats as shown in supplementary figure 9. There was no clinical, biochemical or microscopy data for monkeys presented as mentioned in the results on P.16 of the manuscript. There was also no efficacy data as the experiments did not include HBV-infected or transfected monkeys.

Response: Thanks for raising these points.

- There is a typo in the legend of Supplementary Fig. 9 (labeled as Fig. 9 in revised manuscript). These clinical parameters and body weight data were for cynomolgus monkeys, not for rats. We have corrected it correspondingly. The microscopic changes for monkeys included: basophilic Kupffer cells and hepatocytic vacuolations in the liver, increased foamy macrophages in the lymph nodes, and basophilic macrophages at injection sites, as shown in Fig. R7g and Fig. R8. These microscopic findings were considered KC13-M2G2-related and non-adverse through evaluation by at least two experienced pathologists. We have added these

data to our manuscript (Fig. 9g and Supplementary Fig. 12).

- Accordingly, we have revised the text in Results (Safety profiles of KC13-M2G2 in SD rats and cynomolgus monkeys) from "No test article-related abnormal body weight, food consumption, clinical pathology, and microscopic pathology were observed." to "No test article-related abnormal body weight, clinical pathology was observed (Fig. 9a-e, g). The microscopic observations included: basophilic Kupffer cells and hepatocytic vacuolations in the liver, increased foamy macrophages in the lymph nodes, and basophilic macrophages at injection sites (Fig. 9f and Supplementary Fig. 12). These microscopic findings were considered KC13-M2G2-related and non-adverse after reviewed by at least two experienced pathologists."

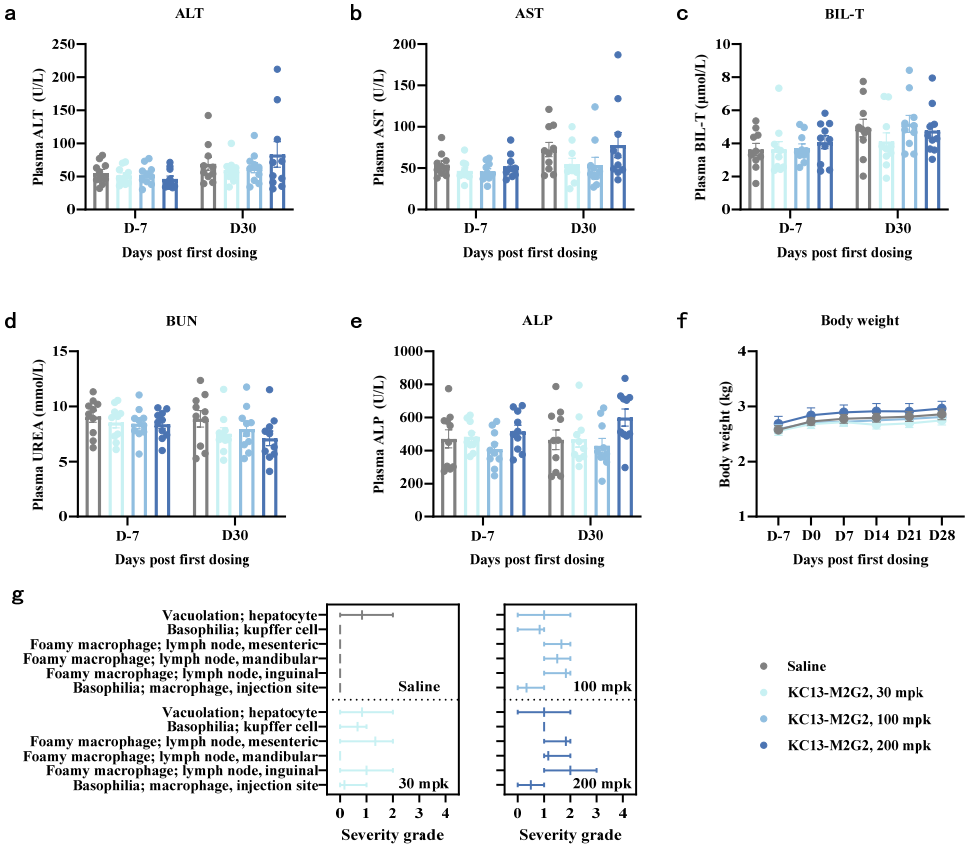

Fig. R7: **Selected clinical pathology parameters, body weights and microscopic findings in cynomolgus monkeys**. The monkeys received three repeat doses of KC13-M2G2 on day 0, 14, and 28. **a-e** Plasma ALT, aspartate aminotransferase (AST), total bilirubin (BIL-T), blood urea nitrogen (BUN) and alkaline phosphatase (ALP) were tested by biochemical analyzer on day -7 and 30. **f** Body weights were measured at certain timepoints. **g** Summary of the range of microscopic findings based on severity grade. Tissues were harvested on day 30, and stained with hematoxylin and eosin

(H&E). Histopathologic evaluation was conducted in a blinded manner by at least two experienced pathologists. Histopathology grades were assigned as minimal (1), mild (2), moderate (3), and severe (4). Data are shown as mean ± SEM (n=10). Only statistically significant differences are indicated (one-way ANOVA test or Kruskal-Wallis test with Dunn's post-hoc test for multiple groups).

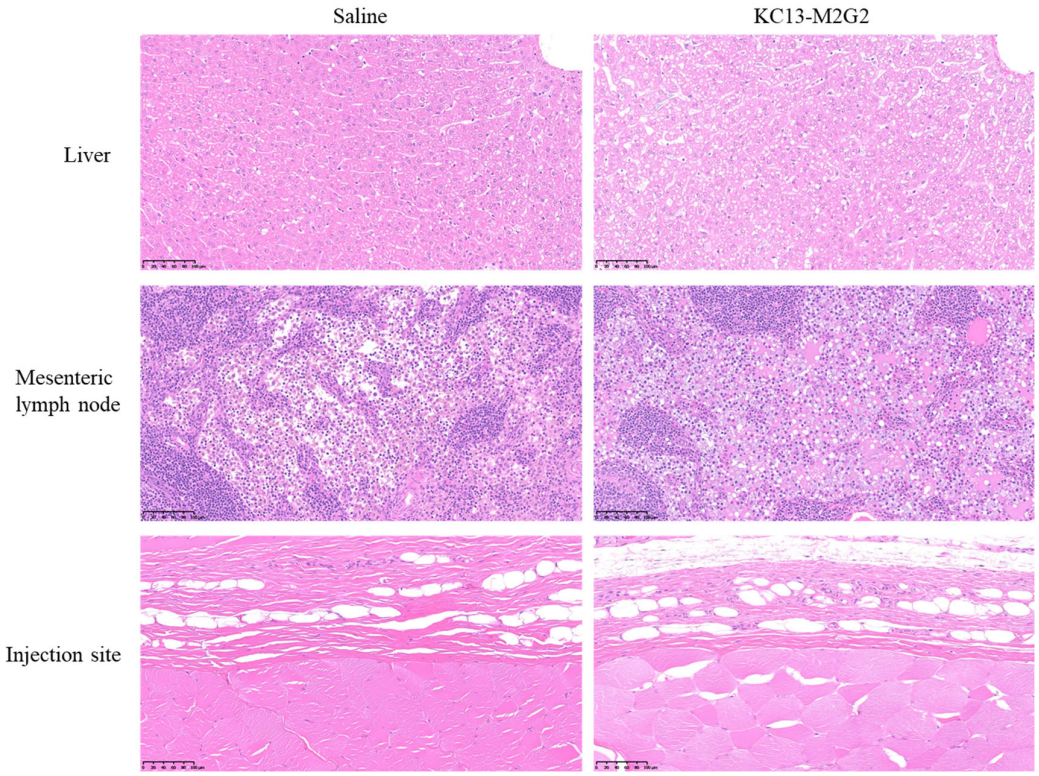

Fig. R8: **Representative H&E-stained liver, mesenteric lymph node, and injection site tissue sections from cynomolgus monkeys treated with KC13-M2G2 or saline.** Monkeys received three repeat doses of KC13-M2G2 on day 0, 14, and 28. Tissues were harvested on day 30, and stained with H&E.

- We agree that efficacy evaluation in monkeys is often preferred. However, HBV exhibits a highly restricted species tropism, primarily infecting humans, chimpanzees, and tree shrews. Among these, chimpanzees have the closest genetic and immunological homology with humans, but the research on chimpanzees is no longer permitted in most countries due to ethical considerations. We also investigated the monkey models for HBV infection. Although woolly monkeys and capuchin monkeys have been found to be naturally infected with HBV, their highly endangered status makes them unsuitable for research[3]. Scientists have also observed that exogenous expression of human sodium taurocholate co-transporting polypeptide (hNTCP) on rhesus macaque hepatocytes *in vivo* achieved only low levels of viral replication and liver dissemination[3,4]. A stringent immunosuppression regimen enabled sustained viral replication in hNTCP rhesus macaques. However, serum virological biomarkers fell precipitously following the

tapering and cessation of immunosuppression[5]. Therefore, it seems additional efforts are needed to establish robust non-human primate models for evaluating the efficacy of HBV therapeutic agents.

- We have added the text to Discussion: "Scientists have devoted many efforts to establishing non-human primate models of chronic HBV infection. Woolly monkeys and capuchin monkeys have been found to be naturally infected with HBV, but their highly endangered status makes them unsuitable for research[43]. Exogenous expression of human sodium taurocholate co-transporting polypeptide (hNTCP) on rhesus macaque hepatocytes *in vivo* achieved only low levels of viral replication and liver dissemination[43,44]. A stringent immunosuppression regimen enabled sustained viral replication in hNTCP rhesus macaques. However, serum virological biomarkers fell precipitously following the tapering and cessation of immunosuppression[45]." The references have been added accordingly (references 43 and 44).

- It has recently been reported that siRNA-induced HBsAg suppression is associated with ameliorated T cell exhaustion in humans (EASL 2025 oral abstract #071). The findings from the current study were opposite to what was reported in humans.

Response: Thanks for raising this important point. Similar phenomenon was also reported that HBsAg reduction mediated by imdusiran was associated with increased activation of HBV-specific T cells and a decrease in exhausted $CD8^+$ T cells (EASL 2022 SAT397). All these findings confirm that siRNA-induced HBsAg suppression contributes to the reconstruction of host immune function.

- In our study, minor responses for HBsAg-specific T cells were observed in rAAV-HBV mice. We have discussed the reasons in Discussion: "Minor changes of HBsAg-specific T cell immune response were observed in our study. This probably because the reactivation of HBsAg-specific T cell had occurred before tissue sampling on day 56. Unlike immunotherapeutic agents which could induce strong cellular immune responses evaluated at 1 or 2 weeks post dosing[37,38], siRNA could not directly trigger immune responses, and it depended on the recognition of antigens by host recovered T cells. Therefore, it seemed difficult to find out the optimal time point for reactivation of cellular immune responses." Considering the differences in susceptibility and immune response to HBV between mouse models and humans, clinical data are more compelling. We prefer to investigate the reconstruction of host immune function during the clinical trials of KC13-M2G2.

- We have revised the text (Discussion) from "AB-729 is another GalNAc-conjugated siRNA that entered phase II clinical study[21,39]. Increased HBV-specific T-cell activation and decreased exhausted CD8 T-cells were observed in some patients with significant reduction of HBsAg mediated by AB-729 treatment, indicating that suppression of HBsAg indeed offer chances for the reconstruction of cellular immune response against HBV[40]." to "In the clinical study of imdusiran, scientists observed that HBsAg reduction mediated by imdusiran was associated

with increased activation of HBV-specific T cells and a decrease in exhausted $CD8^+$ T cells[39]. The clinical study of Daplusiran/Tomligisiran and ARC-520 also showed that siRNA-induced HBsAg suppression is associated with ameliorated T cell exhaustion[40]. These findings confirm that siRNA-induced HBsAg suppression contributes to the reconstruction of host immune function." The references have been updated accordingly (references 39 and 40).

- P.29-30: antiviral activity evaluation in rAAV-HBV mice and HBV-transgenic mice: 'KC13-M2G1' and 'KC13-M1G1' were given instead of 'KC13-M2G2'.

Response: The siRNAs KC13-M2G1 and KC13-M1G1 were used in the immune response evaluation in our early study. Both share the same nucleotide sequence as KC13-M2G2 (KC13), but differ in their GalNAc ligands (G1 vs. G2) and modification patterns (M1 vs. M2).

Reviewer #3 (Remarks to the Author):
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: Thanks for the review on our manuscript.

References

1. Tang, H. *et al*. The transcriptional transactivation function of HBx protein is important for its augmentation role in hepatitis B virus replication. *J Virol.* **79**, 5548-5556 (2005).
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4. Burwitz, B. J. *et al*. Hepatocytic expression of human sodium-taurocholate cotransporting polypeptide enables hepatitis B virus infection of macaques. *Nat Commun*. **8**, 2146 (2017).
5. Biswas, S. *et al*. Long-term hepatitis B virus infection of rhesus macaques requires suppression of host immunity. *Nat Commun.* **13**, 2995 (2022).

Point-by-point Response to Reviewers' Comments:

We sincerely thank all reviewers for their insightful comments, which have improved our manuscript. Our point-by-point responses are provided in purple text below. To distinguish the current revisions from those made in the first round, all new changes in the manuscript text and figures are also highlighted in purple.

Reviewer #1 (Remarks to the Author):

The revised manuscript shows considerable improvement, with added details on siRNA screening, efficacy data, and comparative analyses that enhance the study's completeness. However, several issues remain to be clarified.

While the authors describe the chemical modification types, the exact modification sites and whether systematic comparisons of different modification patterns were performed remain unclear.

Response: We thank the reviewer for raising this important point.

- To address this concern, we provide further explanations of our screening process and results. In the second screening round, we compared various modification patterns applied to the same nucleotide sequences (Fig R1, now Supplementary Fig. 1b). The results demonstrate that the efficacy of a modification pattern is strongly sequence-dependent. For instance, for nucleotide sequence KC09, modification pattern M5 showed the most potent suppression of HBsAg, while M6 showed the least (KC09-M5 vs. KC09-M6). In contrast, this efficacy trend was reversed for nucleotide sequence KC04 (KC04-M5 vs. KC04-M6). For some other nucleotide sequences, such as KC11, KC12, KC13, and KC14, all modification patterns showed potent efficacy under this experimental condition.

- All oligonucleotide sequences used in this study and their modification patterns have been provided in the Supplementary Data file.

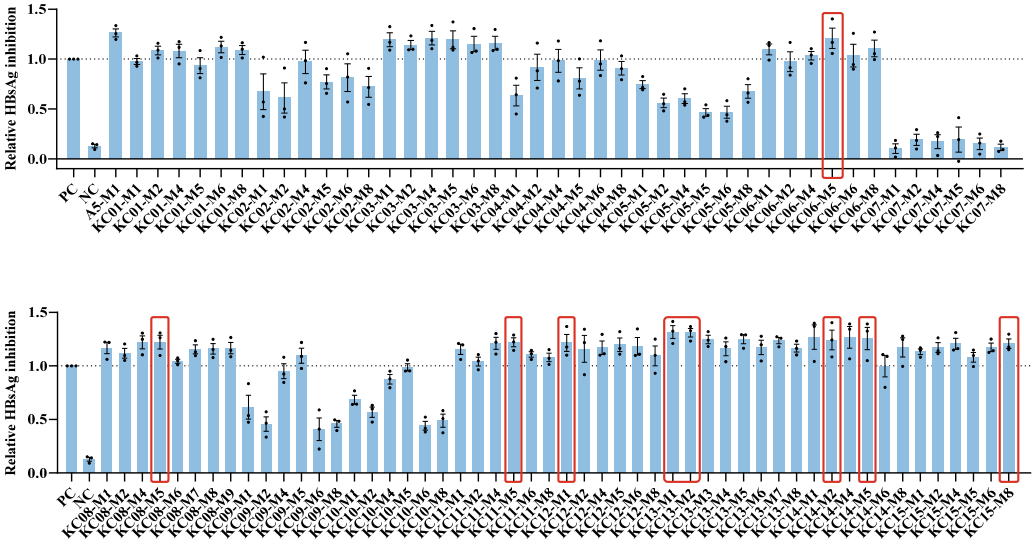

Fig R1: *In vitro* screening of anti-HBV siRNA agents in HepG2.2.15 cells. The second screening round identified optimal modification patterns, from which nine candidates were selected for further evaluation in rAAV-HBV mice. The siRNAs were transfected into cells at 0.3 nM. On day 6 post-transfection, HBsAg in supernatants were quantified using chemiluminescence immunoassay (CLIA) commercial Kits. Data are shown as mean ± SEM (n=3 biologically independent experiments). Source data are provided as a Source Data file.

The efficacy evaluation relies mainly on serum biomarkers, lacking intrahepatic indicators such as HBV RNA, cccDNA or vector DNA residues to evaluate the pattern of viral suppression.

Response: We thank the reviewer for raising this important point. In fact, we assessed the intrahepatic antiviral activity of KC13-M2G2 in rAAV-HBV (genotype D) mice by measuring HBV RNA levels via RT-qPCR on day 70. The results revealed a dose-dependent reduction in intrahepatic HBV S RNA and total HBV RNA levels following KC13-M2G2 treatment (Fig. R2), consistent with the changes observed in serum virological indicators (Fig. 4).

- Accordingly, we have added the text "Analysis of liver tissue by RT-qPCR at the terminal timepoint (Day 70) also revealed a dose-dependent reduction in intrahepatic HBV S RNA and total HBV RNA levels following KC13-M2G2 treatment (Supplementary Fig. 8)." to the Results section, and the text "For measurement of intrahepatic HBV RNA levels, total 50 mg liver tissue was snap frozen in liquid nitrogen and ground into powder. Total RNA was extracted with RNAprep Pure Animal Tissue Total RNA Extraction Kit (TIANGEN) and reverse transcripted into cDNA using GoScript Reverse Transcription Mix (Promega). QPCRs were performed using GoTaq qPCR Master Mix (Promega) with primers listed in Supplementary Table 5. GAPDH was used for normalization." to the Method section.
- Fig R2 has been included in the revised Supplementary Information as Supplementary Fig. 8.

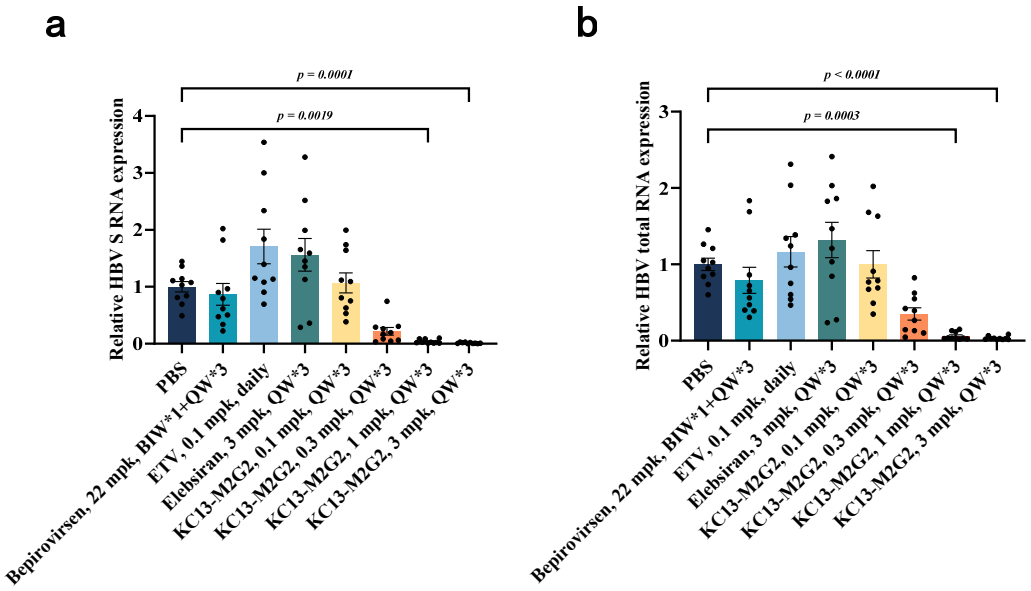

Fig R2: Intrahepatic HBV RNA levels corresponding to Fig. 4. The intrahepatic HBV S RNA **(a)** and total RNA **(b)** levels were measured at the terminal timepoint (Day70) by RT-qPCR. Data are shown as mean ± SEM (n=10 mice). Only statistically significant differences are indicated (Kruskal-Wallis test with Dunn's post-hoc test for multiple groups). Source data are provided as a Source Data file.

The immunogenicity assessment is still limited — only IL-2, IL-6, IL-10, TNF-α, and IFN-γ were measured, without inclusion of type I interferons (IFN-α/β), which are critical for assessing innate immune activation by exogenous nucleic acids.
Additional clarification or expanded discussion would strengthen the work.

Response: We thank the reviewer for raising this insightful point. It is unfortunate that type I interferons were not included in the immunogenicity assessment. Studies had revealed that synthetic RNA can be mistakenly recognized as viral RNA by the host's innate immune receptors, including endosomal and cytosolic pattern recognition receptors such as Toll-like receptor (TLR)-3, TLR-7/8, retinoic acid-inducible gene I-like receptors (RLR), and protein kinase R (PKR)[1-4]. This recognition triggers an antiviral immune response, leading to the production of type I interferons (IFN-α/β). The production of type I interferons was often accompanied by the production of inflammatory cytokines including IL-6 and TNF-α, suggesting general activation of the innate immune response[1,5]. Therefore, although IFN-α/β were not measured here, the levels of these other cytokines provide a reliable view of the innate immune activation status for the purpose of immunotoxicity assessment. Furthermore, our future work will characterize the immune profile of KC13-M2G2 in both healthy and HBV-infected animal models to elucidate its mechanism of action in achieving a functional cure.

- For oligonucleotide-based therapeutics, chemical nucleotide modifications are

typically introduced to effectively prevent immunostimulation. But in the context of HBV treatment, appropriate immunostimulation will improve clinical outcomes. Recent studies have reported that bepirovirsen induced a potent innate immune response in both chronic hepatitis B patients and healthy volunteers, further confirming its immunostimulatory property[6]. Moreover, the clinical B-Fine study data strongly suggested that bepirovirsen triggered a humoral immune response in liver and periphery in treated patients[7]. As described above, clinical trials also showed siRNA treatment was associated with increased HBV-specific T cells. It seems there are different immunostimulatory mechanisms for ASO and siRNA drugs. Although heavily modified siRNAs are theoretically designed to eliminate immunostimulation, more experimental evidences are still needed.

- As suggested by the reviewer, we have expanded the Discussion section to include these points.

Overall, the revision represents substantial progress and clearer data presentation, demonstrating strong HBsAg reduction. Nonetheless, mechanistic insight remains limited, and the current evidence cannot yet distinguish whether the superior efficacy derives from target selection, chemical modification, or delivery optimization, or potentially other contributing factors.

Response: We appreciate the reviewer's valuable suggestion to explore the mechanism in greater depth. To this end, a key focus of our future work will be a detailed investigation of the KC13-M2G2 immune profile in both healthy and HBV-infected animal models, as well as in clinical trials.

References

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7. Andre, B. *et al*. Intrahepatic and peripheral immunophenotyping of participants with chronic hepatitis B receiving bepirovirsen in the B-fine study strongly suggests that bepirovirsen may trigger a humoral response. Abstract 1215, AASLD (2025).

Reviewer #2 (Remarks to the Author):
The authors had revised satisfactorily to the suggestions and comments made previously. I have no further requests.

Response: Thanks again for the review on our manuscript.

Reviewer #3 (Remarks to the Author):
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: Thanks again for the review on our manuscript.