Article type: Meta-analysis

Title: Efficacy and Safety of HBV RNA-Targeting Oligonucleotide Therapies in Nucleos(t)ide Analogue-Suppressed Chronic Hepatitis B: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Clinical Question Box

In patients with virologically suppressed chronic hepatitis B receiving stable nucleos(t)ide analogue therapy, do HBV RNA-targeting oligonucleotides improve treatment outcomes?

HBV RNA-targeting oligonucleotides improve HBsAg responses when added to stable nucleos(t)ide analogue therapy, but they are associated with increased adverse events. The certainty of evidence was moderate for HBsAg loss, achievement of low HBsAg levels, and safety outcomes, but low for quantitative HBsAg reduction. End-of-treatment HBsAg responses should not be interpreted as equivalent to durable functional cure.

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ABSTRACT

Background: RNA-targeting oligonucleotides are being developed to reduce hepatitis B surface antigen (HBsAg) and facilitate functional cure in chronic hepatitis B (CHB). We evaluated the efficacy and safety of small interfering RNA (siRNA) and antisense oligonucleotide (ASO) therapies in patients with virologically suppressed CHB receiving stable nucleos(t)ide analogue (NA) therapy.

Methods: PubMed/MEDLINE, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science were searched from inception through September 20, 2026. Randomized controlled trials comparing an HBV RNA-targeting oligonucleotide plus stable NA therapy with placebo or no active investigational oligonucleotide plus the same NA regimen were eligible. Random-effects meta-analyses used risk differences (RDs) for binary outcomes and mean differences (MDs) for continuous outcomes. Certainty of evidence was assessed using GRADE.

Results: Thirteen publications representing 15 randomized trial units and 2,520 participants were included. RNA-targeting therapy increased HBsAg loss at study-reported assessment time points (RD, 0.21; 95% CI, 0.02 to 0.39; I^2=97%) and achievement of HBsAg <100 IU/mL at study-reported assessment time points (RD, 0.74; 95% CI, 0.66 to 0.82; I^2=83%) and produced greater quantitative HBsAg reduction (MD, -0.85 log10 IU/mL; 95% CI, -1.14 to -0.55; I^2=93%). Any adverse event (RD, 0.19; 95% CI, 0.12 to 0.27; I^2=53%) and serious adverse events (RD, 0.03; 95% CI, 0.01 to 0.04; I^2=0%) were more frequent with RNA-targeting therapy. Certainty was moderate for HBsAg loss and the other key outcomes, but low for quantitative HBsAg reduction.

Conclusions: HBV RNA-targeting oligonucleotides added to stable NA therapy improve HBsAg responses at study-reported time points in virologically suppressed CHB but increase

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adverse events. Substantial heterogeneity and the distinction between HBsAg response and durable functional cure warrant cautious interpretation and longer off-treatment follow-up.

Keywords: chronic hepatitis B; hepatitis B surface antigen; antisense oligonucleotide; small interfering RNA; functional cure; meta-analysis

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a major global public health problem and a leading cause of cirrhosis, hepatic decompensation, and hepatocellular carcinoma.[1] In 2022, an estimated 254 million people worldwide were living with chronic HBV infection, corresponding to approximately 3.3% of the global population. During the same year, approximately 1.2 million new chronic HBV infections occurred and an estimated 1.1 million deaths were attributable to HBV, predominantly through complications of advanced liver disease and hepatocellular carcinoma.[2] These figures underscore the substantial residual disease burden despite widespread vaccination and the availability of effective antiviral therapy.

Oral nucleos(t)ide analogues (NAs) effectively suppress HBV replication and reduce the risk of disease progression, but they generally require prolonged treatment and infrequently result in hepatitis B surface antigen (HBsAg) loss.[3,4] HBsAg loss is associated with durable control of HBV infection and improved clinical outcomes and has therefore become a central therapeutic endpoint in chronic hepatitis B (CHB). Current therapeutic development increasingly focuses on achieving a functional cure, generally defined as sustained HBsAg loss with suppression of HBV DNA after completion of finite therapy.[5] However, functional cure remains uncommon with currently available NA- or pegylated interferon-based treatment, leaving a substantial unmet need for finite therapies capable of producing sustained antigen reduction and virologic control.

Reduction of the viral antigen burden has consequently emerged as an important therapeutic strategy. Persistent production of HBV proteins, particularly HBsAg, originates from transcriptionally active covalently closed circular DNA and, in many patients, integrated HBV DNA, neither of which is directly eliminated by conventional NAs.[6] RNA-targeting oligonucleotides act upstream of viral protein production. Small interfering RNAs (siRNAs)

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induce sequence-specific degradation of HBV RNA through the RNA-induced silencing complex, whereas antisense oligonucleotides (ASOs) bind complementary viral RNA and promote transcript degradation through mechanisms including RNase H activity. These approaches can therefore reduce viral antigen production while background NA therapy continues to suppress HBV replication.[7]

Clinical development has progressed from early proof-of-concept studies to large randomized trials. In the phase 2 B-Clear trial, bepirovirsen produced sustained virologic responses in a subset of participants, and the subsequent B-Well 1 and B-Well 2 phase 3 trials demonstrated functional cure after finite treatment in approximately one fifth of NA-suppressed participants receiving bepirovirsen, compared with none receiving placebo.[8,9] Randomized trials of siRNA-based therapies, including xalnesiran, have likewise demonstrated substantial HBsAg reductions and HBsAg loss in selected patients, although responses have varied according to regimen and baseline characteristics.[10]

Despite this expanding evidence base, the magnitude and consistency of benefit attributable specifically to RNA-targeting oligonucleotides remain difficult to define. Trials differ substantially in molecular class, target, dose, treatment duration, baseline HBsAg concentration, HBeAg status, follow-up, and efficacy endpoints. Some studies have also combined RNA-targeting agents with immunomodulators or other investigational therapies, making the independent contribution of the oligonucleotide difficult to isolate.[11,12] A synthesis restricted to randomized comparisons in virologically suppressed patients receiving stable background NA therapy may therefore provide a more clinically interpretable estimate of the incremental efficacy and safety of these agents.

This systematic review and meta-analysis of randomized controlled trials evaluated the efficacy and safety of HBV RNA-targeting oligonucleotide therapies added to stable nucleos(t)ide analogue treatment in patients with virologically suppressed chronic hepatitis B. Outcomes

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included HBsAg loss, achievement of low HBsAg levels, change in quantitative HBsAg, and treatment-emergent adverse events. Differences in treatment effects between small interfering RNA- and antisense oligonucleotide-based therapies were also explored.

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METHODS

Reporting Guideline

This systematic review and meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement and methodological guidance from the Cochrane Handbook for Systematic Reviews of Interventions.[13,14] The review protocol was prospectively registered with the Open Science Framework (OSF; https://doi.org/10.17605/OSF.IO/KC6F5).[15]

Eligibility Criteria

Inclusion criteria were: (1) prospective randomized controlled trials enrolling adults (≥18 years) with CHB who were virologically suppressed on stable NA therapy; (2) evaluation of a finite-course HBV RNA-targeting oligonucleotide, including siRNA or ASO agents, added to unchanged background NA therapy; (3) comparison with placebo or no active investigational oligonucleotide with the same background NA regimen; and (4) reporting randomized data for at least one prespecified efficacy or safety outcome. Trials enrolling mixed treatment populations were eligible only when data for the NA-suppressed subgroup were separately available. For crossover trials, only randomized pre-crossover data were considered when a concurrent untreated control was required.

Exclusion criteria were: (1) nonrandomized studies, single-arm studies, observational studies, reviews, and preclinical studies; (2) randomized comparisons in which the incremental effect of the RNA-targeting oligonucleotide could not be isolated; and (3) regimens incorporating pegylated interferon or another active immunomodulator as part of the randomized treatment strategy for comparisons intended for the primary synthesis. For multiarm trials, only eligible randomized contrasts were included. No restrictions were applied by language or publication status.

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Search Strategy

PubMed, Embase, CENTRAL, and Web of Science were searched from database inception through September 20, 2026. Search strategies combined controlled vocabulary and free-text terms related to CHB, antisense oligonucleotides, and siRNA. The complete database-specific search strategies and record counts are provided in Supplementary Table S1.

Study Selection

All retrieved records were deduplicated before screening. Two reviewers independently screened titles and abstracts, followed by full-text assessment of potentially eligible reports. Disagreements were resolved by discussion and, when necessary, consultation with a third reviewer. Multiple reports from the same randomized trial were linked and treated as a single study unless they represented independently randomized trial units.

Data Extraction

Two reviewers (M.A. and S.P.) independently extracted data using a standardized form. Extracted variables included trial and registration identifiers, study phase, geographical region, sample size, participant age and sex, baseline HBsAg level, HBeAg status, baseline HBV DNA, prior NA therapy, investigational agent and molecular class, dose and schedule, treatment duration, concomitant therapy, follow-up duration, and prespecified efficacy and safety outcomes. For binary outcomes, event counts and numbers assessed in each randomized group were extracted; for continuous outcomes, means, SDs, and sample sizes were recorded. For multiarm trials, eligible intervention arms were combined when they represented the same prespecified comparison. Shared control groups were handled to avoid double counting. Independently randomized subtrials reported within a single publication were analyzed as separate trial units.

Outcomes and Definitions

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The primary efficacy outcome was HBsAg loss, defined according to the assay-specific threshold used in each trial. Study-reported HBsAg-loss assessments were extracted at selected on-treatment or post-treatment time points. For trials evaluating multiple doses, preference was given to doses supported by subsequent clinical development; otherwise, eligible dose groups were combined, with only one comparison included per randomized trial unit. Secondary efficacy outcomes included HBsAg <100 IU/mL at study-reported assessment time points, quantitative change in HBsAg from baseline, functional cure, off-treatment HBsAg loss, prespecified categorical HBsAg reductions, and eligibility for NA discontinuation. Functional cure was defined according to individual trial criteria and generally required sustained HBsAg loss with suppressed HBV DNA after finite therapy. Functional cure and sustained off-treatment outcomes were considered separately from HBsAg loss. Safety outcomes included treatment-emergent adverse events, serious adverse events, grade ≥3 adverse events, treatment discontinuation due to adverse events, and hepatic adverse events, according to trial-specific definitions. In B-Clear, the HBsAg-loss contrast (bepirovirsen Arm 3 vs. placebo Arm 4) was assessed at Week 12, before the placebo-to-bepirovirsen crossover; later placebo-controlled comparisons were not used.

Risk of Bias Assessment

Two reviewers independently assessed risk of bias using the Cochrane Risk of Bias 2 (RoB 2) tool for randomized trials, considering the effect of assignment to intervention.[16] Assessments covered the five standard RoB 2 domains, with each domain and the overall risk of bias rated as low risk, some concerns, or high risk. Disagreements were resolved by consensus.

Statistical Analysis

Meta-analysis was performed when at least two clinically comparable randomized comparisons reported the same outcome at trial-defined assessment time points. Binary outcomes were

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summarized as risk differences (RDs) and continuous outcomes as mean differences (MDs), each with 95% confidence intervals (CIs). Random-effects models were prespecified because of anticipated clinical heterogeneity across agents, doses, treatment durations, and study phases. Binary outcomes were pooled using the Mantel–Haenszel method, whereas continuous outcomes were synthesized using the inverse-variance method. Studies with zero events in both groups were retained in RD analyses. Statistical heterogeneity was assessed using Cochran's Q and the I^2 statistic. Prespecified subgroup analysis compared siRNA and ASO therapies, with subgroup differences evaluated using formal tests for interaction. All meta-analyses were performed using Review Manager (RevMan), version 5.4.1 (The Cochrane Collaboration).

Reporting Bias and Certainty of Evidence

Funnel plots were used to explore potential small-study effects when at least 10 comparisons were available; for outcomes with fewer than 10 comparisons, funnel plots were considered descriptive only. The certainty of evidence for the primary and key secondary outcomes was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach across the domains of risk of bias, inconsistency, indirectness, imprecision, and publication bias.[17] Certainty was rated as high, moderate, low, or very low.

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RESULTS

Study Selection and Characteristics

The database search identified 2,251 records. After removal of 311 duplicates, 1,940 records underwent title and abstract screening, of which 1,814 were excluded. Among 126 full-text reports assessed for eligibility, 113 were excluded, leaving 13 publications representing 15 independent randomized trial units for inclusion in the meta-analysis (Figure S1). The 15 randomized trial units included 2,520 participants, of whom 1,756 received an RNA-targeting oligonucleotide and 764 received a concurrent control.[8-10,18-27] The included trials evaluated the siRNA agents ARC-520, elebsiran (VIR-2218), and xalnesiran (RG6346), and the ASO agents bepirovirsen, RO7062931, GSK3389404, AHB-137, and ALG-020572, spanning phase 1 through phase 3 development.6-8,13-22 All eligible randomized comparisons involved participants with virologically suppressed CHB receiving stable background NA therapy. Detailed study characteristics are presented in Table 1.

HBsAg Loss

Nine comparisons involving 1,999 participants contributed to the analysis. HBsAg loss occurred in 611 of 1,366 participants (44.7%) receiving an HBV RNA-targeting oligonucleotide and in none of 633 control participants. The pooled RD was 0.21 (95% CI, 0.02 to 0.39; I^2=97%) (Figure 1). In subgroup analyses, the RD was 0.04 (95% CI, −0.02 to 0.11; I^2=0%) for siRNA therapies and 0.27 (95% CI, 0.10 to 0.44; I^2=96%) for ASO therapies, with a statistically significant subgroup interaction (P=.014). The B-Clear contribution was the pre-crossover Week 12 Arm 3 versus Arm 4 comparison (7/68 vs. 0/23). In a post hoc sensitivity analysis, exclusion of B-Clear yielded a similar pooled effect (RD, 0.22; 95% CI, 0.03 to 0.42; I^2=97%; eight comparisons; 1,908 participants), indicating that heterogeneity was not explained by this trial alone.

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Achievement of HBsAg <100 IU/mL at Study-Reported Assessment Times

Six comparisons involving 1,886 participants contributed to this analysis. HBsAg <100 IU/mL was achieved in 1,044 of 1,267 participants (82.4%) receiving RNA-targeting therapy and 7 of 619 participants (1.1%) receiving control treatment. The pooled RD was 0.74 (95% CI, 0.66 to 0.82; I^2=83%) (Figure 2). The RD was 0.60 (95% CI, 0.46 to 0.74; I^2=0%) for siRNA therapies and 0.79 (95% CI, 0.72 to 0.87; I^2=88%) for ASO therapies. The subgroup interaction was significant (P=.01).

Quantitative HBsAg Reduction at Study-Reported Assessment Times

Nine comparisons involving 230 participants contributed to the analysis. RNA-targeting oligonucleotide therapy was associated with a greater reduction in HBsAg than control treatment (MD, −0.85 log10 IU/mL; 95% CI, −1.14 to −0.55; I^2=93%) (Figure S2). The MD was −0.97 log10 IU/mL (95% CI, −1.55 to −0.40; I^2=96%) for siRNA therapies and −0.64 log10 IU/mL (95% CI, −0.92 to −0.36; I^2=77%) for ASO therapies, with no significant subgroup interaction (P=.31).

Any Adverse Event

Thirteen comparisons involving 2,467 participants contributed to this analysis. Adverse events occurred in 1,461 of 1,718 participants (85.0%) receiving HBV RNA-targeting oligonucleotides and 476 of 749 participants (63.6%) receiving control treatment. The pooled RD was 0.19 (95% CI, 0.12 to 0.27; I^2=53%) (Figure 3). The RD was 0.11 (95% CI, −0.04 to 0.27; I^2=33%) for siRNA therapies and 0.23 (95% CI, 0.13 to 0.32; I^2=61%) for ASO therapies, with no significant subgroup interaction (P=.21).

Serious Adverse Events

Thirteen comparisons involving 2,467 participants contributed to this analysis. Serious adverse events occurred in 68 of 1,718 participants (4.0%) receiving HBV RNA-targeting

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oligonucleotides and 10 of 749 participants (1.3%) receiving control treatment. The pooled RD was 0.03 (95% CI, 0.01 to 0.04; I^2=0%) (Supplementary Figure S3). The RD was 0.06 (95% CI, 0.00 to 0.11; I^2=0%) for siRNA therapies and 0.03 (95% CI, 0.01 to 0.04; I^2=0%) for ASO therapies, with no significant subgroup interaction (P=.30). In the phase 3 B-Well trials, functional cure at Week 72 was achieved in 20% and 19% of bepirovirsen-treated participants, respectively, compared with none in the placebo groups. ALT elevations (≥3× ULN) and adverse-event-related treatment discontinuations were also more frequent with bepirovirsen.

Reporting Bias

Funnel plots for HBsAg loss, HBsAg <100 IU/mL, and quantitative HBsAg reduction were interpreted descriptively because fewer than 10 comparisons contributed to these outcomes (Figures S4-S6). Visual inspection of the funnel plots for adverse events and serious adverse events did not indicate clear asymmetry (Figures S7 and S8).

Risk of Bias

Seven of the 15 randomized trial units were judged to be at low overall risk of bias, 7 had some concerns, and 1 was judged to be at high risk (Figure S9). Most concerns reflected incomplete reporting of randomization procedures in early-phase trials, whereas outcome measurement was generally at low risk because efficacy outcomes were based on objective laboratory measurements. The high-risk assessment primarily reflected concerns related to missing outcome data.

Certainty of Evidence

The certainty of evidence was moderate for HBsAg loss, HBsAg <100 IU/mL, any adverse event, and serious adverse events, but low for quantitative HBsAg reduction (Table 2). HBsAg loss was downgraded one level for inconsistency (I^2=97%, variable assessment times and marked between-regimen differences). HBsAg <100 IU/mL and any adverse event were

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downgraded one level for inconsistency; quantitative HBsAg reduction was downgraded for risk of bias and inconsistency, and serious adverse events for imprecision. Publication bias was not considered sufficiently established to warrant additional downgrading.

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DISCUSSION

In this systematic review and meta-analysis of randomized trials in patients with virologically suppressed CHB receiving stable background NA therapy, HBV RNA-targeting oligonucleotides improved HBsAg-related responses compared with control treatment. Higher rates of HBsAg loss and achievement of HBsAg <100 IU/mL were observed, together with greater quantitative HBsAg reduction, although assessment time points differed for HBsAg loss and quantitative HBsAg reduction. These benefits were accompanied by more adverse events and a smaller absolute increase in serious adverse events. By restricting the analysis to randomized comparisons in NA-suppressed patients, the present study more specifically estimates the additional effect of RNA-targeting therapy when viral replication is already effectively controlled.

The magnitude and variability of the HBsAg response may be partly explained by the biology of HBsAg production. During long-term NA therapy, circulating HBsAg may originate not only from transcriptionally active covalently closed circular DNA but also, particularly in some HBeAg-negative patients, from integrated HBV DNA.[28] This distinction is relevant to RNA-targeting therapy because activity depends on whether the selected RNA sequence is retained in transcripts derived from these different viral templates. The experience with ARC-520 provided an early clinical example of this principle, as many integration-derived HBV transcripts lacked the siRNA target sites contained in ARC-520, contributing to the attenuated HBsAg response observed particularly in HBeAg-negative patients.[29] Differences in target sequence, hepatocyte delivery, intracellular persistence, dose, and treatment duration may therefore contribute to the heterogeneity observed across trials and may be more informative than a simple ASO-versus-siRNA classification.[20,30,31]

The clinical significance of profound HBsAg reduction ultimately depends on whether it translates into durable off-treatment control. Current treatment-endpoint consensus therefore

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defines functional cure by sustained HBsAg loss with suppression of HBV DNA after finite therapy, rather than by an on-treatment response alone.[32] This distinction was evident in the phase 3 B-Well trials, in which functional cure was achieved in only a subset of treated patients despite substantial HBsAg reduction.[8] Baseline HBsAg may help identify patients more likely to achieve such responses: in PIRANGA, HBsAg loss occurred only among participants with screening HBsAg levels below 1000 IU/mL, while B-Clear similarly identified lower baseline HBsAg as a predictor of response.[10] Unlike the earlier APASL meta-analysis, which assessed functional cure across various investigational HBV therapies, this review focuses on HBsAg responses to RNA-targeting oligonucleotides in NA-suppressed patients.[33]

The subgroup analyses should also be interpreted within this mechanistic context. Significant subgroup interactions between ASO and siRNA therapies were observed for HBsAg loss and achievement of HBsAg <100 IU/mL, but not for quantitative HBsAg reduction. Because molecular class was closely linked to individual compound, trial phase, treatment duration, and study size across the included trials, the subgroup analysis cannot distinguish a true platform effect from differences in study and treatment characteristics. In addition, some oligonucleotides may have biological effects beyond transcript degradation.[34] Bepirovirsen, for example, has been associated with innate immune activation as well as antisense-mediated suppression of HBV transcripts.[35] Such agent-specific properties further argue against interpreting pooled class comparisons as direct comparisons of molecular mechanisms. An apparent ASO–siRNA subgroup difference does not establish class-level superiority for durable functional cure.

Safety likewise appears to require interpretation at the level of the individual compound. Although overall adverse events were increased, serious adverse events remained uncommon. Hepatic biochemical abnormalities are particularly important because ALT elevations may reflect different processes. Transient ALT elevations accompanying HBsAg decline during

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bepirovirsen treatment have been associated with immune activation, whereas development of ALG-020572 was discontinued after drug-induced liver injury.[22,36] ALT elevation should therefore not be treated as a uniform pharmacodynamic marker. Future trials should distinguish immune-associated hepatic flares from drug-induced hepatotoxicity using their temporal relationship with viral markers, biochemical severity, clinical manifestations, and subsequent treatment response. In B-Well, ALT elevations sometimes accompanied marked HBsAg declines, but aggregate reports cannot establish whether ALT elevation independently predicts HBsAg loss or functional cure; clinically significant ALT elevation also requires assessment for drug-related liver injury.

Several limitations should be considered. The substantial heterogeneity across efficacy outcomes limits interpretation of a single pooled treatment effect, and aggregate study-level data did not permit reliable assessment of potentially important modifiers such as baseline HBsAg, HBV genotype, transcript origin, or prior duration of NA therapy. Several early-phase comparisons were small, and some quantitative HBsAg estimates were derived from published figures. Quantitative HBsAg reduction and HBsAg loss were assessed at varying time points across trials, limiting comparability of pooled efficacy estimates. Comparable randomized off-treatment outcomes were unavailable for many agents, preventing meta-analysis of functional cure as a common endpoint. Finally, serious adverse events were uncommon, limiting the ability of pooled analyses to characterize rare or compound-specific toxicity. These limitations, particularly inconsistency in HBsAg-loss effects, are reflected in the moderate certainty assigned to the primary outcome.

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Conclusion

HBV RNA-targeting oligonucleotides provide substantial additional suppression of HBsAg in patients whose viral replication is already controlled with NA therapy. The magnitude of response appears to depend on the individual agent, viral transcript biology, and patient characteristics rather than on molecular class alone. Future development should determine which patients can convert antigen reduction into sustained off-treatment functional cure while defining the hepatic safety profile of individual compounds.

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Acknowledgement: None

Funding: This research received no external funding.

Author Contributions: M.A.A. and S.M.A. conceived the study; M.A.A., S.P., and S.U.R. conducted the literature search, data extraction, and analysis; M.A.A. and S.P. drafted the manuscript. All authors contributed to manuscript revision and approved the final version.

Competing Interests: The authors declare no competing interests.

Ethics Approval: Not applicable. This systematic review and meta-analysis used aggregate data from previously published studies and did not involve new recruitment of human participants or access to identifiable individual-level data.

Consent to Participate: Not applicable.

Consent for Publication: Not applicable.

Data Availability: All study-level data used in this meta-analysis were derived from published reports and supplementary materials. The data supporting the pooled analyses are presented in the manuscript and supplementary material.

Generative Artificial Intelligence Statement: During preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing and structural refinement. All AI-assisted content was reviewed and verified by the authors, who take full responsibility for the accuracy and integrity of the final manuscript.

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REFERENCES

1. Hsu YC, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. *Nat Rev Gastroenterol Hepatol*. Aug 2023;20(8):524-537. doi:10.1038/s41575-023-00760-9
2. Cui F, Faini D, Razavi-Shearer D, et al. The burden of chronic hepatitis B and C in 2022 and progress towards elimination: a global report. *Lancet Gastroenterol Hepatol*. Jun 2026;11(6):509-520. doi:10.1016/s2468-1253(25)00375-9
3. Liaw YF, Papatheodoridis G. Finite nucleos(t)ide analog therapy for functional cure in HBeAg-negative chronic hepatitis B: Recent development in the paradigm shift. *Hepatology*. Mar 1 2026;83(3):679-692. doi:10.1097/hep.0000000000001107
4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. Aug 2025;83(2):502-583. doi:10.1016/j.jhep.2025.03.018
5. Lim SG, Baumert TF, Boni C, et al. The scientific basis of combination therapy for chronic hepatitis B functional cure. *Nat Rev Gastroenterol Hepatol*. Apr 2023;20(4):238-253. doi:10.1038/s41575-022-00724-5
6. Taddese M, Grudda T, Belluccini G, et al. Transcription of hepatitis B surface antigen shifts from cccDNA to integrated HBV DNA during treatment. *J Clin Invest*. Jan 30 2025;135(6)doi:10.1172/jci184243
7. Hui RW, Mak LY, Fung J, Seto WK, Yuen MF. Prospect of emerging treatments for hepatitis B virus functional cure. *Clin Mol Hepatol*. Feb 2025;31(Suppl):S165-181. doi:10.3350/cmh.2024.0855
8. Hou J, Lim SG, Buti M, et al. Phase 3 Results of Bepirovirsen Treatment for Chronic Hepatitis B Virus Infection. *N Engl J Med*. Jun 25 2026;394(24):2395-2406. doi:10.1056/NEJMoa2515131
9. Yuen MF, Lim SG, Plesniak R, et al. Efficacy and Safety of Bepirovirsen in Chronic Hepatitis B Infection. *N Engl J Med*. Nov 24 2022;387(21):1957-1968. doi:10.1056/NEJMoa2210027
10. Hou J, Zhang W, Xie Q, et al. Xalnesiran with or without an Immunomodulator in Chronic Hepatitis B. *N Engl J Med*. Dec 5 2024;391(22):2098-2109. doi:10.1056/NEJMoa2405485
11. Kennedy PTF, Fung S, Buti M, et al. Peginterferon alpha-2a add-on to siRNA JNJ-73763989 in untreated patients with HBeAg-positive chronic hepatitis B virus (HBV) infection: the phase 2 REEF-IT study. *Gut*. Apr 7 2026;75(5):1043-1054. doi:10.1136/gutjnl-2025-336592
12. Yuen MF, Lim YS, Yoon KT, et al. VIR-2218 (elebsiran) plus pegylated interferon-alfa-2a in participants with chronic hepatitis B virus infection: a phase 2 study. *Lancet Gastroenterol Hepatol*. Dec 2024;9(12):1121-1132. doi:10.1016/s2468-1253(24)00237-1
13. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj*. Mar 29 2021;372:n71. doi:10.1136/bmj.n71
14. Cumpston M, Li T, Page MJ, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. *Cochrane Database Syst Rev*. Oct 3 2019;10(10):Ed000142. doi:10.1002/14651858.Ed000142
15. Open Science Framework. Efficacy And Safety Of RNA-Targeting Oligonucleotides In Virologically Suppressed Chronic Hepatitis B: A Systematic Review And Meta-Analysis Of Randomized Controlled Trials. July 1 2026. September 2026. https://osf.io/kc6f5/overview
16. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *Bmj*. Aug 28 2019;366:l4898. doi:10.1136/bmj.l4898
17. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *Bmj*. Apr 26 2008;336(7650):924-6. doi:10.1136/bmj.39489.470347.AD
18. Gane EJ, Kim W, Lim TH, et al. First-in-human randomized study of RNAi therapeutic RG6346 for chronic hepatitis B virus infection. *J Hepatol*. Nov 2023;79(5):1139-1149. doi:10.1016/j.jhep.2023.07.026
19. Gane E, Lim YS, Kim JB, et al. Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: Results from randomized clinical trials. *J Hepatol*. Oct 2023;79(4):924-932. doi:10.1016/j.jhep.2023.05.023
20. Wooddell CI, Yuen MF, Chan HL, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med*. Sep 27 2017;9(409)doi:10.1126/scitranslmed.aan0241
21. Yuen MF, Schiefke I, Yoon JH, et al. RNA Interference Therapy With ARC-520 Results in Prolonged Hepatitis B Surface Antigen Response in Patients With Chronic Hepatitis B Infection. *Hepatology*. Jul 2020;72(1):19-31. doi:10.1002/hep.31008
22. Agarwal K, Forton D, Zen Y, et al. ALG-020572, an Antisense Oligonucleotide for the Treatment of Chronic Hepatitis B Virus Infection Discontinued for Drug-Induced Liver Injury. *J Viral Hepat*. Sep 2026;33(9):e70224. doi:10.1111/jvh.70224
23. Gane E, Yuen MF, Kim DJ, et al. Clinical Study of Single-Stranded Oligonucleotide RO7062931 in Healthy Volunteers and Patients With Chronic Hepatitis B. *Hepatology*. Oct 2021;74(4):1795-1808. doi:10.1002/hep.31920
24. Gane EJ, Hsu YC, Chen CY, et al. A First-in-Human study of AHB-137, an unconjugated antisense oligonucleotide, in healthy subjects and patients with chronic hepatitis B. *Hepatology*. Aug 12 2026;doi:10.1097/hep.0000000000001835
25. Wang W, Mai J, Hou J, et al. Safety, pharmacokinetics and antiviral activity of AHB-137 in healthy volunteers and chronic hepatitis B patients: a phase 1a/1b study. *Hepatol Int*. Aug 2026;20(4):1035-1046. doi:10.1007/s12072-026-11102-7
26. Yuen MF, Heo J, Jang JW, et al. Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: a phase 2 randomized controlled trial. *Nat Med*. Oct 2021;27(10):1725-1734. doi:10.1038/s41591-021-01513-4
27. Yuen MF, Heo J, Kumada H, et al. Phase IIa, randomised, double-blind study of GSK3389404 in patients with chronic hepatitis B on stable nucleos(t)ide therapy. *J Hepatol*. Oct 2022;77(4):967-977. doi:10.1016/j.jhep.2022.05.031
28. Suslov A, Matter MS, Calabrese D, El Taher A, Wieland S, Heim MH. Serum HBsAg cannot distinguish cccDNA activity from integrated HBV DNA expression in chronic hepatitis B virus infection. *J Hepatol*. Aug 29 2026;doi:10.1016/j.jhep.2026.08.018
29. Wooddell CI, Sanders D, Xu Z, et al. Characterization of Hepatitis B Virus Transcripts in Chronically HBV-Infected Chimpanzees and Patients Treated with ARC-520 siRNA Demonstrates Transcriptional Silencing of cccDNA. *Viruses*. Dec 19 2024;16(12)doi:10.3390/v16121943
30. Iannacone M, Beccaria CG, Allweiss L, et al. Targeting HBV with RNA interference: Paths to cure. *Sci Transl Med*. Jul 2 2025;17(805):eadv3678. doi:10.1126/scitranslmed.adv3678
31. Qin ZX, Zuo L, Zeng Z, et al. GalNac-siRNA conjugate delivery technology promotes the treatment of typical chronic liver diseases. *Expert Opin Drug Deliv*. Apr 2025;22(4):455-469. doi:10.1080/17425247.2025.2466767
32. Ghany MG, Buti M, Lampertico P, Lee HM. Guidance on treatment endpoints and study design for clinical trials aiming to achieve cure in chronic hepatitis B and D: Report from the 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference. *J Hepatol*. Nov 2023;79(5):1254-1269. doi:10.1016/j.jhep.2023.06.002
33. Chen J, Ji D, Jia J, et al. Functional cure with new antiviral therapy for hepatitis B virus: a systematic review and meta-analysis. *Hepatol Int*. Aug 2025;19(4):773-795. doi:10.1007/s12072-025-10823-5
34. Delahaye JL, You S, Fu K, et al. Bepirovirsen induces innate immune proteins independent of HBV. *Hepatol Int*. Aug 4 2026;doi:10.1007/s12072-026-11134-z

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35. Ermler ME, Delahaye JL, Jordan W, et al. Bepirovirsen induces innate immune activation in the liver potentially through TLR8 signaling. *JHEP Rep*. Sep 2026;8(9):101923. doi:10.1016/j.jhepr.2026.101923
36. Joshi S, Freudenberg JM, Singh JM, et al. Immunomodulation by bepirovirsen may induce killing of infected hepatocytes (B-Together study). *Hepatol Int*. Feb 2026;20(1):46-58. doi:10.1007/s12072-025-10917-0

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Table 1. Characteristics of the Randomized Trial Units Included in the Meta-analysis

Study	Agent, class (phase)	Population	Comparison	N (I/C)	Age (y)	Male (%)
siRNA therapies						
Gane et al, 2023 (RG6346)	Xalnesiran, GalNAc-siRNA (1)	NUC-suppressed CHB	Xalnesiran vs placebo	12/6	45.3 (8.7)	50.0
Gane et al, 2023 (VIR-2218-1001)	Elebsiran, GalNAc-siRNA (1/2)	Noncirrhotic, NRTI-suppressed CHB	Elebsiran vs placebo	24/8	43.4 (10.1)	59.4
Hou et al, 2024 (PIRANGA)	Xalnesiran, GalNAc-siRNA (2)	NA-suppressed CHB	Xalnesiran vs NA alone	60/35	42.2 (10.3)	80.2
Wooddell et al, 2017 (Heparc-2001)	ARC-520, siRNA (2)	NUC-experienced, suppressed CHB	ARC-520 vs placebo	30/10	43.4 (7.2)	70.0
Yuen et al, 2020 (Heparc-2002)	ARC-520, siRNA (2)	HBeAg−, NUC-suppressed CHB	ARC-520 vs placebo	38/20	46.1 (10.3)	56.9
Yuen et al, 2020 (Heparc-2003)	ARC-520, siRNA (2)	HBeAg+, NUC-suppressed CHB	ARC-520 vs placebo	21/11	42.0 (11.2)	68.8
ASO therapies						
Agarwal et al, 2026	ALG-020572, GalNAc-ASO (1)	Noncirrhotic, HBeAg−, NA-suppressed CHB	ALG-020572 vs placebo	6/2	48.4 (5.1)	100
Gane et al, 2021	RO7062931, GalNAc-ASO (1)	NA-suppressed CHB	RO7062931 vs placebo	44/15	45.2 (8.1)	88.1
Gane et al, 2026	AHB-137, unconjugated ASO (1)	HBeAg−, NA-suppressed CHB	AHB-137 vs placebo	16/4	48.2 (7.8)	90.0
Hou et al, 2026 (B-Well 1)	Bepirovirsen, ASO (3)	Noncirrhotic, NA-suppressed CHB	Bepirovirsen vs placebo	653/328	49.9 (11.4)	70.1
Hou et al, 2026 (B-Well 2)	Bepirovirsen, ASO (3)	Noncirrhotic, NA-suppressed CHB	Bepirovirsen vs placebo	571/286	48.9 (10.7)	71.9
Wang et al, 2026	AHB-137, unconjugated ASO (1b)	HBeAg−, NA-suppressed CHB	AHB-137 vs placebo	16/4	47.6 (7.5)	75.0
Yuen et al, 2021	Bepirovirsen, ASO (2a)	Stable NA-treated CHB	Bepirovirsen vs placebo	5/2	45.1 (8.3)	71.4
Yuen et al, 2022 (B-Clear)	Bepirovirsen, ASO (2b)	Stable NA-treated CHB	Bepirovirsen vs placebo*	204/23	47.7 (11.7)	72.7
Yuen et al, 2022 (GSK3389404)	GSK3389404, GalNAc-ASO (2a)	Stable NA-treated CHB	GSK3389404 vs placebo	56/10	49.4 (9.8)	71.2

ASO, antisense oligonucleotide; CHB, chronic hepatitis B; ETV, entecavir; GalNAc, N-acetylgalactosamine; HBeAg, hepatitis B e antigen; I/C, intervention/control; NA, nucleos(t)ide analogue; NRTI, nucleos(t)ide reverse-transcriptase inhibitor; NUC, nucleos(t)ide analogue; siRNA, small interfering RNA.

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Table 2. Summary of Findings and GRADE Certainty of Evidence

Outcome	Participants (comparisons)	Effect estimate	Certainty
HBsAg loss[a]	1,999 (9)	RD 0.21 (95% CI, 0.02 to 0.39)	Moderate ⊕⊕⊕◯
HBsAg <100 IU/mL[a]	1,886 (6)	RD 0.74 (95% CI, 0.66 to 0.82)	Moderate ⊕⊕⊕◯
Quantitative HBsAg reduction[b]	230 (9)	MD −0.85 $\log_{10}$ IU/mL (95% CI, −1.14 to −0.55)	Low ⊕⊕◯◯
Any adverse event[a]	2,467 (13)	RD 0.19 (95% CI, 0.12 to 0.27)	Moderate ⊕⊕⊕◯
Serious adverse event[c]	2,467 (13)	RD 0.03 (95% CI, 0.01 to 0.04)	Moderate ⊕⊕⊕◯

[a] Downgraded one level for inconsistency ; [b] Downgraded for risk of bias and inconsistency ; [c] Downgraded for imprecision. CI, confidence interval; GRADE, Grading of Recommendations Assessment, Development and Evaluation; HBsAg, hepatitis B surface antigen; MD, mean difference; RD, risk difference.

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Figure Legends

Figure 1. HBsAg loss at study-reported assessment times with HBV RNA-targeting oligonucleotide therapy.

Figure 2. Achievement of HBsAg <100 IU/mL at study-reported assessment times with HBV RNA-targeting oligonucleotide therapy.

Figure 3. Any adverse event with HBV RNA-targeting oligonucleotide therapy.

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