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CLINICAL LIVER

Antiviral Activity, Safety, and Pharmacokinetics of Capsid Assembly Modulator NVR 3-778 in Patients with Chronic HBV Infection

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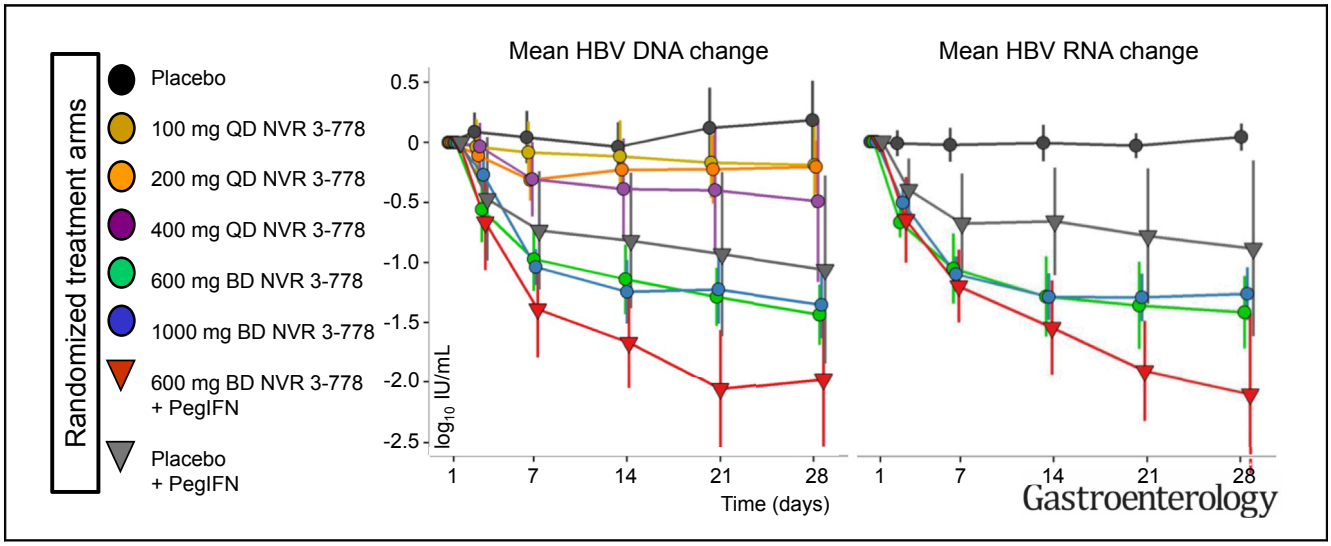

See Covering the Cover synopsis on page 1225.

BACKGROUND & AIMS: NVR 3-778 is a first-in-class hepatitis B virus (HBV) capsid assembly modulator that can inhibit HBV replication. We performed a proof-of-concept study to examine the safety, pharmacokinetics, and antiviral activity of NVR 3-778 in patients with chronic HBV infection. **METHODS:** We performed a phase 1 study in 73 hepatitis B envelope antigen (HBeAg)-positive patients with chronic HBV infection without cirrhosis. In a 2-part study (part 1 in New Zealand and part 2 in Hong Kong, Singapore, Taiwan, Korea, and the United States), patients were randomly assigned to groups that were given oral NVR 3-778 (100 mg, 200 mg, or 400 mg daily or 600 mg or 1000 mg twice daily) or placebo for 4 weeks. Additional groups received combination treatment with pegylated interferon (pegIFN) and NVR 3-778 (600 mg twice daily) or pegIFN with placebo. **RESULTS:** Reductions in serum levels of HBV DNA and HBV RNA were observed in patients receiving ≥1200 mg/d NVR 3-778. The largest mean reduction in HBV DNA was observed in the group given NVR 3-778 plus pegIFN (1.97 $\log_{10}$ IU/mL), compared with the groups given NVR 3-778 or pegIFN alone (1.43 $\log_{10}$ IU/mL and 1.06 $\log_{10}$ IU/mL, respectively). The mean reduction in HBV RNA was also greatest in the group given NVR 3-778 plus pegIFN (2.09 $\log_{10}$ copies/mL), compared with the groups given NVR 3-778 or pegIFN alone (1.42 $\log_{10}$ copies/mL and 0.89 $\log_{10}$ copies/mL, respectively). There was no significant mean reduction in HBsAg during the 4-week treatment period. There were no discontinuations and no pattern of dose-related adverse effects with NVR 3-778. **CONCLUSIONS:** In a phase 1 study of HBeAg-positive patients with chronic HBV infection without cirrhosis, NVR 3-778 was well tolerated and demonstrated antiviral activity.

The agent reduced serum levels of HBV DNA and HBV RNA, to the greatest extent in combination with pegIFN. The observed reductions in HBV RNA confirmed the novel mechanism of NVR 3-778. Clinicaltrials.gov no. NCT02112799 (single-center) and NCT02401737 (multicenter).

Keywords: Encapsidation; Capsid Inhibitor; Hepatitis B Treatment; Clinical Trial.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

NVR 3-778 is a first in class hepatitis B virus (HBV) capsid assembly modulator (CAM) that can inhibit HBV replication. We performed a phase 1 study of its safety, pharmacokinetics, and antiviral activity in HBeAg-positive patients with chronic HBV infection without cirrhosis.

NEW FINDINGS

NVR 3-778 was well tolerated and demonstrated antiviral activity. The agent reduced serum levels of HBV DNA and HBV RNA—to the greatest extent in combination with pegylated interferon.

LIMITATIONS

This was a phase 1 study and did not compare NVR 3-778 to other antiviral agents.

IMPACT

CAMs like NVR 3-778 may prove useful for treating patients with chronic HBV infection, most likely in combination with other therapies.

Chronic viral hepatitis due to hepatitis B virus (HBV) remains a leading cause of premature mortality worldwide, with an estimated 600,000 to 1 million deaths per year associated with HBV infection.[1,2] Improved treatment options are needed to better manage chronic HBV infection and reduce the risk of severe liver disease and death.

Current therapies for HBV infection, comprising nucleoside or nucleotide analogs or pegylated interferon (pegIFN), can suppress HBV replication in many patients, but therapeutic responses that are durable posttreatment are achieved only by a minority of patients. To maintain suppression of HBV replication and mitigate HBV-related liver inflammation, prolonged, often life-long treatment with HBV nucleoside/nucleotide analogs is usually used, with potential long-term issues of safety, tolerability, patient adherence, and viral resistance.[3,4]

The primary goal for improving treatment outcomes for HBV infection is therefore to increase the rate of therapeutic responses that are durable posttreatment; that is, durable hepatitis B surface antigen (HBsAg)-positive inactive carrier states or HBsAg-negative partial or functional cures with reduced risks for long-term morbidity and mortality. Highly potent combination treatment regimens could result in eventual cures when the rate of infection of new hepatocytes falls below the rate of disappearance of infected hepatocytes due to natural or treatment-enhanced infected cell turnover.

The HBV viral core protein (HBc) is an attractive target for developing novel HBV therapeutics. HBc-mediated encapsidation of the viral polymerase and pregenomic HBV RNA (pgRNA) by multimerically assembled viral core (capsid) protein molecules is essential for the production of HBV nucleocapsids and subsequent release of enveloped infectious virus particles from infected cells.[5–7] Small molecules have been identified that bind to HBc and can misdirect the assembly of HBc molecules into defective capsid structures, thereby inhibiting pgRNA encapsidation and formation of nucleocapsids and infectious virions.[8–11] This class of compounds can also inhibit replenishment of intranuclear covalently closed circular DNA (cccDNA) over time, and may have immunomodulatory properties.[12–14] HBc modulators could therefore provide important contributions to increasing the loss of infected hepatocytes and increase durable treatment responses, most likely as a component of new combination treatment regimens for HBV-infected patients.

NVR 3-778 is a novel, orally bioavailable, small-molecule agent that induces nonfunctional HBV capsid devoid of HBV DNA and RNA, thereby inhibiting HBV replication as a capsid assembly modulator (CAM). Significant reductions in serum and intrahepatic HBV DNA and serum HBV RNA levels have been observed in HBV-infected humanized mice receiving 42 days of NVR 3-778.[15] NVR 3-778 was well tolerated and achieved high plasma exposures in a dose-ranging study in healthy human volunteers.[16] Here we report the antiviral activity, safety, and pharmacokinetics of NVR 3-778 in adults with chronic HBV infection in a multicenter phase 1 study, which evaluated 4 weeks of treatment with NVR 3-778 at various dose levels, alone and in combination with pegIFN.

Methods

Phase 1 Program

The objectives of the phase 1 clinical trial program for NVR 3-778 were to assess the dose-related safety, pharmacokinetics, and preliminary antiviral effects of NVR 3-778, a first-in-class HBV capsid assembly modulator, in patients with high-viremic chronic HBV infection who were cared for in outpatient clinics. The phase 1b data in this report derive from 2 completed phase 1b clinical studies: (1) a 2-part single-center clinical protocol completed in New Zealand (ClinicalTrials.gov NCT02112799), in which part I (phase 1a) was a dose-

Abbreviations used in this paper: **AE, adverse event; ALT, alanine aminotransferase; BD, twice daily; CAM, capsid assembly modulators; cccDNA, covalently closed circular DNA; EC_{50}, median effective concentration; HBc, HBV core protein; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pegIFN, pegylated interferon; pgRNA, pregenomic RNA; QD, once daily; SAE, serious adverse event; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.**

Most current article

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ranging study of NVR 3-778 in healthy volunteers,[16] with a subsequent part II (phase 1b) dose-ranging trial in adults with high-viremic chronic HBV infection; and (2) a multicenter phase 1b protocol (ClinicalTrials.gov NCT02401737), similar to Part II of the New Zealand protocol, which was implemented concurrently with Part II of the New Zealand trial to expand phase 1b patient enrollment to clinical sites outside New Zealand (Hong Kong, Singapore, Taiwan, Korea, United States). These 2 phase 1b protocols were the same in all respects (patient eligibility criteria, randomized treatment assignments, clinical and laboratory evaluations) and were conducted concurrently from November 2014 to July 2016. Before screening, all patients gave written informed consent for their participation in the study, in accordance with Good Clinical Practice standards and the Declaration of Helsinki. The study protocols were approved by the ethics committees/institutional review boards for all participating study centers. All authors had access to the study data and have reviewed and approved the final manuscript.

Patient Enrollment Criteria

Eligible patients were ages 18 to 65 years, male or female, with clinical histories and serologic profiles consistent with chronic HBV infection according to Centers for Disease Control and Prevention criteria[3,4]: HBsAg-positive at a previous screen and at screen, negative for antibody to HBsAg, and negative for immunoglobulin M anti-core antibody. Patients were required to be hepatitis B envelope antigen (HBeAg)-positive with serum HBV DNA >20,000 IU/mL, a level of HBV viremia associated with significant risk for liver injury.[3,4,17] Patients were required to be noncirrhotic, without evidence of cirrhosis on hepatic imaging studies, and with no history of signs of hepatic decompensation. Also, they could not have been treated with an HBV nucleos(t)ide therapy within 3 months before screening or pegIFN within 6 months before screening. For the 2 pegIFN-treated cohorts, patients were required to be fully treatment-naïve and have serum alanine aminotransferase (ALT) levels above the upper limit of normal (ULN). For all cohorts, screening ALT was required to be less than 7-fold above the ULN. Additional enrollment criteria are described in the Supplementary Materials.

Study Drugs

NVR 3-778 was provided in 100-mg capsules with matching placebo capsules. Pegylated interferon alpha-2a (pegIFN, Pegasys; Hoffman-La Roche AG, Basel, Switzerland) was administered as 180-μg subcutaneous injections weekly in cohorts J and K.

Study Design

For the 7 treatment cohorts in this study, the treatment period was 4 weeks (study days 1–28), with 4 weeks of post-treatment follow-up (to study day 56). The first 4 cohorts (F, G, H, and I) were sequential monotherapy dose-escalation cohorts, with patients randomized to blinded treatment with active NVR 3-778 capsules (10 patients per cohort in cohorts F and G, 8 per cohort in cohorts H and I) or matching placebo capsules (2 patients in each of cohorts F–I). The 4 dose-escalation cohorts (F, G, H, and I) received NVR 3-778 monotherapy doses of 100, 200, or 400 mg once daily (QD), or 600 mg twice daily (BD), respectively. After satisfactory safety data were available for the first 4 cohorts, the fifth and sixth cohorts (J and K) were recruited and randomized in parallel, providing a parallel controlled comparison of combination treatment for cohort J (NVR 3-778 600 mg BD + pegIFN 180 μg subcutaneous weekly) to blinded pegIFN monotherapy for cohort K (pegIFN + placebo capsules). Cohort L explored a higher monotherapy dose of NVR 3-778 (1000 mg BD); this seventh cohort enrolled 9 patients, randomized 7:2 to active NVR 3-778 capsules and matching placebo capsules.

Randomization was managed centrally by Clinical Network Services (Brisbane, Australia), who assigned the relevant randomization number and assigned appropriate investigational product bottle numbers. All randomization was done via a computer-generated randomization list. Patients and all clinical site personnel were blinded to study drug assignment.

The NVR 3-778 monotherapy cohorts were screened and enrolled in sequence, with protocol-mandated safety reviews by the clinical investigator group before enrollment of subsequent higher-dose cohorts. Cohorts J and K were enrolled after review of cohort I (600 mg BD NVR 3-778) safety data.

After study treatment completion, patients could receive follow-on treatment with a regulatory-approved HBV therapy (HBV nucleos[t]ide therapy or pegIFN), at patient and investigator discretion.

Clinical and Laboratory Assessments

Safety-related clinical and laboratory evaluations were conducted at each study visit during treatment and follow-up, including interim history of any adverse events (AEs); physical examinations to evaluate any new clinical symptoms or signs; vital signs; repeated blood counts, serum chemistry panels, and urinalyses; and repeat electrocardiograms at baseline (day 1), on day 3, day 29 (1 day after last treatment dose), and day 56 (study completion).

Clinical AEs and laboratory abnormalities were graded by a standard National Institutes of Health/National Institute of Allergy and Infectious Diseases AE grading table adapted for use in hepatitis patients, in whom aminotransferase abnormalities are commonly present pretreatment. To facilitate AE analyses, raw terms in the investigator recordings were coded into standardized terms using the regulatory-standard Medical Dictionary of Regulatory Activities coding dictionary.

Virologic assessments were performed at central laboratories, and included the following evaluations: qualitative assays for HBsAb and HBeAb, at screening and day 56 (study completion); quantitative assays for HBsAg and HBeAg at all study visits except day 3 of treatment; serum HBV DNA levels assessed with the HBV TaqMan v2.0 polymerase chain reaction assay (Roche Diagnostics, Pleasanton, CA) at all study visits; and serum HBV RNA and hepatitis B core-related antigen (HBcrAg) levels assessed at all visits for the last 4 cohorts (I, J, K, L). Quantitative serum HBsAg and HBeAg levels were assessed with the Abbott Architect assays, and HBV RNA and HBcrAg levels were assessed with validated investigational assays. Virologic assay details are provided in the Supplementary Materials.

To assess possible viral resistance to NVR 3-778, the potential presence of amino acid substitutions in the HBV core protein was assessed at baseline and day 29 by deep sequencing using Illumina technology (MiSeq System; Illumina, San Diego, CA) performed by DDL Diagnostic Laboratory (Rijswijk, The Netherlands). Amino acid substitutions were

Table 1. Baseline Demographics and Disease Characteristics of Study Patients, by Treatment Group

Patient group	Pooled placebo	100 mg QD Cohort F	200 mg QD Cohort G	400 mg QD Cohort H	600 mg BD Cohort I	600 mg BD + pegIFN Cohort J	Placebo + pegIFN Cohort K	1000 mg BD Cohort L	All groups
n	10	10	10	8	8	10	10	7	73
Age									
Mean (SD)	31.1 (7.3)	32.7 (6.3)	34.2 (9.1)	42.9 (10.9)	44.6 (11.4)	39.9 (11.7)	32.6 (8.4)	32 (8.2)	36 (10.1)
Median (range)	33 (21–41)	31.0 (26–44)	32.5 (21–52)	40.5 (29–58)	44.5 (27–58)	41.5 (24–61)	32.5 (19–47)	32 (21–42)	34 (19–61)
Sex (M:F)	10:0	10:0	9:1	7:1	7:1	9:1	10:0	7:0	69:4
BMI (kg/m^2)									
Mean (SD)	26.8 (4.0)	25.1 (4.8)	25.6 (4.3)	23.6 (2.9)	23.6 (3.5)	24.9 (4.7)	23.4 (3.3)	22.9 (3.0)	24.6 (3.9)
Median (range)	25.7 (21.7–32.4)	24.9 (18–33.7)	25.2 (19.6–34)	23.4 19.4–27.8)	23.4 (19.1–29.1)	23.5 (19.3–34.4)	23.4 (19.3–28.7)	23.7 (19–27.8)	23.9 (18–34.4)
Race (n)									
Asian	8	8	9	7	8	10	10	7	67
White	0	0	0	1	0	0	0	0	1
Native Hawaiian or other Pacific Islander	2	2	1	0	0	0	0	0	5
Genotype[a]	B=4; C=5; nd=1	B=5; C=3; D=1; nd=1	B=3; C=7	A=1; B=3; C=4	B=3; C=3; nd=2	B=5; C=5	B=5; C=4; nd=1	B=2; C=5	A=1; B=30; C=36; D=1; nd=5
HBeAg+ n (%)[b]	10 (100)	10 (100)	10 (100)	8 (100)	8 (100)	10 (100)	10 (100)	7 (100)	73 (100)
HBV DNA level (log_{10} IU/mL) Mean (SD)	7.70 (1.17)	7.83 (0.92)	8.30 (0.78)	8.10 (0.31)	7.37 (1.47)	7.44 (1.19)	7.93 (0.85)	8.39 (0.32)	7.85 (0.98)
HBV RNA level (log_{10}copies/mL) Mean (SD)	6.48 (1.19)	nd	nd	nd	6.28 (1.30)	6.07 (1.32)	6.52 (1.13)	7.23 (0.53)	6.45 (1.15)
HBsAg level (log_{10} IU/mL) Mean (SD)	4.44 (0.53)	4.59 (0.57)	4.75 (0.33)	4.60 (0.28)	3.71 (0.86)	3.75 (0.88)	4.30 (0.66)	4.76 (0.25)	4.35 (0.69)
HBeAg level, PEI (log_{10} U/mL) Mean (SD)	2.75 (1.04)	2.69 (1.14)	3.06 (0.29)	3.01 (0.27)	2.07 (1.11)	2.60 (0.82)	2.71 (0.69)	3.01 (0.35)	2.74 (0.81)
HBcrAg level, (log_{10} IU/mL) Mean (SD)	5.43 (0.92)	nd	nd	nd	4.47 (1.33)	5.16 (0.64)	5.13 (0.69)	5.57 (0.50)	5.18 (0.86)
ALT level, (U/L) Mean (SD)	48 (25)	34 (21)	46 (33)	43 (23)	151 (276) 54 (29)[c]	85 (49)	197 (241) 87 (46)[c]	72 (70)	84 (137)

nd, not determined; PEI, Paul Ehrlich Institute; SD, standard deviation.
[a]Patient genotype data determined using Abbott Real Time HCV Genotype II assay.
[b]Qualitative HBeAg test performed at the screening time point.
[c]With baseline ALT outlier excluded.

defined as changes compared with genotype-specific reference sequences. Treatment-emergent amino acid substitutions in HBV core protein were defined as substitutions not detected at baseline (<1% frequency) and present at ≥10% frequency on day 29.[18]

Pharmacokinetic Assessments

Predose plasma concentration (C_{trough}) levels of NVR 3-778 were determined at all treatment visits using a validated liquid-chromatography tandem-mass-spectrometry assay (details in Supplementary Materials). Intensive plasma sampling was performed over 8 hours after each patient's first dose of study drug on day 1. On day 29, plasma samples were taken at 12 to 24 hours after the last dose of study drug on day 28. Wash-out plasma concentrations were assessed at 2 posttreatment visits, days 35 and 42.

Pharmacokinetic parameters were calculated by non-compartmental analyses of plasma concentration results using WinNonlin software (Certara, Princeton, NJ).

Statistical Analyses

No formal power calculation was performed, with the number of patients included determined arbitrarily. Results on continuous variables were expressed as means with standard deviation or median with range as appropriate. Group comparisons of HBV DNA, HBV RNA, HBsAg, HBeAg, and HBcrAg were performed using Student t tests. Relationships between HBV DNA and HBV RNA levels and between baseline ALT and DNA response were evaluated with Pearson correlation analyses. A 2-tailed $P < .05$ was considered to be statistically significant. Statistical analyses

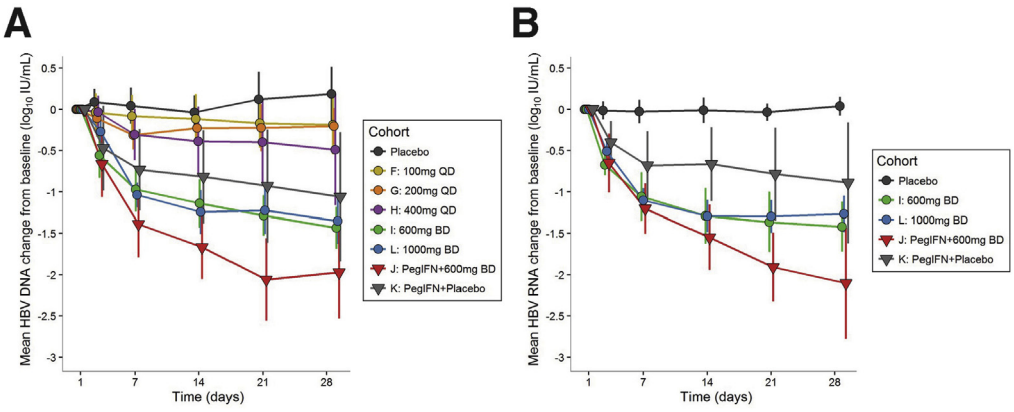

Figure 1. On-treatment changes from baseline in (*A*) mean (±SE) serum HBV DNA and (*B*) mean (±SE) serum HBV RNA levels.

were performed using R 3.2.3 (R core team 2015) and SAS 9.4 (SAS Institute Inc., Cary, NC). HBV RNA was analyzed retrospectively in an exploratory fashion, with analyses focusing on cohorts in which a DNA decline was seen.

Results

Patient Enrollment and Disposition

The total study population for the first in-patient phase 1b studies with NVR 3-778 comprised 73 patients. Details of patient disposition and randomization are presented in Supplementary Figure 1. There were no premature treatment discontinuations or treatment modifications, and no premature study discontinuations. Study conduct proceeded sequentially as described in the protocol, with full enrollment of the planned treatment cohorts, and no study advancement concerns arising in the intercohort safety reviews.

Baseline patient characteristics are presented in Table 1. A cumulative total of 10 patients were randomized to blinded placebo treatment, 2 from each of the 5 NVR 3-778

Table 2. Mean Change in Viral Parameters From Baseline at Day 28, and C_{trough} Levels of NVR 3-778 Across Cohorts

Patient group	Pooled placebo	100 mg QD Cohort F	200 mg QD Cohort G	400 mg QD Cohort H	600 mg BD Cohort I	600 mg BD + pegIFN Cohort J	Placebo + pegIFN Cohort K	1000 mg BD Cohort L
Serum HBV DNA (log_{10} IU/mL) mean (SD) [range]	0.19 (0.33) [0.95,−0.13]	−0.19 (0.36) [0.26, −0.95]	−0.20 (0.22) [0.09, −0.59]	−0.49 (0.67) [0.48, −1.59]	−1.43 (0.25)[a] [−1.06, −1.74]	−1.97 (0.56) [−1.13, −2.88]	−1.06 (0.78) [0.00, −2.28]	−1.35 (0.28) [−0.85,−1.61]
Serum HBV RNA (log_{10}copies/mL) mean (SD) [range]	0.04 (0.11) [0.22, −0.10]	nd	nd	nd	−1.42 (0.30)[b] [−0.92, −1.88]	−2.10 (0.67) [−1.55, −3.28]	−0.89 (0.73) [0.04, −2.03]	−1.27 (0.22) [−0.94, −1.54]
HBsAg (log_{10} IU/mL) mean (SD) [range]	0.02 (0.06) [0.14,−0.06]	−0.03 (0.06) [0.06,−0.12]	−0.03 (0.07) [0.11,−0.13]	0.03 (0.06) [0.09,−0.1]	0.02 (0.06)[c] [0.09,−0.09]	−0.08 (0.12) [0.12,−0.25]	−0.02 (0.22) [0.22,−0.51]	1.0 (0.08) [0.14,−0.11]
HBeAg (log_{10} U/mL) mean (SD) [range]	0.09 (0.22) [0.36,−0.33]	0.02 (0.09) [0.24,−0.09]	−0.04 (0.14) [0.19,−0.21]	−0.04 (0.22) [0.14,−0.56]	−0.09 (0.15)[d] [0.05,−0.37]	−0.21 (0.26) [0.16,−0.75]	−0.29 (0.47) [0.32,−1.25]	0.07 (0.21) [0.35,−0.19]
HBcrAg (log_{10} IU/mL) mean (SD) [range]	−0.01 (0.13) [0.26,−0.18]	nd	nd	nd	−0.10 (0.44)[e] [0.72,−0.44]	−0.10 (0.22) [0.17,−0.57]	−0.28 (0.45) [0.14,−1.26]	−0.13 (0.08) [−0.02,−0.22]
C_{trough}[f] (μg/mL) mean (SD) [range]	n/a	0.39 (0.14) [0.18, 0.59]	0.98 (0.77) [0.22, 1.35]	1.6 (0.82) [0.71, 2.90]	8.39 (3.71) [4.42, 12.3]	7.78 (3.29) [3.70, 12.6]	n/a	11.8 (5.90) [6.34, 20.7]

na, not applicable; nd, not determined; SD, standard deviation.
[a]Response outlier (−3.71 log_{10} IU/mL) excluded from the analysis; mean (SD) −1.72 (0.84) with outlier included.
[b]Response outlier (−3.96 log_{10} IU/mL) excluded from the analysis, mean (SD) −1.79 (1.07) with outlier included.
[c]Response outlier (−0.41 log_{10} IU/mL) excluded from the analysis, mean (SD) −0.03 (0.16) with outlier included.
[d]Response outlier (−1.73 log_{10} U/mL) excluded from the analysis, mean (SD) −0.29 (0.60) with outlier included.
[e]Response outlier (−2.08 log_{10} IU/mL) excluded from the analysis, mean (SD) −0.38 (0.85) with outlier included.
[f]C_{trough} calculated from determinations at Days 7, 14, 21, and 29.

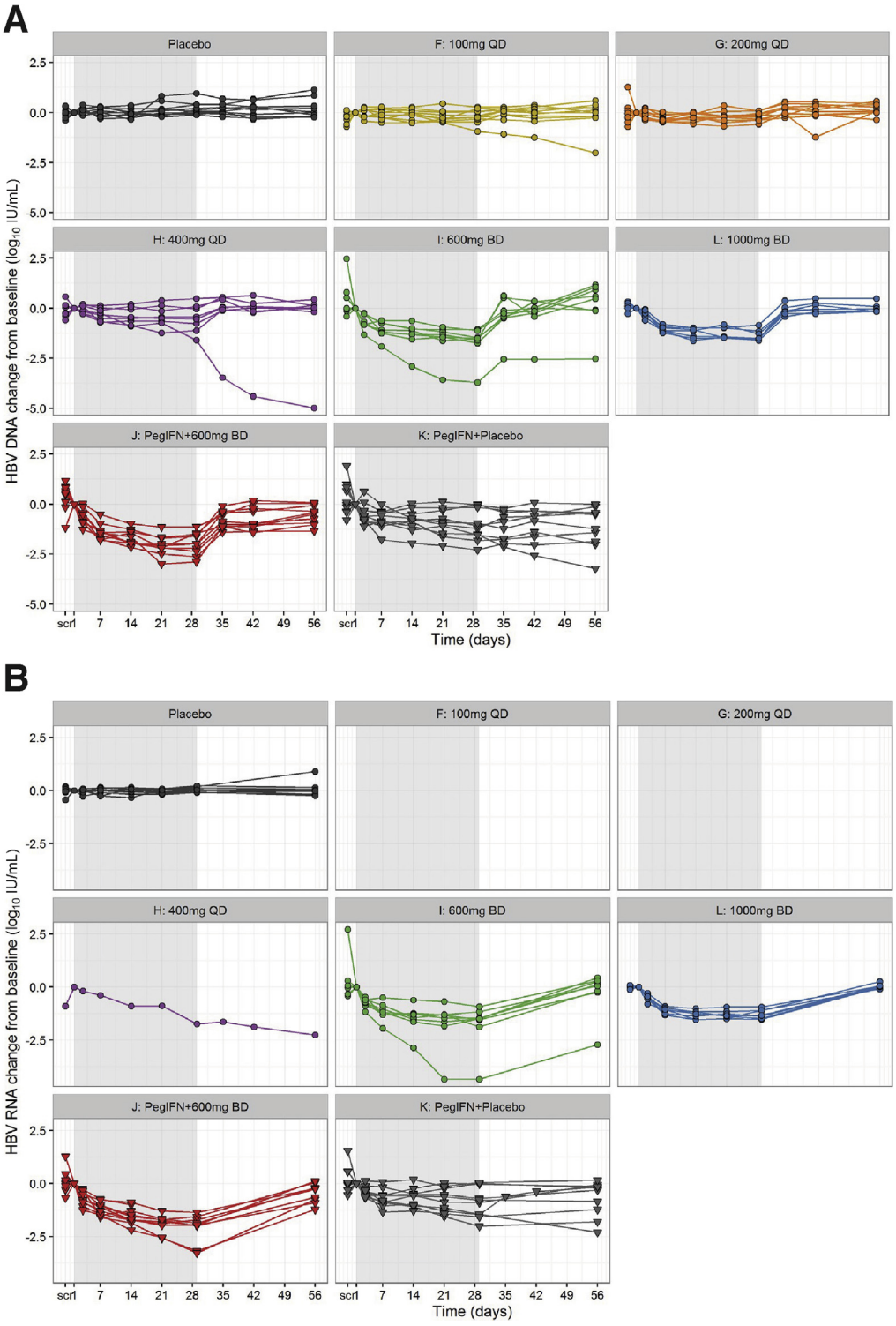

Figure 2. Individual profiles across all time points: (*A*) HBV DNA and (*B*) HBV RNA. The gray-shaded area shows the treatment period. Pretreatment screening values and posttreatment follow-up values are shown.

monotherapy cohorts. Mean patient age was 36 years (range 19–61 years). Most patients were male and infected with HBV genotypes B or C. All enrolled patients were HBsAg- and HBeAg-positive at the screening visit. Mean serum HBV DNA and RNA levels were above 7 $\log_{10}$ IU/mL and 6 $\log_{10}$ copies/mL, respectively, across the cohorts. Mean ALT levels at baseline ranged from 34 to 197 U/L. Three patients showed a >2-fold increase in ALT between screening and baseline, with baseline ALT >10× ULN (pretreatment ALT flare); 1 in cohort I (600 mg BD) going from 279 U/L at screening to 830 U/L at baseline, and 2 in cohort K (pegIFN monotherapy) going from 130 and 167 U/L at screening to 514 and 755 U/L at baseline, respectively (Supplementary Figure 2). Baseline mean ALT values are shown in Table 1 with and without these outlier patients with pretreatment ALT "flares."

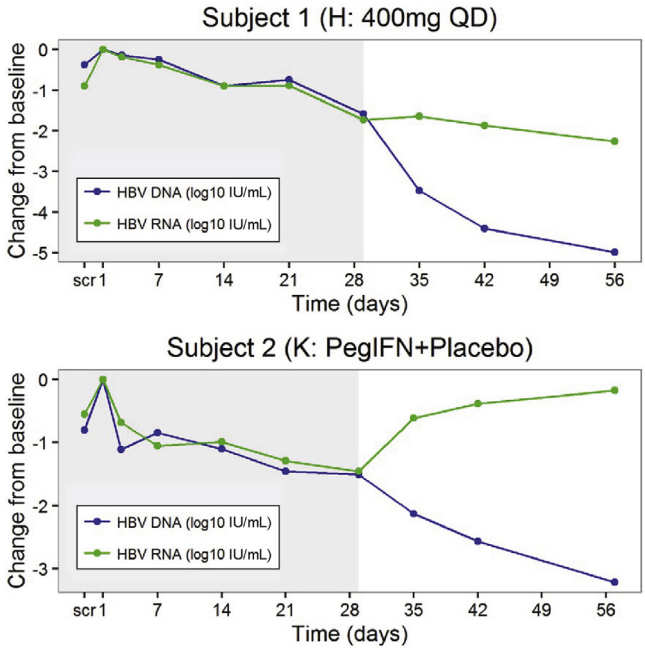

Figure 3. Individual HBV DNA and HBV RNA change kinetics for the 2 subjects of the study who switched to treatment with TDF monotherapy on day 30, after the end of 28-day treatment with either 400 mg QD NVR 3-778 (subject 1) or with pegIFN (subject 2). The gray-shaded area shows the treatment period. Pretreatment screening values and posttreatment follow-up values are shown.

Antiviral Activity Observations

Changes in patients' mean serum HBV DNA and RNA are shown graphically in Figure 1 and summarized in Table 2. Individual treatment responses for serum HBV DNA and RNA are shown in Figure 2. With placebo treatment, there was no change in serum HBV DNA or HBV RNA levels during the 28-day study treatment period. Mean HBV DNA decline was minimal with low once-daily doses of NVR 3-778 (100, 200, and 400 mg QD), but HBV DNA reductions became substantial when daily dosing was increased to ≥1200 mg/d and administered as BD divided daily dosing to increase NVR 3-778 plasma trough concentrations. Cohort I (600 mg BD NVR 3-778) exhibited a mean HBV DNA reduction of 1.72 $\log_{10}$ IU/mL. There was one outlier patient in cohort I, with a pretreatment ALT flare (Figure 2*A*). This was the aforementioned patient whose ALT increased from 279 IU/L at screening to 830 IU/L at baseline. There was a concomitant HBV DNA decrease from 8.71 to 6.24 $\log_{10}$ IU/mL between screening and baseline (ie, a pretreatment HBV DNA decline of 2.47 $\log_{10}$ IU/mL). When this outlier patient was excluded, cohort I achieved a mean 1.43 $\log_{10}$ IU/mL decrease in serum HBV DNA from baseline. Viral kinetics are shown in Figure 1*A* with this outlier patient excluded. Cohort L (1000 mg BD NVR 3-778) reached a similar mean serum HBV reduction from baseline of 1.35 $\log_{10}$ IU/mL. All patients in cohorts I and L displayed serum HBV DNA reductions >0.5 $\log_{10}$ IU/mL from baseline (Figure 2*A*).

HBV DNA reduction appeared more pronounced after dosing with either 600 or 1000 mg BD NVR 3-778 as compared with pegIFN monotherapy (cohort K), which showed a mean HBV DNA reduction of 1.06 $\log_{10}$ IU/mL during treatment (Table 2). This difference did not reach statistical significance ($P > .05$). Three of the 10 patients treated with pegIFN monotherapy (cohort K) did not respond with HBV DNA reductions (Figure 2*A*). These 3 patients had serum HBV DNA changes from baseline <0.2 $\log_{10}$ IU/mL after 28 days of treatment.

The largest mean reduction in serum HBV DNA levels was achieved with the combination of NVR 3-778 and pegIFN (−1.97 $\log_{10}$ IU/mL) (cohort J). All patients in this combination cohort displayed >1 $\log_{10}$ IU/mL HBV DNA reductions from baseline. This response was significantly higher than that obtained with NVR 3-778 or pegIFN alone (both *t* tests $P < .05$).

There were 2 other patients with unusual HBV DNA response profiles: 1 in the 100-mg QD cohort F and 1 patient in cohort H (400 mg QD) (Figure 2*A*). Due to the negligible HBV DNA responses for these 2 lower-dose cohorts, these individual profiles had no impact on the treatment response analysis and are described in detail in the Supplementary Materials.

The kinetics of the serum HBV RNA and HBV DNA declines during treatment were very similar (Figure 1): NVR 3-778 monotherapy treatment with BD dosing (cohorts I and L) resulted in a larger but not statistically significant effect on HBV RNA declines compared with pegIFN monotherapy (cohort K) (Figure 1*B*). As with HBV DNA, HBV RNA response was significantly higher with the NVR 3-778 and pegIFN combination (cohort J) compared with NVR 3-778 or pegIFN alone (both *t* tests $P < .05$). Mean on-treatment responses are shown in Figure 1*B*, and all time points for all patients are shown in Figure 2*B*. There was 1 outlier patient in cohort I (600 mg BD NVR 3-778), as described previously. The mean reduction of HBV RNA in this cohort was 1.42 $\log_{10}$ copies/mL when this outlier is excluded. The 1000-mg BD NVR 3-778 cohort L achieved a similar mean HBV RNA reduction of 1.27 $\log_{10}$ copies/mL during treatment. All patients treated with 600 mg or 1000 mg BD NVR 3-778 for 28 days had serum HBV RNA reductions >0.5 $\log_{10}$ IU/mL.

In comparison, a lower mean HBV RNA reduction of 0.89 $\log_{10}$ copies/mL was achieved during pegIFN monotherapy (Figure 1*B*). There were 3 nonresponders in the pegIFN monotherapy group (Figure 2*B*), which showed HBV RNA reductions of <0.1 $\log_{10}$ copies/mL from baseline. These were the same 3 patients who showed nonresponse in serum HBV DNA levels.

The combination of 600 mg BD NVR 3-778 and pegIFN resulted in a mean HBV RNA reduction of 2.06 $\log_{10}$ copies/mL during treatment (Figure 1*B*). All patients in this combination therapy cohort showed HBV RNA reductions >1 $\log_{10}$ copies/mL, and 1 patient achieved undetectable HBV RNA by the end of treatment (Figure 2*B*).

After the end of treatment, serum HBV DNA and RNA levels returned to baseline levels for all patients in the NVR 3-778 BD monotherapy cohorts (I, L) with the exception of the outlier with pretreatment ALT flare (Figure 2). In the pegIFN cohorts, 11 and 8 of the 20 patients had plasma HBV DNA and HBV RNA at levels of 0.5 log IU/mL or more below

Table 3. Summary of Clinical Adverse Events and Grade 3–4 Laboratory Abnormalities

Event	Pooled placebo (n = 10)	NRV 3-778 (100 mg) (n = 10)	NVR 3-778 (200 mg) (n = 10)	NVR 3-778 (400 mg) (n = 8)	NVR 3-778 (600 mg) (n = 8)	NVR 3-778 (600 mg) + pegIFN (n = 10)	Placebo + pegIFN (n = 10)	NVR 3-778 (1000 mg) (n = 7)
Clinical adverse events, n (%)[a]								
Subjects ≥1 TEAE	7 (70.0)	6 (60.0)	5 (50.0)	3 (37.5)	3 (37.5)	7 (70.0)	8 (80.0)	5 (71.4)
Dry mouth	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (10.0)	1 (10.0)	1 (14.3)
Fatigue	0 (0.0)	0 (0.0)	1 (10.0)	1 (12.5)	0 (0.0)	3 (30.0)	3 (30.0)	0 (0.0)
Influenza-type illness	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	2 (20.0)	0 (0.0)
Injection site erythema	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	1 (10.0)	0 (0.0)
Malaise	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	1 (10.0)	0 (0.0)
Pyrexia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	2 (20.0)	0 (0.0)
Influenza	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (10.0)	1 (10.0)	0 (0.0)
Upper respiratory tract	1 (10.0)	1 (10.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)
Headache	1 (10.0)	1 (10.0)	0 (0.0)	1 (12.5)	0 (0.0)	1 (10.0)	2 (20.0)	1 (14.3)
Oropharyngeal pain	1 (10.0)	0 (0.0)	2 (20.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Eczema	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	2 (28.6)
Grade 3 or 4 laboratory abnormalities, n (%)								
Gamma glutamyl Transferase	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)
Triglycerides	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

TEAE, treatment-emergent adverse event.
[a]All clinical adverse events occurring in at least 3 patients in either treatment group are listed regardless of whether they were attributed or not to the study drug.

baseline at the end of the follow-up period, respectively. All but 1 of these 11 patients also had ALT levels above 100 U/L during or after treatment (Supplementary Figure 2). The other 9 patients in these cohorts had plasma HBV DNA levels returning to baseline levels. All but 1 of these 9 patients had end of treatment ALT levels below 100 IU/L.

There was a strong and significant correlation between changes in serum HBV DNA and RNA values across all patients and all time points (Supplementary Figure 3), for both absolute values (Pearson correlation coefficient = 0.91, $P < .0001$) and for changes from baseline (Pearson correlation coefficient = 0.87, $P < .0001$).

There were 6 outlier data points obtained from 2 patients at posttreatment time points (days 35, 42, and 56) (highlighted in green in Supplementary Figure 4). The full kinetic profiles for serum HBV DNA and HBV RNA for these 2 patients are shown in Figure 3. These 2 patients started follow-on treatment with tenofovir disoproxil fumarate (TDF) on study day 30, 2 days after completing study treatment with NVR 3-778 400 mg QD (subject 1, a cohort H patient) or pegIFN (subject 2, a cohort K patient). Initiation of follow-on treatment, 2 days or more after the completion of study treatment but before completion of the 4-week posttreatment study period, was allowed by the study protocols at investigator and patient discretion, with consideration for any investigator concerns about potential posttreatment flares, which have been previously observed with HBV therapies. Only these 2 patients initiated an approved HBV therapy within 25 days after the end of treatment in this study.

The data for the 2 patients receiving prompt follow-on treatment with TDF provided interesting ancillary observations potentially related to different mechanisms of action for 3 anti-HBV agents: 2 study drugs (NVR 3-778 and pegIFN) and TDF. The data for subjects 1 and 2 indicate that HBV DNA and HBV RNA levels declined in parallel during study treatment and, as expected, serum HBV DNA levels continued to decline after switching to follow-on TDF treatment. In contrast, HBV RNA levels did not respond appreciably to TDF treatment, and the correlation between HBV DNA and RNA responses was lost after study treatment with NVR 3-778 or pegIFN. These results likely reflect the different mechanism of action of HBV nucleo(s)tide treatment, which inhibits polymerase-mediated reverse transcription of encapsidated HBV RNA to DNA but does not prevent continued formation and secretion of pgRNA-containing pseudo-virion particles.

Assessments of quantitative serum levels of HBsAg, HBeAg, and HBcrAg did not indicate significant changes in these markers within the 28-day treatment period in the NVR 3-778 monotherapy cohorts. Individual response profiles for these markers are shown for all cohorts in Supplementary Figures 4–6, and individual profiles are described in the Supplementary Materials.

Mean changes in serum HBeAg were <0.1 $\log_{10}$ U/mL across all NVR 3-778 monotherapy cohorts, when the one outlier from the 600-mg BD cohort was excluded. The pegIFN cohorts J and K showed mean reductions in serum HBeAg from baseline of 0.21 and 0.29 $\log_{10}$ U/mL, respectively. Individual responses showed a large variation (Table 2).

Mean reductions from baseline in serum HBcrAg were small (<0.2 $\log_{10}$ IU/mL) across all cohorts. The 1 outlier in the 600-mg BD NVR 3-778 cohort and 1 outlier in the pegIFN monotherapy cohort are apparent from the individual response profiles in Supplementary Figure 6.

Resistance Analyses

Deep sequencing analyses were performed on samples from all patients obtained at baseline and day 29. No treatment-emergent amino acid substitutions in the HBV core protein sequences were apparent at day 29 in any of the patients analyzed. There were no cases of on-treatment breakthrough of HBV DNA levels in any of the patients receiving NVR 3-778 alone or in combination with pegIFN, consistent with the HBV DNA sequencing results. Viral breakthrough was defined in this short-treatment-duration study as an increase of ≥0.5 $\log_{10}$ IU/mL in HBV DNA from nadir.

ALT Analyses

In the overall study population, there were 36 patients with ALT levels above ULN (45 U/L) at pretreatment baseline (day 1). The distribution of these patients is as follows: placebo, n = 4; cohorts F, n = 1; G, n = 4; H, n = 3; I, n = 4; J, n = 9; K, n = 9; and L, n = 2. Three patients had baseline ALT levels above 10× ULN, 1 in the 600-mg BD NVR 3-778 and 2 in the pegIFN monotherapy cohorts.

There were no on-treatment or posttreatment ALT flares in this study, defined here as ALT increase ≥2× above baseline ALT and reaching ≥10× ULN. Four patients in this study had ≥2× increases in ALT above baseline during the treatment or follow-up period that resulted in their ALT levels being at least 2× ULN but less than 10× ULN. Two of these patients participated in cohort K (pegIFN monotherapy), 1 in cohort J (NVR 3-778 600 mg BD plus pegIFN), and 1 in cohort F (NVR 3-778 100 mg QD). There was 1 additional patient in the pegIFN monotherapy group with >2× ALT on-treatment increase from baseline, but this patient's highest ALT level remained below 2× ULN. Finally, there was 1 additional patient in the 400-mg QD NVR 3-778 cohort with an apparent on-treatment ALT increase across multiple timepoints, but this change remained at below 2-fold difference from baseline. All individual ALT profiles are shown in Supplementary Figure 2.

Comparing all patients with elevated baseline ALT (>45 U/L) with those with normal ALT, there was no significant correlation between elevated baseline ALT and either HBV DNA or HBV RNA response. Among the 3 patients with baseline ALT >10× ULN, the 1 from cohort I (600 mg BD) showed favorable outlier responses for all virologic endpoints. One such patient from cohort K (pegIFN monotherapy) showed nonresponse in HBV DNA and RNA (0.00 and 0.01 $\log_{10}$ change from baseline), and the other one from cohort K showed DNA and RNA responses above the mean of the cohort.

Pharmacokinetic Analyses

Mean peak plasma concentration (C_{max}) and mean exposure (area under the plasma concentration-time curve) increased approximately linearly up to a dose of 600 mg. Plasma concentrations were similar in the 600 mg NVR 3-778 BD monotherapy group and in the group receiving this dose in combination with weekly pegIFN. Mean C_{trough} levels determined predose on days 7, 14, 21, and 29 were 5.3-fold higher in the 600-mg BD cohort as compared with the 400-mg QD cohort (Table 2). Mean C_{trough} levels for all individual patients in the 600-mg BD cohort ranged between 4.4 and 12.3 μg/mL. Mean C_{trough} levels were 1.4-fold higher in the 1000-mg BD cohort as compared with the 600-mg BD cohort. The slightly higher C_{trough} in the 1000-mg group did not translate into a more pronounced HBV DNA reduction. The NVR 3-778 pharmacokinetic profile in patients with HBV was similar overall to that observed in phase 1a healthy volunteers.[16]

Safety Observations

Study treatments were well tolerated, and all patients completed the study with no premature treatment discontinuations or modifications. Clinical AEs were generally transient and mild or moderate in intensity, and most (78%) were not attributed to study treatment by the clinical investigators. There was only 1 serious adverse event (SAE) in this study; 1 patient developed a protracted rash that eventually resolved, as summarized later in this article.

All clinical AEs that occurred in at least 3 study patients (regardless of attribution to study treatment), and all observed grade 3–4 abnormalities in safety-related clinical laboratory parameters, are summarized in decreasing order of frequency in Table 3. The safety data in Table 3 and the overall study data show no appreciable pattern of treatment-related adverse clinical or laboratory effects (active NVR 3-778 vs placebo) or any clear pattern of adverse effects related to NVR 3-778 dose.

Clinical chemistry and hematological abnormalities in this study were predominantly mild (grade 1) or moderate (grade 2) in intensity, and transient. Of note, there were no grade 3 or 4 liver function test abnormalities (eg, ALT, aspartate transaminase, gamma-glutamyl transferase, albumin) during the treatment period in patients given multiple doses of NVR 3-778 for 28 days. There were 3 isolated and transient grade 3 laboratory abnormalities: a grade 3 elevation of gamma-glutamyl transferase level (a cohort J patient, NVR 3-778 + pegIFN combination cohort), a grade 3 elevation of triglyceride level (a cohort F patient, 100 mg QD NVR 3-778) and a grade 3 neutropenia (a cohort H patient, 400 mg QD NVR 3-778). One grade 4 neutropenia treatment-emergent AE was reported in a cohort K patient (pegIFN alone).

There was 1 SAE during this study, involving a 42-year-old male patient in cohort F, the lowest-dose cohort (100 mg NVR 3-778 QD). On day 22 of study dosing, he developed a severe (grade 3) papulovesicular rash with a predominantly acral distribution involving his hands, arm, side of neck, and 1 leg (palmar plantar erythrodysesthesia), which was categorized as possibly related to study drug treatment. There were no perioral or mucosal lesions, no ecchymotic skin involvement, no bullae, and no systemic manifestations or hematological abnormalities. Eosinophil count at the time

was 0.11 × 10^9/L. Viral serology evaluations were negative (enterovirus antibodies, and others). This patient completed his 28 days of dosing shortly after onset of the rash. An initial dermatology consultation treated the patient for possible eczema with topical betamethasone, oral prednisone, and cetirizine on an outpatient basis, during follow-up. The rash was subsequently managed with a psoriasis-like treatment regimen of psoralen, ultraviolet light, and topical steroid ointment during outpatient follow-up and resolved after approximately 6 months.

Five other patients reported with minor skin rashes during the study (including posttreatment rashes); these events were categorized as probably related to the treatment in 3 patients from cohort J (600 mg NVR 3-778 BD + pegIFN) and unrelated to the treatment in 2 patients from cohort L (1000 mg NVR 3-778 BD). These rashes were predominantly localized eczematoid rashes, sometimes occurring in patients with a known history of eczema. There were no other patients with rashes similar to the SAE rash.

In summary, serial clinical and safety-related laboratory evaluations, conducted for all patients at every study visit, did not reveal any appreciable pattern of treatment-related or dose-related clinical AEs or laboratory abnormalities for NVR 3-778 treatment of HBV patients. For the patients in the 2 cohorts that received pegIFN treatment, safety observations revealed the flu-like adverse effects and hematologic effects expected with interferon therapies.

Discussion

At present, the major unmet medical need for patients with HBV infection is the lack of treatment options that can deliver an effective antiviral response that is durable after the end of a finite treatment regimen in a large proportion of patients.[19] One strategy to achieve durable posttreatment responses, or potentially even virologic cures in chronically HBV-infected patients, is to intensify antiviral suppression of virus replication to a level in which only very few or no new infectious virus particles are formed in infected hepatocytes. As a result, the rate of infection of new hepatocytes in the liver should fall below the rate of natural death and turnover of already infected hepatocytes, resulting in progressive reduction in the number of infected hepatocytes over time.

HBV CAMs have the potential to interfere with several aspects of HBV replication and persistence. There are 2 types of CAMs: those that form adherent core protein aggregates, and those that form functionally defective capsids (such as NVR 3-778). For both types, the preclinically best-characterized mechanism of action is the inhibition of encapsidation of viral pgRNA and polymerase, which prevents the formation of HBV nucleocapsids and infectious virions. This mechanism of action inhibits infection of hepatocytes in hepatic lobules and formation of cccDNA via either new cell infection or intracellular cccDNA replenishment cycles in cells that are already HBV-infected, phenomena that depend on the production of HBV DNA-containing nucleocapsids.[5,7,8,10–12,20,21]

NVR 3-778 appears to have been the first CAM to complete clinical proof-of-concept studies in HBV-infected patients. The phase 1b study results reported here show consistent and concurrent dose-related reductions in serum HBV DNA and RNA levels, as predicted by its novel mechanism of action (ie, inhibition of pgRNA encapsidation). The magnitude of HBV DNA and RNA reductions appeared greater than those achieved by pegIFN monotherapy, but it is especially notable that direct anti-HBV activities (HBV DNA and HBV RNA reductions) appeared greatest for NVR 3-778 + pegIFN combination treatment. These results are consistent with results previously obtained in a humanized mouse model of HBV infection.[15] When comparing the HBV DNA reduction after 28 days of treatment, NVR 3-778 monotherapy at a dose of 600 mg BD (mean 1.43 $\log_{10}$ reduction from baseline) had less effect than entecavir (mean 2.81 $\log_{10}$ reduction), TDF (mean 2.68 $\log_{10}$ reduction) and 25 mg tenofovir alafenamide fumarate (mean 2.81 $\log_{10}$ reduction).[22,23] More potent CAMs, such as JNJ-56136379, have shown HBV DNA reductions comparable to those seen with entecavir.[24–26]

There was also an apparent reduction in slope of HBV DNA reduction between weeks 2 and 4 as compared with slopes of the first 2 weeks. This pharmacokinetic result suggests that dosing with NVR 3-778 may be approaching systemic target saturation at doses ≥1200 mg/d. Achieving additional efficacy through oral dosing formulations with increased bioavailability might be feasible if such formulations could be devised. Also, greater direct antiviral efficacy may be observed with more potent CAMs that are currently in early development.[24-26] Unlike nucleos(t)ide analogs, and consistent with the mechanism of action of HBV RNA encapsidation, NVR 3-778 showed suppression of plasma HBV RNA with similar effect size compared with the HBV DNA response.

Treatment with pegIFN also reduced both serum HBV DNA and RNA levels in HBV patients consistent with previous observations.[27] There was a high correlation between serum HBV DNA and HBV RNA levels in patients treated with NVR 3-778 or pegIFN. This observation is consistent with both drugs inhibiting viral replication at or upstream of pgRNA encapsidation. In contrast, switching from these treatments to the nucleotide TDF resulted in the loss of correlation between serum HBV DNA and HBV RNA markers.

Although modest HBV RNA reduction has been reported in some patients after 3 months of nucleos(t)ide analog treatment, which is probably attributable to indirect effects,[28] the lack of serum HBV RNA suppression in most nucleos(t)ide-treated patients has been described in another recent study.[29] This is because nucleos(t)ide analogs do not inhibit release of HBV RNA-containing particles.[15,20,27,30] The response of 2 patients in the current study who received follow-on treatment with the nucleotide analog TDF is consistent with these previous observations. During follow-on TDF treatment, serum HBV DNA levels continued to decrease, as expected; but serum HBV RNA levels did not respond to TDF treatment, remaining high in both, and increasing appreciably in one of the patients (Figure 3). These results demonstrated that NVR 3-778 and pegIFN, but not the HBV polymerase inhibitor TDF, can

directly inhibit the production of HBV RNA-containing particles.

Serum HBV RNA has also been reported as an early marker to predict the smaller subgroup of nucleos(t)ide-treated patients who achieve HBeAg seroconversion.[28] The molecular mechanism underlying the progression of this subgroup of patients to HBeAg loss remains to be determined. The additional effects on HBV RNA suppression by CAMs may optimize viral control if CAMs can be added to patients receiving nucleos(t)ide analogs. The long-term use of CAMs in combination of nucleos(t)ide analogs needs to be explored to determine long-term antiviral efficacy and safety, and possible emerging resistance to CAMs. Although neither baseline amino acid substitutions associated with in vitro resistance to NVR 3-778, nor emerging resistant variations were observed in this 4-week study, long-term studies should include careful monitoring of viral DNA levels and genomic sequencing to assess possible resistance. Longer-term studies also will be needed to determine if combination therapy (eg, CAMs with nucleos[t]ide analogs, novel antiviral agents and immunomodulatory agents) will produce progressive reductions in HBsAg levels and HBsAg clearance, toward enhancing the proportion of patients to achieve posttreatment durable responses.

The observation of negligible antiviral effects for the low-dose, NVR 3-778 QD monotherapy cohorts may be related to insufficient systemic exposures to NVR 3-778. The antiviral median effective concentration (EC_{50}) of NVR 3-778 in stable HBV-replicating HepG2 cells was determined as approximately 250 ng/mL. In the presence of 40% human serum, the antiviral EC_{50} value was increased to approximately 3 μg/mL. The mean C_{trough} levels were 392 ng/mL in the 100-mg QD and 1597 ng/mL in the 400-mg QD cohorts, below the protein-adjusted inhibitory concentrations. In contrast, all patients in the 600-mg and 1000-mg BD cohorts achieved mean C_{trough} levels above the 3 μg/mL protein-adjusted EC_{50} concentration, and all patients in these cohorts responded to treatment with serum HBV DNA reductions larger than 0.5 $\log_{10}$ IU/mL.

NVR 3-778 was generally well tolerated in all cohorts with no discontinuations. Most AEs were mild and not attributed to the study drug. Skin rashes were observed and graded as drug-related in 1 patient as an SAE receiving NVR 3-778 100 mg BD, and in 3 patients receiving 600 mg BD and pegIFN. These observations occurred in the lowest-dose cohort and the cohort with concomitant pegIFN.

With the short phase 1 treatment period, there were no meaningful effects on other virologic markers (HBsAg, HBeAg, HBcrAg) in any of the cohorts. The principal activity of HBc inhibitors does not necessarily involve direct inhibition of cccDNA formation, although inhibition of nucleocapsid formation might result in reduced cccDNA in HBV-infected cells over time. Studies with longer treatment durations will be needed to assess the ability of NVR 3-778 treatment to reduce the number of HBV-infected cells in the liver via antiviral or secondary immunomodulatory activities, leading to HBsAg reductions over time.

In conclusion, this study provides a first clinical demonstration that substantial inhibition of viral production can be achieved in patients with HBV by a novel mechanism, inhibition of encapsidation of pregenomic HBV RNA, by an orally bioavailable antiviral agent targeted to the HBV core protein. Further to this, other more potent CAMs are now undergoing clinical development.[24–26]

NVR 3-778 treatment for 28 days, up to a dose of 1000 mg BD, was generally well tolerated. Substantial and correlated reductions in serum HBV DNA and HBV RNA levels were observed consistently with the higher-dose cohorts, and were notably greatest for combination treatment with NVR 3-778 and pegIFN. Assessments of other potential activity effects of HBV core inhibitors, such as serum HBsAg and HBeAg reductions, immunomodulatory effects, and effects on cccDNA persistence, will require longer treatment periods.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2018.12.023.

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Conflicts of interest
These authors disclose the following: Man Fung Yuen is an advisor for AbbVie, Arbutus, Arrowhead, Biocartis, Gilead Sciences, GlaxoSmithKline, Ionis, Roche, and Vir Biotechnology; and received speaker and grant supports from AbbVie, Arrowhead, Bristol Myers Squibb, Fujirebio, Gilead Sciences, Merck Sharp & Dohme, Novartis Pharmaceuticals, Sysmex. Henry Lik Yuen Chan is an advisor for AbbVie, Aptorum, Arbutus, Altimmune, Gilead, GRAIL, Intellia, Janssen, MedImmune, Roche, and Vir Biotechnology; and a speaker for AbbVie, Gilead, and Roche. George Hartman, Sandy Liaw, Klaus Klumpp, and Nathaniel Brown were employees of Novira Therapeutics Inc, part of the Janssen Pharmaceutical Companies. Osvaldo Flores was an employee of Novira Therapeutics Inc, part of the Janssen Pharmaceutical Companies, at the time that this study was undertaken, but is no longer employed by this company. Oliver Lenz and Willem Talloen are employees of Janssen Pharmaceutica NV. Thomas N. Kakuda is an employee of Janssen Biopharma, a Janssen Pharmaceutical Company, and holds stocks in Johnson & Johnson. The remaining authors disclose no conflicts.

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Supplementary Materials and Methods

Additional Patient Enrollment Criteria

Patients were required to have a body mass index of 18 to 35 kg/m^2, systolic blood pressure <160 and diastolic blood pressure <95 mm Hg, and no clinically significant abnormalities on 12-lead electrocardiograms at screening. Eligible hematologic profiles were hemoglobin >12 g/dL (men) or >11 g/dL (women), total white cell count >4000/mm^3 with absolute neutrophil count >1800/mm^3, and platelets >100,000/mm^3. Serum albumin, bilirubin, creatinine, blood urea nitrogen, and prothrombin time were required to be normal.

Patients with known cirrhosis or with clinical or laboratory evidence of potentially decompensating cirrhosis were excluded by the following protocol criteria: a history of clinical signs of hepatic decompensation (ascites, bleeding varices, jaundice, encephalopathy); physical stigmata of portal hypertension; low serum albumin (<3.5 g/dL); elevated serum direct bilirubin; elevated prothrombin time or International Normalized Ratio; or evidence of cirrhosis on a previous liver biopsy; transient hepatic elastography (Fibroscan) score exceeding 10.5 kPa, or evidence of cirrhosis on a hepatic imaging study (magnetic resonance imaging or ultrasound) if a Fibroscan was unavailable. Additional exclusions included human immunodeficiency virus or hepatitis C virus coinfection, confounding medical or psychosocial conditions, and treated diabetes mellitus or hemoglobin A1c >7%. Serum alpha fetoprotein >100 ng/mL was exclusionary; an alpha fetoprotein elevated value >ULN but <100 ng/mL required a hepatic imaging study to rule-out focal lesions suspicious of hepatocellular carcinoma. Eligible patients consented to the required use of adequate birth control during the study, and eligible women were required to have negative pregnancy tests at screening and at pretreatment baseline (day 1).

Study Visit Schedule

Before screening, all patients gave written informed consent for their participation in the study, in accord with Good Clinical Practice standards and the Declaration of Helsinki. After the screening visit, eligible patients were to attend a baseline visit (day 1) within 28 days of screening. Subsequent study visits included 5 during the 28-day treatment period (days 3, 7, 14, 21, and 29), and 3 post-treatment visits (days 35, 42, 56).

Clinical and Laboratory Assessments: Safety and Efficacy

Quantitative HBsAg and HBeAg assays were performed with serum samples from all cohorts and all visits except days 3 and 42; and HBcrAg levels were assessed at all visits for the last 4 cohorts (I, J, K, L). Serum HBV DNA was assessed quantitatively with the Food and Drug Administration–approved COBAS TaqMan HBV Test v2.0 (Roche Diagnostics, Pleasanton, CA), which has been validated to quantify HBV DNA from serum samples for diverse HBV genotypes (A–H), with a reported lower limit of quantification of 20 IU/mL and a linear dynamic range of quantitation of 20 to 1.7 $\times$ 10^8 IU/mL, using the World Health Organization pooled serum reference standard for quantitation.

Serum levels of HBV RNA were quantitatively assessed using an investigational quantitative reverse transcriptase polymerase chain reaction (PCR) assay for HBV RNA, which was developed by DDL Diagnostic Laboratory (Rijswijk, The Netherlands), based on the method by van Bommel et al.[1] The lower limit of quantification for the quantitative serum HBV RNA assay is 4.04 $\log_{10}$ copies/mL (158 copies/PCR) and limit of detection (95% positivity rate) is 2.49 $\log_{10}$ copies/mL (5 copies/PCR). For the exploratory analyses presented in this article, quantitative values were used for all measurements for which target was detected. Serum levels of HBeAg and HBsAg were quantitatively assessed using the Abbott Architect assays (Abbott Diagnostics; Abbott Park, IL). The HBcrAg chemiluminescence immunoassay detects circulating HBc, p22cr, and HBeAg and was performed as previously described.[2]

Supplementary Results: Efficacy Observations

Remarkable Individual HBV DNA Response Profiles

There was 1 patient with a remarkable HBV DNA response in the 100-mg QD cohort G. This patient reached a viral load reduction of 0.95 $\log_{10}$ IU/mL at the end of treatment, and HBV DNA levels further decreased after the end of treatment to reach a reduction of 2.01 $\log_{10}$ IU/mL from baseline by the end of the follow-up period (Figure 3*A*). The ALT profile for this patient showed a continuous up to 2.7-fold ALT increase from baseline over several time points during the treatment period, with the highest ALT of 106 U/L on Day 29. This ALT profile differentiated this patient from the other patients in this cohort (Supplementary Figure 2). The ALT data are described in more detail as follows. This patient was infected with a genotype B HBV and did not show unusual plasma concentrations of NVR 3-778 as compared with the other patients in the cohort.

Another remarkable HBV DNA response profile was from 1 patient in cohort H (400-mg QD), who showed the largest reduction of HBV DNA from baseline by the end of treatment in this cohort (1.59 $\log_{10}$ IU/mL) and further reduction during the follow-up period to reach a reduction of 4.99 $\log_{10}$ IU/mL by the end of the follow-up period. This patient was the only one in this cohort to have HBV DNA reduction >1 $\log_{10}$ IU/mL at the end of the treatment period. The ALT profile for this patient showed a continuous up to 1.97-fold ALT increase from baseline over several time points during the treatment period, with the highest ALT of 171 U/L on day 29. This ALT profile differentiated this patient from the other patients in this cohort (Supplementary Figure 2). This patient was infected with genotype A HBV and did not show unusual plasma

concentrations of NVR 3-778 as compared with the other patients in the cohort. After the end of treatment with NVR 3-778, this patient switched to treatment with the nucleotide analog TDF on day 30 and showed substantial further reduction of serum HBV DNA during the follow-up period (Figure 3*A*).

Remarkable Individual HBsAg Response Profiles

Mean serum HBsAg levels changed less than 0.1 $\log_{10}$ IU/mL across all cohorts, including those receiving pegIFN. One outlier response was apparent from the individual HBsAg kinetics profiles. One patient in the 600-mg BD cohort I had a remarkable HBsAg reduction from baseline to end of treatment of serum HBsAg (0.41 $\log_{10}$ IU/mL). This patient also had a large 1.59 $\log_{10}$ IU/mL reduction in HBsAg between screening and pretreatment baseline time points. These results correlate with a pretreatment ALT flare in this patient and with outlier responses in serum HBV DNA and RNA reductions, as described previously. There were also 2 unusual HBsAg profiles in the pegIFN monotherapy group. One patient in this cohort showed a single HBsAg value on day 29 that was 0.51 $\log_{10}$ IU/mL below baseline. HBsAg levels were between 0.09 and 0.18 $\log_{10}$ IU/mL above baseline at all other on-treatment and follow-up time points for this patient. One other patient in this cohort showed a slight but continuous reduction of HBsAg across all on-treatment time points to achieve an end of treatment reduction in HBsAg of 0.28 $\log_{10}$ IU/mL from baseline (Supplementary Figure 3). This patient also had a pretreatment ALT flare with a 4-fold increase in ALT from 130 U/L at screening to 514 U/L pretreatment on day 1. ALT levels remained between 3 and 11× ULN until the end of the follow-up period (142 U/L on day 56). This patient had a nonresponder profile of unchanged serum HBV DNA or RNA levels after 28 days of pegIFN treatment.

Remarkable Individual HBeAg Response Profiles

Mean changes in serum HBeAg were <0.1 $\log_{10}$ PEI U/mL across all NVR 3-778 monotherapy cohorts, with the exception of the 600-mg BD cohort, which had a mean reduction of 0.29 $\log_{10}$ U/mL from baseline. This was driven by 1 outlier response of 1.73 $\log_{10}$ U/mL in this cohort, from the 1 patient with similar outlier responses in all virological markers (Supplementary Figure 4). When this patient was excluded from the analysis, the mean reduction from baseline in this cohort was also <0.1 $\log_{10}$ U/mL. One patient in the 400-mg QD NVR 3-778 cohort showed a slight but continuous response across all on-treatment time points reaching an HBeAg reduction of 0.56 $\log_{10}$ U/mL from baseline at the end of treatment. HBeAg continued to decrease after the end of the treatment period. This was 1 of the 2 patients who switched to nucleotide treatment with TDF 2 days after the end of NVR 3-778 treatment.

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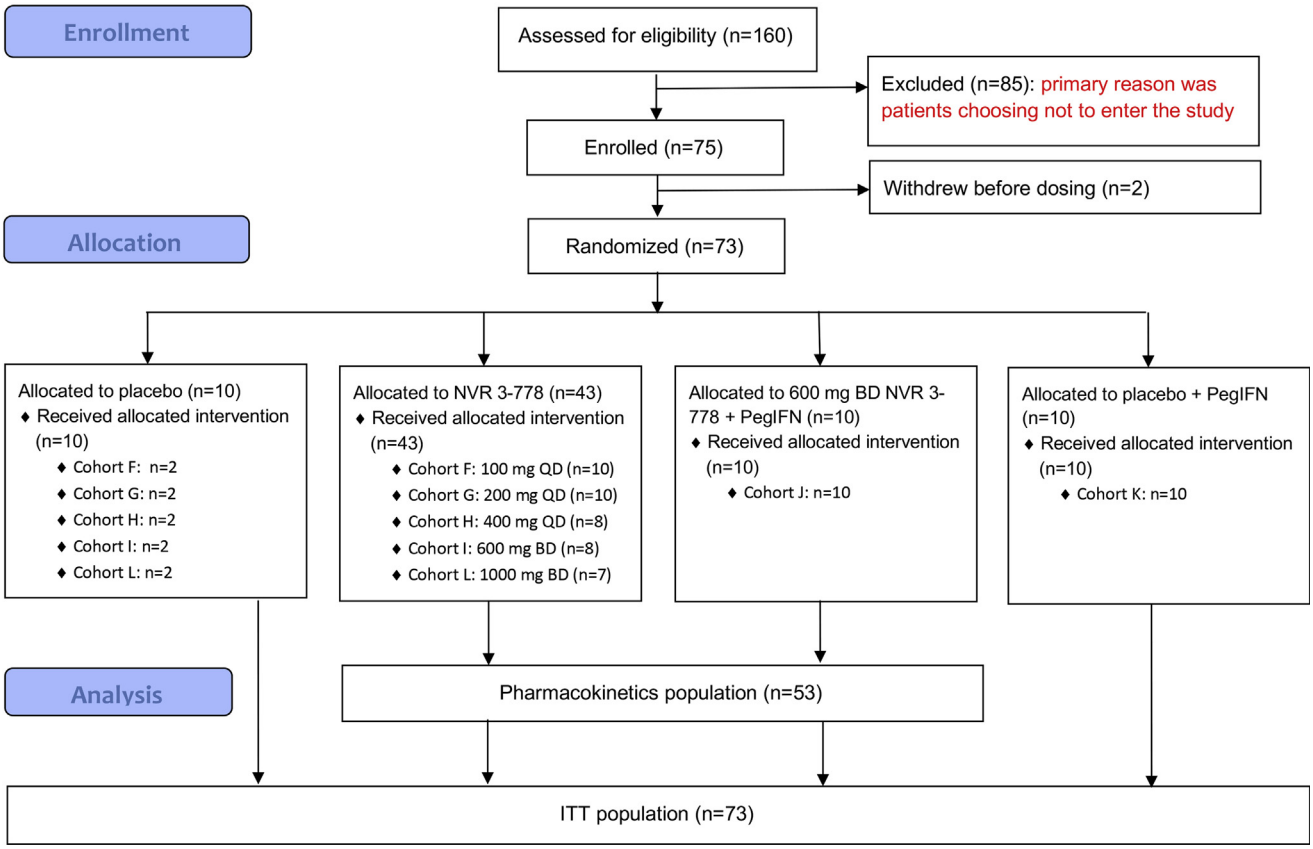
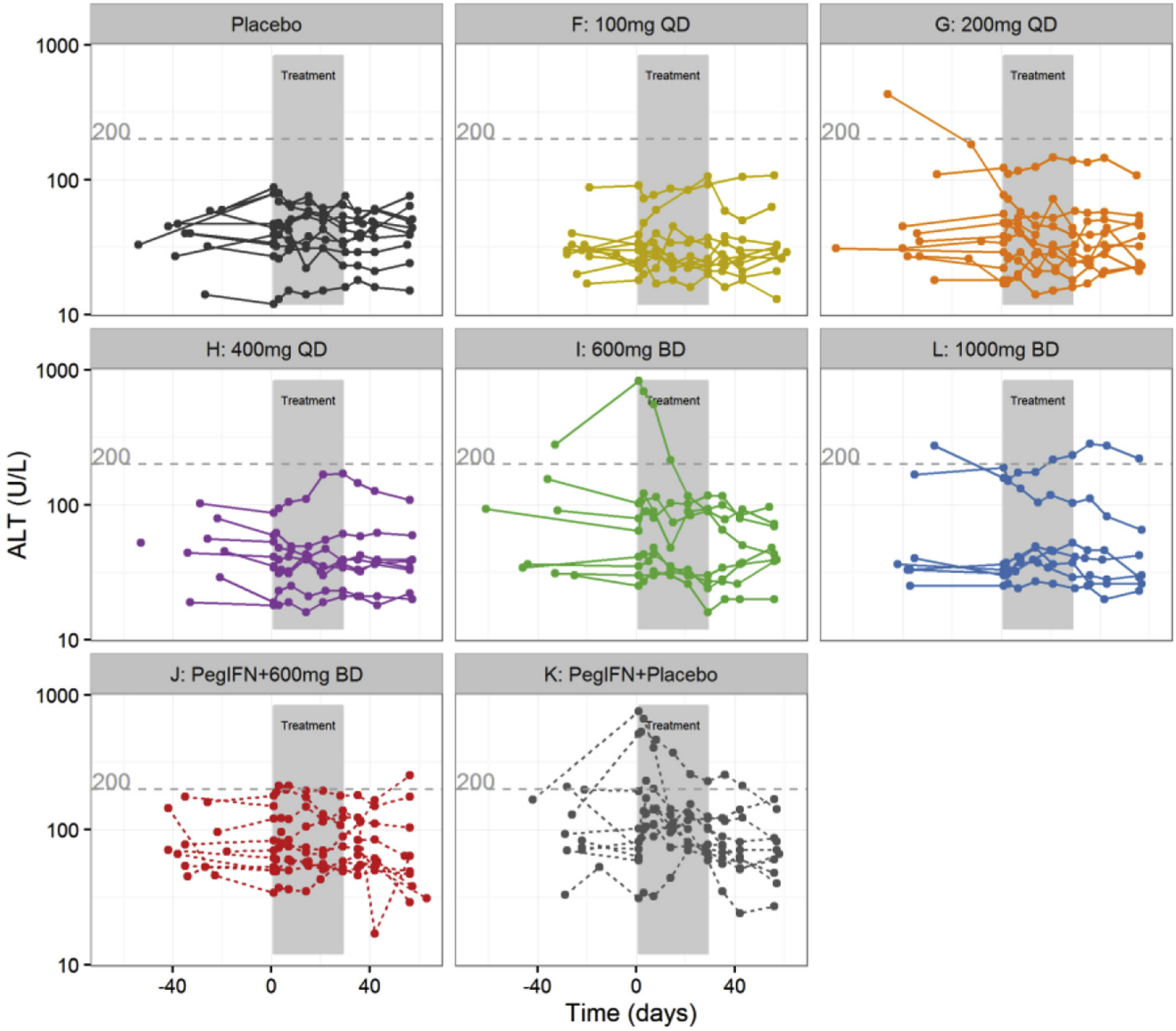
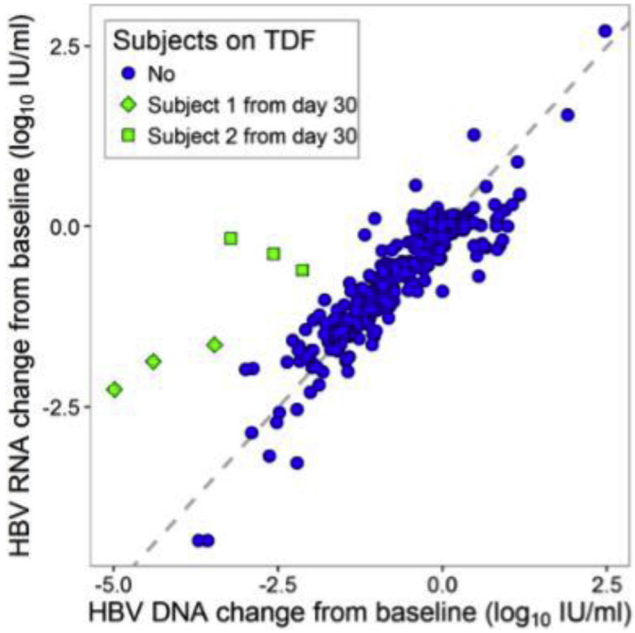


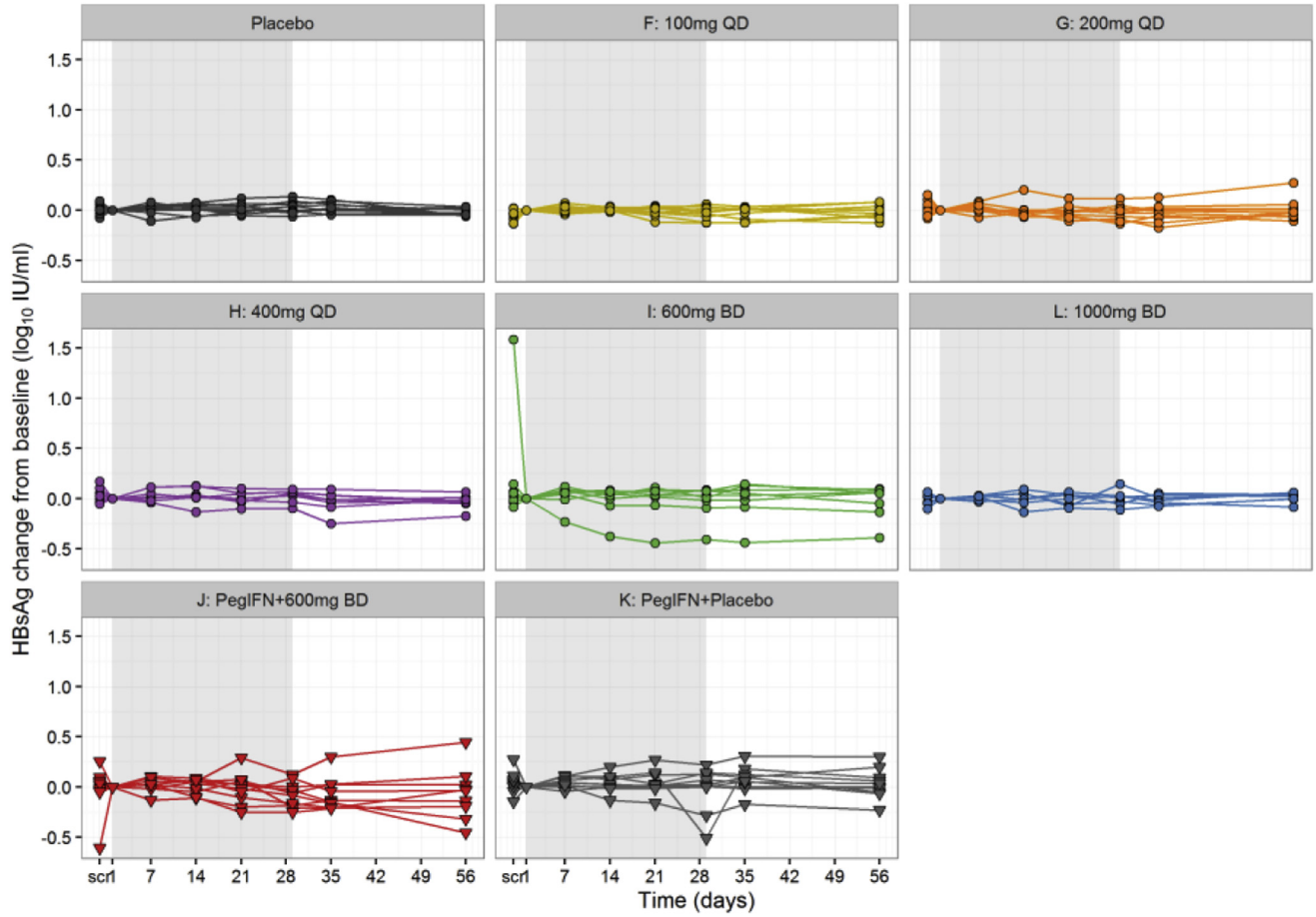
Supplementary Figure 1. Study populations.



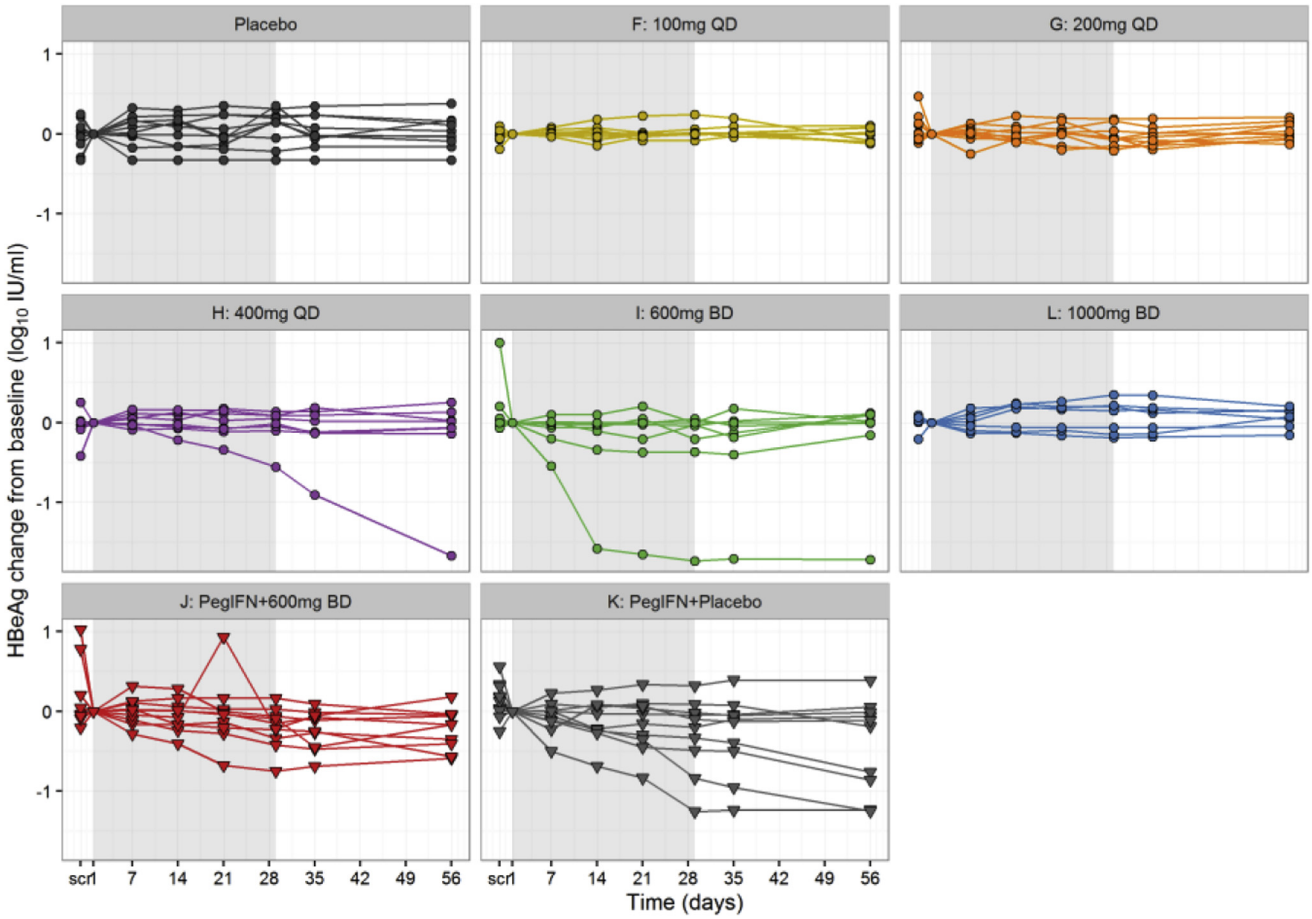
Supplementary Figure 2. Individual ALT profiles across all time points. The gray-shaded area shows the treatment period. Pretreatment screening values and posttreatment follow-up values are shown. The dotted line indicates 200 U/L ALT for orientation.



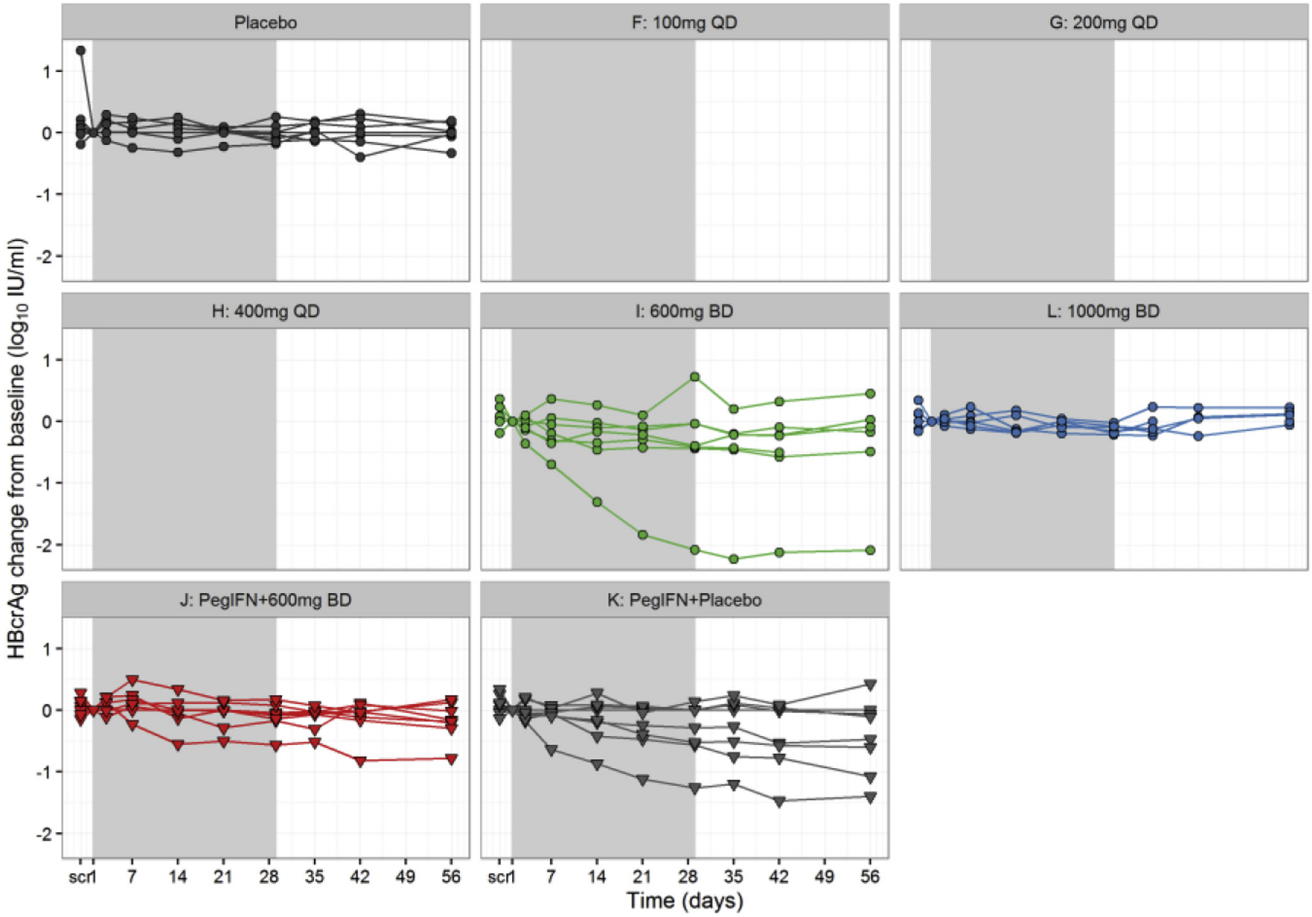
Supplementary Figure 3. Correlation analysis between serum HBV DNA and HBV RNA measurements: data from all patients and time points.



Supplementary Figure 4. Individual HBsAg profiles across all time points. The gray-shaded area shows the treatment period. Pretreatment screening values and posttreatment follow-up values are shown.



Supplementary Figure 5. Individual HBeAg profiles across all time points. The gray-shaded area shows the treatment period. Pretreatment screening values and posttreatment follow-up values are shown.



Supplementary Figure 6. Individual HBcrAg profiles across all time points. The gray-shaded area shows the treatment period. Pretreatment screening values and posttreatment follow-up values are shown. HBcrAg was not determined in patients from cohorts F, G, and H.