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RNA Interference Therapy With ARC-520 Results in Prolonged Hepatitis B Surface Antigen Response in Patients With Chronic Hepatitis B Infection

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BACKGROUND AND AIMS: ARC-520, the first an RNA interference (RNAi) therapeutic, was designed to reduce all RNA transcripts derived from covalently closed circular DNA, leading to a reduction in viral antigens and hepatitis B virus (HBV) DNA.

APPROACH AND RESULTS: We aimed to evaluate the depth of hepatitis B surface antigen (HBsAg) decline in response to multiple doses of ARC-520 compared to placebo (PBO) in two randomized, multicenter studies in nucleoside/nucleotide analogue reverse-transcriptase inhibitor (NUC)–experienced patients with hepatitis B early antigen (HBeAg)–negative (E-neg) or HBeAg-positive (E-pos) disease. A total of 58 E-neg and 32 E-pos patients were enrolled and received four monthly doses of PBO (n = 20 E-neg, 11 E-pos), 1 mg/kg ARC-520 (n = 17 E-neg, 10 E-pos), or 2 mg/kg ARC-520 (n = 21 E-neg, 11 E-pos) concomitantly with NUC. HBsAg change from baseline to 30 days after the last ARC-520 dose were compared to PBO. Both E-neg and E-pos high-dose groups significantly reduced HBsAg compared to PBO, with mean reductions of 0.38 and 0.54 log IU/mL, respectively. HBsAg reductions persisted for approximately 85 days and >85 days after the last dose in E-neg and E-pos patients, respectively. The low-dose groups did not reach statistical significance in either study. E-pos patients showed a dose-dependent reduction in HBeAg from baseline. Mean maximum reduction was 0.23 and 0.69 log Paul Ehrlich IUs/mL in the low-dose and high dose ARC-520 groups respectively. ARC-520 was well tolerated, with only two serious adverse events of pyrexia possibly related to study drug observed.

CONCLUSIONS: ARC-520 was active in both E-neg and E-pos, NUC-experienced HBV patients; but absolute HBsAg reductions were moderate, possibly due to expression of HBsAg from integrated HBV DNA, indicating the need for RNAi therapeutics that can target viral transcripts regardless of origin. (Hepatology 2020;72:19-31).

Infection with hepatitis B virus (HBV) is a significant global health problem. The World Health Organization reports that an estimated 257 million people worldwide are chronically infected with HBV.[1] It is estimated that during their lifetimes 15%-40% of chronic HBV (CHB) patients may develop serious sequelae of infection such as chronic

Abbreviations: AE, adverse event; API, active pharmaceutical ingredient; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; ECG, electrocardiogram; E-neg, HBeAg-negative; EOS, end of study; E-pos, HBeAg-positive; ETV, entecavir; HBeAg, hepatitis B early antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IgE, immunoglobulin E; ITT, intent to treat; NUC, nucleoside/nucleotide analogue reverse-transcriptase inhibitor; PBO, placebo; PEIU, Paul Ehrlich international unit; PK, pharmacokinetic; qHBsAg, quantitative HBsAg; RNAi, RNA interference; SAE, serious adverse event; siRNA, short interfering RNA; TDF, tenofovir disoproxil fumarate; TEAE, treatment emergent AE.

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hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).[2-5] Functional cure characterized by a sustained loss of hepatitis B surface antigen (HBsAg) with or without antibody seroconversion has emerged as a consensus endpoint for new therapies for CHB.[4,6] Current therapies for CHB, although effective at suppressing viral DNA and reducing liver inflammation, rarely result in sustained functional cures off therapy. Multiple studies have highlighted the importance of viral load in predicting the risk of cirrhosis and HCC.[7,8] However, sustained HBsAg loss has been identified as an additional, independent predictor of further reductions in the risk of HCC development.[9-11]

HBV persists in the nucleus of hepatocytes as a minichromosome, covalently closed circular DNA (cccDNA), which serves as the template for five overlapping viral transcripts including pregenomic RNA.[12,13] All HBV transcripts are encoded in overlapping reading frames, with a common 3′ end, and use the same polyadenylation signal (PAS). These transcripts serve as templates for the translation of all viral proteins, i.e., precore (HBV early antigen [HBeAg]); core; polymerase; L, M, and S surface antigens (collectively HBsAg); and X protein. These viral proteins play an important role in the production of new viral particles and the viral life cycle; however, HBeAg and HBsAg are often produced at large excess, and both can be detected at high levels in the liver and serum of many patients. HBeAg and HBsAg have been implicated in the maintenance of viral infection through induction of exhaustion or impairment of cluster of differentiation 8–positive T-cell immune responses.[14,15] More recently, a single-dose study with ARC-520 in patients with CHB combined with studies in chimpanzees chronically infected with HBV showed that HBsAg was expressed not only from the episomal cccDNA minichromosome but also from transcripts arising from HBV DNA integrated into the host genome and was the dominant source of HBsAg in HBeAg-negative (E-neg) or nucleos(t)ide analogue reverse-transcriptase inhibitor (NUC)–experienced patients.[16]

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Because high antigen load is believed to play a key role in maintaining chronic viral infection, there is interest in new therapies that can reduce both viral loads and antigens as a means to restore immune control of the infection. Therapies using RNA interference (RNAi) as a mechanism of action can directly target HBV mRNA transcripts with high specificity, profoundly reducing the production of viral proteins, including HBsAg. RNAi utilizes small, noncoding RNAs to regulate the expression of genetic information.[17] ARC-520 injection was the first RNAi-based therapeutic to enter the clinic. It consists of two synthetic short interfering RNAs (siRNAs) conjugated to cholesterol, which enhances delivery of the siRNAs to hepatocytes. ARC-520 injection uses a polymer-based excipient (ARC-EX1) that enables endosomal escape of the siRNAs into the cytoplasm where RNAi occurs.[18] The mRNA target sites for ARC-520 are located 118 and 71 bases upstream, respectively, of the conventional HBV PAS, within the open reading frame of the HBV X protein. Because all cccDNA-derived HBV transcripts overlap in this region, the siRNAs in ARC-520 could target for degradation all cccDNA-derived viral transcripts, thus preventing translation of all cccDNA-derived viral proteins. They are, however, not expected to cleave most transcripts resulting from integrated HBV DNA due to loss of the target site located in the DR1/DR2 region of the virus, which is commonly deleted upon HBV integration.[16]

We have previously reported on the safety, tolerability, and pharmacokinetics of ARC-520 in healthy volunteers[19] as well as single-dose pharmacodynamics of ARC-520 in a phase 2 study in patients with CHB.[16] Here, we report on two double-blinded, phase 2 multidose studies of ARC-520 in NUC-experienced, E-neg, or HBeAg-positive (E-pos) patients with CHB in combination with tenofovir (TDF) or entecavir (ETV).

Participants and Methods

STUDY DESIGN

Two multicenter, randomized, double-blind, placebo-controlled, multidose, phase 2 studies were conducted in parallel to evaluate the level of HBsAg reduction following intravenous administration of the investigational product ARC-520 injection to a population of adults with CHB infection. The two studies were similar in design, with the difference being that one study was conducted in E-neg patients (the Heparc-2002 study, Clinicaltrials.gov registration no. NCT02604199), and the other study was conducted in E-pos patients (the Heparc-2003 study, Clinicaltrials.gov registration no. NCT02604212). For Heparc-2002 and Heparc-2003 it was planned to enroll 60 patients and 48 patients, respectively, into four treatment groups at a 1:1:2:2 ratio (placebo [PBO] low dose plus ETV or TDF, PBO high dose plus ETV or TDF, 1 mg/kg ARC-520 plus ETV or TDF, 2 mg/kg ARC-520 plus ETV or TDF, respectively). Patients who had signed an institutional review board/independent ethics committee approved informed consent and had met all the protocol eligibility criteria were randomized to a treatment by a centralized, electronic system. Blinding to treatment assignment was maintained throughout the study period. The studies were conducted at multiple sites in Hong Kong, the Republic of Korea, and Germany, with recruitment of subjects starting in November 2015, early termination of study in November 2016, and last patient visit in December 2016. The studies were terminated early due to monkey deaths occurring in a nonclinical study with another RNAi investigational product and attributed to the ARC-EX1 delivery agent also used in ARC-520. This toxicity was not associated with the siRNA used in ARC-520.

Eligible patients were to be enrolled in parallel into one of four treatment groups: low-dose PBO (n = 10 for Heparc-2002, n = 8 for Heparc-2003), high-dose PBO (n = 10 for Heparc-2002, n = 8 for Heparc-2003), low-dose ARC-520 injection (1 mg/kg, n = 20 for Heparc-2002, n = 16 for Heparc-2003), or high-dose ARC-520 injection (2 mg/kg, n = 20 for Heparc-2002, n = 16 for Heparc-2003). All patients continued their daily oral ETV or TDF throughout the study. Each patient was assigned to either active (ARC-520 injection) or PBO (0.9% normal saline) treatment using a block randomization algorithm. Final confirmation of eligibility was checked on day 1. Other than pharmacists and staff involved in randomization, dispensing and preparation of the study drug remained blinded.

A subset of 12 patients were to be enrolled into each study who were designated pharmacokinetic (PK) patients. Blood samples were collected predose and at specified times after dosing to determine PK

parameters (PK patients only) and pharmacodynamic and safety endpoints. Two hours prior to treatment administration, all patients were pretreated with oral antihistamine.

Visits to the clinical facilities occurred at screening and on days 1 (first dose), 2, 15, 29 (second dose), 30, 43, 57 (third dose), 58, 71, 85 (fourth dose), 86, 99, and 113 (end of study [EOS]). Patients randomized to the PK portion of the study were to have additional visits on days 3 and 87. The final follow-up visit for patients not enrolled into a planned extension study was to occur on day 169. Adverse events (AEs) were followed until resolution or grade 1 status was achieved, until the condition stabilized, until the event was otherwise explained, or until the patient was lost to follow-up. The study was performed in accordance with the 2008 Declaration of Helsinki[20] and good clinical practice guidelines. The study was approved by the institutional review board/independent ethics committee of the participating institutions. All subjects gave written informed consent before screening. Additionally, the sponsor appointed an independent data safety monitoring board to provide independent oversight of the study and assure patient safety.

PARTICIPANTS

Potential patients underwent screening to confirm eligibility and were randomized during the 60 days prior to the scheduled dosing date. Patients with CHB, 18-75 years in age, were eligible to participate. The Heparc-2002 study required a diagnosis of E-neg, immune active (indicated by elevated liver enzyme level, mentioned below), CHB infection (HBsAg-positive for >6 months, confirmed by two assays >6 months apart), >2 months of continuous daily oral ETV (0.5 or 1.0 mg/day) or TDF (300 mg/day), and HBV DNA <200 IU/mL at screening. For the Heparc-2003 study, E-pos CHB patients with the same inclusion criteria were eligible to participate. The full inclusion and exclusion criteria for both Heparc-2002 and Heparc-2003 studies are listed in Supporting Table S1.

TREATMENTS

Test Formulation

ARC-520 injection was administered by intravenous infusion at a dose of 1.0 or 2.0 mg/kg. Arrowhead Pharmaceuticals, Inc. (Pasadena, CA), supplied the ARC-520 injection as two sterile 10-mL vials containing ARC-520 active pharmaceutical ingredient (API; siRNAs AD0009 and AD0010) and ARC-EX1 (the delivery excipient). The API is composed of a 1:1 molar mixture of two synthetic, double-stranded, cholesterol-conjugated RNA oligonucleotides. ARC-EX1 is a masked, hepatocyte-targeted polymeric amine (polymeric amine = L-melittin-derived peptide; masking group = carboxydimethyl maleic anhydride N-acetyl galactosamine melittin-like peptide).[21] Prior to dosing subjects, a study pharmacist mixed one vial of ARC-EX1 with one vial of API to yield the ARC-520 injection.

Reference Formulation

The PBO was normal saline (0.9%).

Antihistamine

An oral antihistamine (diphenhydramine 50 mg or cetirizine 10 mg or chlorpheniramine 8 mg or hydroxyzine 50 mg) was supplied to all patients; all antihistamine doses were given at 2 ± 0.5 hours prior to study drug or placebo administration.

Patients received four doses of ARC-520 injection or PBO every 4 weeks, administered intravenously by clinical staff at the infusion rate of 0.4 mL/minute concomitantly with 3.6 mL/minute of normal saline. The dose administered throughout the trial was based on patient weight at screening.

SAFETY ASSESSMENTS

The safety analysis included all patients who were randomized and received study medication. Safety measures included (1) AEs, (2) physical examinations (3) vital signs (resting heart rate, semisupine systolic/diastolic blood pressure, respiratory rate, and temperature); (4) electrocardiogram (ECG) measurements (readings taken after the subject was supine for at least 3 minutes); (5) clinical laboratory tests (hematology, biochemistry, coagulation, and urinalysis); (6) use of concomitant medications, and (7) recording reasons for treatment discontinuation due to toxicity.

Abnormalities in laboratory findings or other assessments that were deemed clinically significant by the principal investigators and were initially detected

during the study or present at baseline and significantly worsened during the study were reported as AEs, whether or not they were considered drug-related.

VIROLOGIC PARAMETERS

Whole-blood samples were processed for serum and samples stored at −80°C until transfer to the bioanalytical laboratory. Quantitative HBV serology parameters assessed were HBV DNA (Cobas AmpliPrep/Cobas TaqMan, v2.0; Roche Diagnostics), HBsAg (Elecsys HBsAg II quant; Roche Diagnostics), HBeAg (Liaison; DiaSorin; E-pos patients only). Qualitative assessments also included the presence of antibody to HBsAg and antibody to HBeAg (E-pos patients only) as well as HBV genotyping and sequencing of archival samples, where available.

ADDITIONAL ASSESSMENTS

Descriptions of PK, cytokine, and complement assessments are provided in the Supporting Information.

OBJECTIVES

The primary objective of the study was to evaluate the depth of HBsAg decline in response to multiple doses of ARC-520 compared to PBO in patients with CHB as a measure of drug activity. Secondary objectives were the determination of incidence and frequency of AEs possibly or probably related to treatment as a measure of safety and tolerability of ARC-520 and to evaluate multidose PK of ARC-520 in patients with HBV when coadministered with a fixed dose of ETV or TDF. There were also multiple additional exploratory objectives, which were excluded from the analysis due to the early termination of the studies.

STATISTICAL ANALYSIS

All study patients who received at least one dose of study drug and had valid quantitative HBsAg (qHBsAg) values at baseline and at least one time point on or after day 15 were included in the intent-to-treat (ITT) population. Patients who were randomized to either the PBO low-dose or PBO high-dose group were pooled into one single PBO group for efficacy and safety analyses.

The primary efficacy analysis compared the change from baseline to day 113 in the $\log_{10}$ of the qHBsAg between each ARC-520 dose group and the pooled PBO group in the ITT population. Baseline for efficacy measures was defined as the mean of screening (two time points) and day 1 (predose) log qHBsAg values, with a minimum of one valid screening and a valid day 1 (predose) value required. Change from baseline used a restricted maximum likelihood–based repeated measures mixed effects model that included observations from all regularly scheduled visits for each patient. A similar analysis was performed on the available quantitative HBeAg data in E-pos patients. The HBeAg analysis set included a subset of patients with evaluable data as some patients had missing HBeAg data due to early termination of the study. A detailed description of the statistical methods is provided in the Supporting Information.

Results

A total of 58 out of 60 and 32 out of 48 planned subjects were enrolled and randomized in the Heparc-2002 and Heparc-2003 studies, respectively (Table 1). A total of 93 and 61 patients were screened for Heparc-2002 and Heparc-2003, respectively (Supporting Figs. S1 and S2). The demographic data for both Heparc-2002 and Heparc-2003 are depicted in Table 2.

For Heparc-2002, 9 patients were randomized to the PBO low-dose group, 11 to the PBO high-dose group, 17 to the ARC-520 injection 1 mg/kg group, and 21 to the ARC-520 injection 2 mg/kg group (Table 2). Fifty-two of 58 subjects completed the study, 4 patients were discontinued due to study termination by the sponsor, 1 patient withdrew due to pregnancy, and 1 patient withdrew consent.

For Heparc-2003, of the 32 patients who enrolled in this study, 10 received ARC-520 injection 1.0 mg/kg, 11 received ARC-520 injection 2.0 mg/kg, 6 received PBO low dose, and 5 received PBO high dose (Table 2). Twenty-two of 32 randomized subjects completed the study, 14 in the ARC-520 injection groups and 8 in the PBO groups. Nine of 10 subjects who discontinued were due to early study termination by the sponsor, and 1 withdrew consent.

Most participants in the Heparc-2002 study and all in the Heparc-2003 study were Asian. The remainder

TABLE 1. Patient Disposition (All Randomized Patients)

	Heparc-2002				Heparc-2003			
	Placebo		ARC-520 Injection		Placebo		ARC-520 Injection	
Category	Low Dose n (%)	High Dose n (%)	1 mg/kg n (%)	2 mg/kg n (%)	Low Dose n (%)	High Dose n (%)	1 mg/kg n (%)	2 mg/kg n (%)
Patients randomized	9	11	17	21	6	5	10	11
Patients completed	9 (100)	8 (72.7)	17 (100)	18 (85.7)	4 (66.7)	4 (80.0)	6 (60.0)	8 (72.7)
Patients discontinued	0	3 (27.3)	0	3 (14.3)	2 (33.3)	1 (20.0)	4 (40.0)	3 (27.3)
AE	0	0	0	0	0	0	0	0
Lost to follow-up	0	0	0	0	0	0	0	0
Physician decision	0	0	0	0	0	0	0	0
Pregnancy	0	0	0	1 (4.8)	0	0	0	0
Study terminated by sponsor	0	2 (18.2)	0	2 (9.5)	2 (33.3)	1 (20.0)	3 (30.0)	3 (27.3)
Withdrawal by patient	0	1 (9.1)	0	0	0	0	1 (10.0)	0
Death	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0

of the patients enrolled in the Heparc-2002 study were of Caucasian and African descent (Table 2). Median age ranged from 37.5 to 48 years across cohorts in both studies. HBV genotype information for patients in these studies was not available because HBV DNA levels were too low for genotyping at screening as all patients had been on long-term NUC therapy prior to study entry. Historical genotype information was not available for any patients either as this is not typically done as part of standard clinical practice. Based on the location of study sites and the ethnicity of patients (Table 2), it can be assumed that the majority of patients had HBV of either genotype B or C.

VIROLOGIC RESPONSES

As required per protocol, concomitant NUC medication included either TDF (300 mg) or ETV (0.5 mg or 1 mg). In all treatment groups, the majority of patients received ETV (0.5 mg) as concomitant NUC therapy for HBV infection.

All but one E-neg patient entering the Heparc-2002 study were reported to be NUC-experienced, with a mean duration of prior NUC therapy of 5.2-5.4 years (Table 3). Heparc-2002 cohorts were well matched with regard to their prior years of NUC therapy and HBV DNA levels on day 1 and EOS. Only 2 patients had measurable HBV DNA levels prior to ARC-520, 1 PBO patient with a titer of 23 IU/mL at screening and 31 IU/mL on day 1 and 1 ARC-520, 1 mg/kg, patient with a titer of 28 IU/mL at screening and 71,665 IU/mL on day 1. The latter patient was reported to be on TDF therapy for 5 months prior to entering the study. No patients had measurable titers at the EOS visit.

E-pos patients entering the Heparc-2003 study were all NUC-experienced, with a mean duration of prior NUC therapy of 2.8-3.7 years (Table 3). Heparc-2003 cohorts were well matched with regard to their prior years of NUC therapy and HBV DNA levels on day 1 and EOS. Three patients had measurable HBV DNA titers, 2 on day 1 and 1 at EOS. All reported titers were close to the lower limit of quantitation of the assay of 20 IU/mL. Baseline HBsAg levels were well matched across cohorts, with mean log HBsAg between 2.6 and 3.7 log IU/mL (Table 3); and baseline HBsAg was similar in E-neg and E-pos patients. Absolute log HBsAg values over the study time are shown in Supporting Figs. S3 and S4.

TABLE 2. Patient Demographics at Baseline and ARC-520 Treatment Exposure

	Heparc-2002				Heparc-2003			
	Placebo		ARC-520 Injection		Placebo		ARC-520 Injection	
Category	Low Dose n (%)	High Dose n (%)	1 mg/kg n (%)	2 mg/kg n (%)	Low Dose n (%)	High Dose n (%)	1 mg/kg n (%)	2 mg/kg n (%)
Age (years)								
n	9	11	17	21	6	5	10	11
Mean (SD)	46.2 (12.1)	48.7 (9.5)	45.0 (10.5)	45.7 (10.3)	39.8 (9.5)	45.0 (10.7)	42.1 (12.6)	41.6 (12.1)
Min, Max	31, 65	34, 63	28, 62	30, 65	26, 53	33, 57	28, 66	21, 54
Sex (n, %)								
Male	7 (77.8)	4 (90.9)	10 (58.8)	12 (57.1)	3 (50.0)	4 (80.0)	10 (100.0)	5 (45.5)
Female	2 (22.2)	1 (9.1)	7 (41.2)	9 (42.9)	3 (50.0)	1 (20.0)	0	6 (54.5)
Ethnicity								
Chinese	3 (33.3)	2 (18.2)	6 (35.3)	8 (38.1)	1 (16.7)	1 (20.0)	3 (30.0)	4 (36.4)
Korean	2 (22.2)	8 (72.7)	5 (29.4)	8 (38.1)	5 (83.3)	4 (80.0)	7 (70.0)	6 (54.5)
Vietnamese	0	0	0	2 (9.5)	0	0	0	1 (9.1)
Black or African	1 (1.1)	0	1 (5.9)	0	0	0	0	0
Hispanic	0	0	1 (5.9)	0	0	0	0	0
Non-Hispanic Caucasian	3 (33.3)	1 (9.1)	4 (23.5)	3 (14.3)	0	0	0	0
Other	0	0	0	0	0	0	0	0
Body mass index (kg/m^2)								
Mean (SD)	25.4 (2.7)	24.6 (3.0)	23.9 (2.8)	23.2 (2.9)	23.5 (4.5)	21.8 (2.7)	23.3 (2.3)	24.6 (3.7)
Min, Max	22.1, 29.7	20.0, 29.8	18.1, 29.4	19.0, 29.1	19.7, 29.5	19.0, 25.9	20.2, 27.9	19.1, 29.8
Number doses received (n, %)								
1	0	0	0	0	1 (16.7)	0	1 (10.0)	1 (9.1)
2	0	1 (9.1)	0	1 (4.8)	1 (16.7)	1 (20.0)	0	0
3	0	0	0	0	0	0	3 (30.0)	2 (18.2)
4	9 (100.0)	10 (90.9)	17 (100.0)	20 (95.2)	4 (66.7)	4 (80.0)	6 (60.0)	8 (72.7)

E-neg patients enrolled in the Heparc-2002 study showed a dose-dependent reduction in HBsAg values from baseline. The low-dose group had mean reductions of <0.2 log IU/mL from day 1 through day 113, whereas the high-dose group showed mean HBsAg reductions of 0.2-0.4 log IU/mL (Fig. 1A). Reductions in HBsAg were seen after the first dose, with additional reductions after the second dose in the low-dose group and after the second and third doses in the high-dose group. Nadir for both treatment groups was reached at day 99, 15 days after the last dose. A gradual return of HBsAg levels was observed after nadir, with both groups approaching HBsAg levels observed in the placebo group by 70 days after nadir. Primary endpoint

TABLE 3. NUC Therapy, HBsAg and HBeAg Levels at Baseline, and HBV DNA Levels Pretherapy and Posttherapy

	Heparc-2002			Heparc-2003		
		ARC-520 Injection			ARC-520 Injection	
Category	Placebo	1 mg/kg	2 mg/kg	Placebo	1 mg/kg	2 mg/kg
NUC-experienced patients	19 of 20	17 of 17	21 of 21	11 of 11	10 of 10	11 of 11
Mean (min, max) years of prior NUC therapy	5.4 (1.5, 8.5)	5.4 (2.5, 7.4)	5.2 (1.4, 8.8)	3.7 (1.4, 7.3)	3.5 (1.2, 5.8)	2.8 (1.0, 6.7)
Baseline log HBsAg						
Mean (min, max) Log HBsAg (log IU/mL)	3.3 (2.0, 4.5)	3.2 (0.4, 4.2)	2.6 (0.5, 3.8)	3.7 (2.8, 4.4)	3.3 (2.4, 4.1)	3.3 (2.7, 4.1)
Baseline HBeAg						
Mean (min, max) Log HBeAg (log PEIU/mL)	N/A	N/A	N/A	0.7 (–1.1, 1.6)	–0.1 (–0.8, 1.0)	–0.2 (–1.7, 1.1)
HBV DNA day 1						
>20 IU/mL	1	1	0	0	1	1
<20 IU/mL	9	5	4	7	2	5
Not detected	10	10	17	4	7	5
Missing	0	1	0	0	0	0
HBV DNA day 113 EOS						
>20 IU/mL	0	0	0	1	0	0
<20 IU/mL	5	6	5	4	2	6
Not detected	12	11	13	4	4	2
Missing/early term	3	0	3	2	4	3

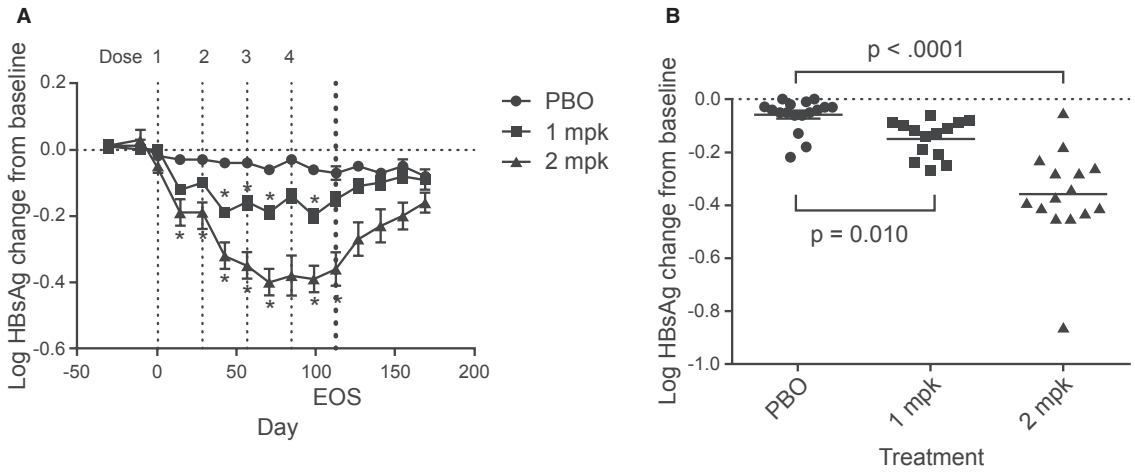

FIG. 1. Mixed effect model repeat measurement analysis of log HBsAg change from baseline to day 113 in the ITT analysis set in the Heparc-2002 study. (A) Mean change from baseline over time. (B) Individual and mean log HBsAg change from baseline to day 113. *P < 0.05 versus placebo; error bars indicate standard error; baseline = average of screen and day 1 visits.

analysis was performed at day 113, 30 days after the last dose (Supporting Table S2). The high-dose ARC-520 group showed a highly significant reduction in HBsAg ($P < 0.0001$) compared to placebo, with a mean reduction of 0.379 log IU/mL from baseline (Fig. 1B). However, the low-dose ARC-520 group was not statistically lower than PBO at day 113 ($P = 0.081$), with an average reduction of 0.157 log IU/mL from baseline.

E-pos patients enrolled in the Heparc-2003 study also showed a dose-dependent reduction in HBsAg values from baseline. The low-dose group had mean reductions of <0.25 log IU/mL from day 1 through day 113, whereas the high-dose group showed mean HBsAg reductions of 0.2-0.6 log IU/mL (Fig. 2A). Reductions in HBsAg were seen after all doses in the high-dose group but not in the low-dose group, which maintained HBsAg reductions throughout the dosing period. Nadir for the low-dose and high-dose treatment groups was reached at days 71 and 99, respectively. A gradual return of HBsAg levels was observed after nadir, with the low-dose group approaching HBsAg levels observed in the placebo group by 30 days after the last dose. The high-dose group still showed significant reductions in HBsAg 70 days after reaching nadir. Primary endpoint analysis was performed at day 113, 30 days after the last dose (Supporting Table S3). The high-dose ARC-520 group showed a highly significant reduction in HBsAg ($P > 0.0001$) compared to PBO, with a mean reduction of 0.539 log IU/mL from baseline (Fig. 2B). However, the low-dose ARC-520 group was not statistically lower than PBO at day 113 ($P = 0.5910$), with an average reduction of 0.143 log IU/mL from baseline.

There were no correlations between baseline HBsAg levels and magnitude of HBsAg reduction for both E-pos and E-neg patients (Supporting Fig. S5).

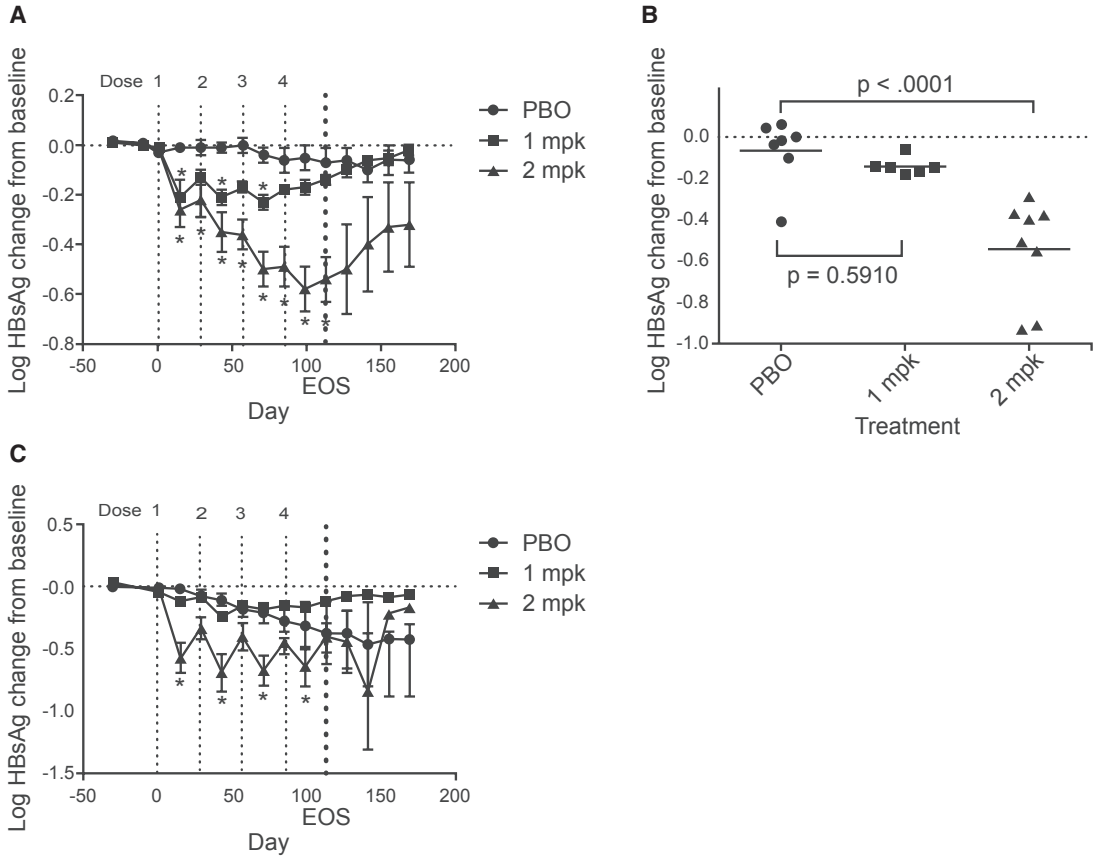

FIG. 2. Log HBsAg and HBeAg change from baseline to day 113 in the Heparc-2003 study. (A) Mixed effect model repeat measurement analysis of log HBsAg change from baseline to day 113 in the ITT analysis set. (B) Individual and mean log HBsAg change from baseline to day 113. (C) Mean log HBeAg change from baseline over time. *$P < 0.05$ versus placebo; error bars indicate standard error; baseline = average of screen and day 1 visits.

E-pos patients also showed a dose-dependent reduction in HBeAg from baseline (Fig. 2C). Mean maximum reduction in the low-dose ARC-520 group was 0.27 log Paul Ehrlich international units (PEIUs)/mL on day 43, with HBeAg levels gradually returning to baseline after the last dose. Mean maximum reduction in the high-dose ARC-520 group was 0.72 log PEIU/mL on day 43, with a sawtooth reduction and recovery observed after each dose. Interestingly, mean HBeAg levels were gradually decreasing in the placebo group, driven by 1 patient who had an increase in serum alanine aminotransferase from 28 to 64 U/L between days 29 and 85, concomitant with a 2.2 log PEIU/mL drop in HBeAg. All other placebo patients showed minimal changes in HBeAg from baseline. Statistical analysis showed a significant reduction of HBeAg for the high-dose group at 15 days after each dose but not at day 113 EOS or other time points. The low-dose group showed no significant reduction in HBeAg compared to placebo at any time point (Fig. 2C; Supporting Table S4).

SAFETY AND TOLERABILITY

Overall, in the Heparc-2002 study, the study treatment was found to be safe and well tolerated. There were no deaths in the study, the majority of treatment emergent AEs (TEAEs) were mild, and none led to discontinuation from the study treatment or the study (Table 4). There were three serious AEs (SAEs) in 3 patients reported during the study: 2 SAEs of pyrexia and 1 SAE of cholangiocarcinoma (Table 4). There was one discontinuation due to pregnancy. Additionally, there were no significant safety issues with regard to vital signs, ECGs, or adverse changes in safety laboratory tests.

Similarly, in the Heparc-2003 study, the study treatment was safe and well tolerated. There were no deaths, pregnancies, SAEs, or severe TEAEs reported in this study. The majority of TEAEs were mild, and no TEAEs led to discontinuation (Table 4). Additionally, there were no significant safety issues with regard to vital signs, ECGs, or adverse changes in safety laboratory tests.

The SAE of cholangiocarcinoma was reported in a 61-year-old male patient with a history of CHB, liver cirrhosis, osteoarthritis, and hypertension. It was first detected during routine ultrasound and confirmed by abdominal computed tomographic scan after the last dose of ARC-520 and was considered unrelated to study drug by the investigator.

The two cases of pyrexia had onset within 1 hour of study infusion and were treated successfully with acetaminophen. Both patients were kept in the hospital overnight for observation without recurrence of fever, causing classification as SAEs considered possibly related.

Because the ARC-EX1 component was derived from honey bee venom, allergenicity was measured by changes in bee venom immunoglobulin E (IgE) levels from predose to EOS. There were no clinically significant increases in bee venom–specific IgE levels after dose administration in either study. A total of 3 ARC-520 patients showed an increase in IgE levels from predose to postdose, while 2 ARC-520 patients showed a decrease. A similar rate of changes was seen in PBO patients. Patients experiencing small changes in IgE did not demonstrate associated clinical symptoms of hypersensitivity.

Discussion

ARC-520 injection was the first RNAi therapeutic targeting HBV to enter clinical testing. It was designed to reduce all RNA transcripts derived from viral cccDNA, leading to a reduction in viral antigens, as well as HBV DNA. Viral antigens, especially HBsAg, have been implicated in the suppression of the immune system due to immune exhaustion, leading to persistence of chronic viral infection. RNAi therapy leading to reductions in viral antigens may allow for immune reconstitution and functional cure.

ARC-520 has been shown to have a favorable tolerability profile in a single-dose study in healthy volunteers.[19] It has also been evaluated in a single-dose phase 2 study in patients with CHB.[16] In that study, HBsAg was strongly reduced in treatment-naive patients positive for HBeAg but was reduced significantly less in patients who were E-neg or had received long-term therapy with NUCs regardless of HBeAg status. This differential response was attributed to the finding in chimpanzees that HBsAg was expressed not only from the episomal cccDNA minichromosome but also from transcripts arising from HBV DNA integrated into the host genome, which may have been an important or even dominant source

TABLE 4. Incidence of AEs in the Heparc-2002 and Hepac-2003 Studies

	Heparc-2002				Heparc-2003			
	Placebo		ARC-520 Injection		Placebo		ARC-520 Injection	
Category	Low Dose n (%)	High Dose n (%)	1 mg/kg n (%)	2 mg/kg n (%)	Low Dose n (%)	High Dose n (%)	1 mg/kg n (%)	2 mg/kg n (%)
Overview of AEs								
Number patients in safety population	9	11	17	21	6	5	10	11
Patients with at least one TEAE	4 (44.4)	4 (36.4)	6 (35.3)	12 (57.1)	4 (66.7)	0	5 (50.0)	3 (27.3)
Patients with at least one serious TEAE	0	0	2 (11.8)	1 (4.8)	0	0	0	0
SAEs								
Pyrexia	0	0	1 (5.9)	1 (4.8)	0	0	0	0
Cholangiocarcinoma	0	0	1 (5.9)	0	0	0	0	0
TEAEs in more than 1 patient across both studies regardless of relationship								
Chest discomfort	0	1 (9.1)	0	1 (4.8)	0	0	0	0
Chills	0	0	1 (5.9)	1 (4.8)	0	0	1 (10.)	0
Fatigue	0	0	1 (5.0)	2 (9.5)	1 (16.7)	0	1 (10.0)	0
Influenza-like illness	0	0	1 (5.0)	1 (4.8)	0	0	0	0
Malaise	1 (11.1)	0	0	1 (4.8)	0	0	0	0
Pyrexia	0	0	2 (11.8)	3 (14.3)	0	0	1 (10.0)	0
Nasopharyngitis	0	1 (9.1)	0	0	1 (16.7)	0	0	2 (18.2)
Upper respiratory tract infection	1 (11.1)	1 (9.1)	1 (5.9)	3 (14.3)	0	0	0	1 (9.1)
Blood creatine phosphokinase increase	1 (11.1)	0	0	0	0	0	0	1 (9.1)
Headache	0	0	1 (5.9)	1 (4.8)	0	0	1 (10.0)	0

There were no deaths and no treatment or study discontinuations due to TEAEs in either study.

of HBsAg in E-neg and Nuc-experienced E-pos patients.

Here, we report on two double-blinded, phase 2 multidose studies of ARC-520 in NUC-experienced, E-neg or E-pos patients with CHB in combination with TDF or ETV.

The primary objective of the studies was to evaluate the depth of HBsAg decline in response to multiple doses of ARC-520 compared to PBO as a measure of drug activity. The 2 mg/kg high-dose groups met the primary endpoint of statistically significant reduction in HBsAg compared to PBO 30 days after the fourth dose in both E-neg and E-pos patients, while the 1 mg/kg groups did not. The absolute reductions in HBsAg were relatively modest, 0.38 and 0.54 log IU/mL for E-neg and E-pos patients, respectively, with reductions slightly larger in the E-pos cohort. This may be an indication of a larger amount of HBsAg expressed from cccDNA in NUC-experienced E-pos patients. Overall, reductions in HBsAg were still significantly smaller than those observed in NUC-naive, E-pos subjects.[16] This is consistent with the mode of action and design of ARC-520, which targets all transcripts expressed from cccDNA but cannot cleave most transcripts resulting from integrated HBV DNA due to loss of the target site located in the DR1/DR2 region of the virus, which is commonly deleted upon HBV integration.[16] It is also consistent with reports of reductions of cccDNA upon long-term NUC treatment, regardless of HBeAg status.[22] HBsAg levels persisting after multiple doses of ARC-520 may therefore represent a "floor" of HBsAg expression that cannot be addressed by therapies, including ARC-520, that only target cccDNA-derived viral transcripts.

HBsAg reductions persisted for a prolonged period of time after multiple doses of ARC-520, approximately 85 days after the last dose in E-neg and >85 days after the last dose in E-pos patients. This prolonged persistence of activity is due to the unique RNAi mechanism in which a small amount of siRNA guide strand can persist and be active within the RNA-induced silencing complex in the cytoplasm of target cells for extended periods of time. A long duration of activity of more than 4 weeks has also been demonstrated after a single dose of ARC-520 injection in preclinical animal studies[21] and in patients with CHB.[23]

ARC-520 was well tolerated in this study, in which all patients were pretreated with an oral antihistamine. Antihistamine pretreatment was implemented based on results from nonclinical and phase 1 studies indicating that ARC-520 could induce histamine release. It was subsequently shown that the ARC-EX1 delivery agent induced histamine release through mast cell degranulation.[19] Only two SAEs that were possibly related to study drug were reported in both studies, both for pyrexia and both occurring shortly after dosing with ARC-520 in two separate patients. Both patients went on to receive additional doses of ARC-520 without subsequent drug-related AEs.

In summary, ARC-520 injection was well tolerated in two randomized phase 2 multidose studies in E-pos and E-neg, NUC-experienced patients with CHB infection. Multiple doses of 2 mg/kg ARC-520 significantly reduced HBsAg in both patient groups compared to PBO, and antigen reductions were sustained for a long period of time; however, absolute reductions were generally moderate. Most likely, the limited pharmacologic activity was due to the high level of HBsAg expression from integrated HBV DNA in these populations combined with the inability of ARC-520 to target mRNA resulting from integrated HBV DNA. This indicates the need for the development of RNAi therapeutics that can target all viral transcripts, regardless of their origin, as RNAi is the only clinical-stage technology currently available that can target HBsAg resulting from integrated HBV DNA. Clinical studies with RNAi molecules that can be subcutaneously administered and target viral transcripts from all sources, such as ARO-HBV, are now in progress.

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Authors Contributions: Investigation: M.-F.Y., I.S., J.-H.Y., J.H.A., J.H., J.H.K., H.L.Y.C., K.T.Y., H.K., M.M., J.P.; Formal Analysis: M.-F.Y., T.S., J.H., C.-L.L., S.A.L., B.D.G.; Conceptualization: T.S., J.H., R.G.G., B.D.G.; Writing – Original Draft: T.S., J.H., R.G.G., B.D.G.; Writing – Review & Editing: T.S., B.D.G., M.-F.Y., C.-L.L., S.A.L.; Visualization: T.S.

REFERENCES

1) World Health Organization. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017.
2) Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661-662.
3) Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-2128.
4) European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370-398.
5) Seto WK, Lo YR, Pawlotsky JM, Yuen MF. Chronic hepatitis B virus infection. Lancet 2018;392:2313-2324.
6) Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: from discovery to regulatory approval. J Hepatol 2017;67:847-861.
7) Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
8) Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ; Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-686.
9) Fung J, Cheung KS, Wong DK, Mak LY, To WP, Seto WK, et al. Long-term outcomes and predictive scores for hepatocellular carcinoma and hepatitis B surface antigen seroclearance after hepatitis B e-antigen seroclearance. Hepatology 2018;68:462-472.
10) **Yuen MF**, **Wong DK**, Fung J, Ip P, But D, Hung I, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology 2008;135:1192-1199.
11) Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Batrla-Utermann R, et al. Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. Gut 2014;63:1648-1657.
12) Bock CT, Schwinn S, Locarnini S, Fyfe J, Manns MP, Trautwein C, et al. Structural organization of the hepatitis B virus minichromosome. J Mol Biol 2001;307:183-196.
13) Gish RG, Given BD, Lai CL, Locarnini SA, Lau JY, Lewis DL, et al. Chronic hepatitis B: virology, natural history, current management and a glimpse at future opportunities. Antiviral Res 2015;121:47-58.
14) Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012;61:1754-1764.
15) Ferrari C. HBV and the immune response. Liver Int 2015;35 (Suppl. 1):121-128.
16) **Wooddell CI**, **Yuen MF**, Chan HL, Gish RG, Locarnini SA, Chavez D, 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 2017;9:eaan0241.
17) Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell 2009;136:642-655.
18) **Rozema DB**, **Lewis DL**, Wakefield DH, Wong SC, Klein JJ, Roesch PL, et al. Dynamic polyconjugates for targeted *in vivo* delivery of siRNA to hepatocytes. Proc Natl Acad Sci USA 2007;104:12982-12987.
19) Schluep T, Lickliter J, Hamilton J, Lewis DL, Lai CL, Lau JY, et al. Safety, tolerability, and pharmacokinetics of ARC-520 injection, an RNA interference–based therapeutic for the treatment of chronic hepatitis B virus infection, in healthy volunteers. Clin Pharmacol Drug Dev 2017;6:350-362.
20) World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Ferney-Voltaire, France: World Medical Association; 2008.
21) **Wooddell CI**, **Rozema DB**, Hossbach M, John M, Hamilton HL, Chu Q, et al. Hepatocyte-targeted RNAi therapeutics for the treatment of chronic hepatitis B virus infection. Mol Ther 2013;21:973-985.
22) **Lai CL**, **Wong D**, Ip P, Kopaniszen M, Seto WK, Fung J, et al. Reduction of covalently closed circular DNA with long-term nucleos(t)ide analogue treatment in chronic hepatitis B. J Hepatol 2017;66:275-281.
23) Yuen M, Chan H, Given B, Hamilton H, Schluep T, Lewis DL, et al. Phase II, dose ranging study of ARC-520, a siRNA-based therapeutic, in patients with chronic hepatitis B virus infection. Hepatology 2014;60(Suppl. 1):LB-21.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.31008/suppinfo.