Journal Pre-proof

Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy

Michel Bazinet, Victor Pântea, Gheorghe Placinta, Iurie Moscalu, Valentin Cebotarescu, Lilia Cojuhari, Pavlina Jimbei, Liviu Iarovoi, Valentina Smesnoi, Tatiana Musteata, Alina Jucov, Ulf Dittmer, Adalbert Krawczyk, Andrew Vaillant

PII: S0016-5085(20)30320-6
DOI: https://doi.org/10.1053/j.gastro.2020.02.058
Reference: YGAST 63264

To appear in: *Gastroenterology*
Accepted Date: 27 February 2020

Please cite this article as: Bazinet M, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, Jimbei P, Iarovoi L, Smesnoi V, Musteata T, Jucov A, Dittmer U, Krawczyk A, Vaillant A, Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy, *Gastroenterology* (2020), doi: https://doi.org/10.1053/j.gastro.2020.02.058.

Therapy	Response rates during therapy	Response rates during treatment-free follow-up
TDF pegIFN	**LOW** HBsAg loss HBsAg seroconversion	**LOW** Functional cure
TDF pegIFN **NAPs**	**HIGH** HBsAg loss HBsAg seroconversion Asymptomatic transaminase flares	**HIGH** Functional cure Normal liver function

Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy

Short title: NAP combination therapy in chronic HBV

Michel Bazinet[1], Victor Pântea[2], Gheorghe Placinta[2], Iurie Moscalu[3], Valentin Cebotarescu[2], Lilia Cojuhari[2], Pavlina Jimbei[4], Liviu Iarovoi[2], Valentina Smesnoi[4], Tatiana Musteata[4], Alina Jucov[2,3], Ulf Dittmer[5], Adalbert Krawczyk[5,6], and Andrew Vaillant[1]*

1. Replicor Inc. Montreal, Canada
2. Department of Infectious Diseases, Nicolae Testemiţanu State University of Medicine and Pharmacy, Chișinău, Republic of Moldova
3. ARENSIA Exploratory Medicine, Republican Clinical Hospital Chișinău, Moldova
4. Toma Ciorbă Infectious Clinical Hospital, Chișinău, Republic of Moldova
5. Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
6. Department of Infectious Diseases, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

*Corresponding author

Supported by Replicor Inc.

AE, adverse event; AUC, area under the curve; CMV, cytomegalovirus; ECG, electrocardiogram; G-CSF, granulocyte colony stimulating factor; GGT, glutamyl aminotransferase; HIV, human immunodeficiency virus; INR, international normalized ratio; IU, international units; LLOQ, lower limit of quantification; LMS, liver median stiffness; NAP., nucleic acid polymer; pegIFN, pegylated interferon; TDF, tenofovir disoproxil fumarate; TND, target not detected; ULN, upper limit of normal; WBC, white blood cell

Contact information:

Andrew Vaillant
Chief Scientific Officer
Replicor Inc.
6100 Royalmount Avenue

Montreal, Quebec, Canada H8Y 3E6
+1 513 733-1998
availlant@replicor.com

Disclosures:

MB and AV are employees and shareholders of Replicor Inc. and inventors of patents assigned to Replicor. The Institute for Virology received compensation from Replicor for performing virologic diagnostics. All other authors have no interests to declare.

Author contributions:

AV and MB designed the study, VP, GP, IM, VC, LC, PJ, LI, VS, TM and AJ collected data and AK and UD supervised virologic assessments. AV performed the data analysis and wrote the manuscript with assistance from all authors.

Abstract

Background & Aims:

Nucleic acid polymers (NAPs) inhibit assembly and secretion of hepatitis B virus (HBV) subviral particles. We performed an open-label, phase 2 study of the safety and efficacy of the NAPs REP 2139 or REP 2165 combined with tenofovir disoproxil fumarate (TDF) and pegylated interferon alfa-2a (pegIFN) in patients with negative chronic HBV infection who were negative for HB e antigen (HBeAg).

Methods:

Following 24 weeks TDF therapy, 40 patients were randomly assigned to groups that received 48 weeks of experimental therapy (TDF + pegIFN + REP 2139-Mg or REP 2165-Mg) or 24 weeks of control therapy (TDF + pegIFN) followed by 48 weeks experimental therapy. Patients were then followed for a treatment-free period of 48 weeks. Primary outcomes were the safety and tolerability of REP 2139-Mg or REP 2165-Mg in combination with TDF + pegIFN compared to TDF + pegIFN alone through the first 48 weeks of therapy and subsequently throughout 48 weeks of NAP-based combination therapy (treatment weeks 24-72 in the experimental group and weeks 48-96 in the control group). Secondary outcomes reductions in HBsAg in control and experimental groups over the first 48 weeks of the study and throughout 48 weeks of combination therapy and virologic control (HBsAg positive, HBV DNA below 2000 IU/mL, normal level of alanine aminotransferase) or functional cure (HBsAg below 0.05 IU/mL, HBV DNA target not detected, normal level of alanine aminotransferase) after removal of all therapy.

Results:

Levels of HBsAg, anti-HBs, and HBV DNA did not differ significantly between the groups given REP 2139 vs REP 2165. PegIFN-induced thrombocytopenia (P=.299 vs controls) and neutropenia (P=.112 vs controls) were unaffected by NAPs (REP 2139 vs REP 2165). Increases in levels of transaminases were significantly more frequent (P<.001 vs controls) and greater (P=.002 vs controls) in the NAP groups (but did not produce symptoms), correlated with initial decrease in HBsAg, and normalized during therapy and follow up. During the first 24 weeks of TDF and pegIFN administration, significantly higher proportions of patients in NAP groups had

decreases in HBsAg to below 1 IU/mL (*P*<.001 vs control) and HBsAg seroconversion (*P*=.046 vs control). At the time patients completed the TDF + pegIFN + NAP regimen, HBsAg levels were 0.05 IU/mL or lower in 24/40 participants (all with seroconversion up to 233,055 mIU/mL). During 48 weeks of treatment-free follow up, virologic control persisted in 13/40 participants (2 lost to follow up after 24 weeks), whereas functional cure persisted in with 14/40 participants (all completing 48 weeks of follow-up) with persistent HBsAg seroconversion. One participant had a viral rebound during follow up with hepatic decompensation and was placed on TDF therapy.

Conclusions:

In a phase 2 randomized trial, we found that addition of NAPs to TDF + pegIFN did not alter tolerability and significantly increased rates of HBsAg loss and HBsAg seroconversion during therapy and functional cure after therapy. Clinicaltrials.gov no: NCT02565719.

Keywords: HBV, HBsAg, functional cure, nucleic acid polymer

Introduction

Chronic HBV infection causes progressive liver fibrosis, cirrhosis and hepatocellular carcinoma[1]. With 292 million persons infected worldwide[2] and 800,000 deaths annually[3], this disease presents a significant global medical burden. Approved therapies do not result in optimal outcomes: NUCs suppress viral replication and related liver disease[4] but require lifelong therapy[5] whereas pegIFN is associated with HBsAg loss sustained after therapy in a small proportion (up to 10%) of patients[6], even when combined with NUCs[7].

The NAP REP 2139 blocks the assembly of SVPs in hepatocytes harboring cccDNA or integrated HBV DNA via an as yet unidentified host target[8, 9]. This effect blocks the release of HBsAg and lowers intracellular HBsAg[8]. REP 2139 treatment *in vivo* in DHBV-infected ducks is accompanied by reduction in viral replication in the liver within two weeks[10], rapid clearance of serum HBsAg with delayed clearance of serum HBV DNA and control of infection in the liver (elimination of HBsAg and multilog reductions in HBV DNA and cccDNA) maintained after the removal of therapy[11].

Monotherapy with the REP 2139 precursor REP 2055[10, 12] in HBeAg positive HBV chronic HBV infection was accompanied by HBsAg reduction, HBsAg and HBeAg seroconversion and HBV DNA clearance, however long term virologic control similar to inactive chronic HBV (HBV DNA < 2000 IU/mL, normal ALT) occurred in only 3/8 participants, persisting to 5 years in 2/8 participants with one of these participants additionally experiencing functional cure (HBV DNA target not detected, HBsAg < LLOQ, normal ALT)[12]. REP 2139-Ca monotherapy was accompanied by similar antiviral responses but the addition of a 13 week course of pegIFN or thymosin α1 rapidly cleared HBsAg to < LLOQ in all participants, increased anti-HBs levels and the incidence of transaminase flares and established functional control in 8/9 patients[12]. In chronic HBeAg negative HBV / HDV co-infection, HBsAg declines during REP 2139-Ca + pegIFN therapy were accompanied by HDV RNA loss[9]. Functional control of HBV infection and functional cure of HDV infection (HDV RNA target not detected, normal ALT) has persisted in 7/12 patients for 2 years with 3 of these patients further achieving functional cure of HBV infection[13].

In *in vitro* studies, suppression of intracellular HBsAg and inhibition of HBsAg release by REP 2139 was not accompanied by any toxic effects[8]. In preclinical studies in cynomolgus monkeys, REP 2139 was not accompanied by alteration in liver function or histopathology, even at doses 10x those used in the clinic[10]. Moreover *in vivo*, HBsAg declines were not associated with any detectable changes in liver function. However, in all previous clinical studies, transaminase flares were observed but only in those participants where HBsAg was cleared to < 1 IU/mL and self resolved during treatment. Liver function was normal throughout these flares, which were otherwise asymptomatic and correlated with the establishment of virologic control (HBsAg positive, HBV DNA ≤ 2000 IU/mL, normal ALT) or functional cure (HBsAg < 0.05 IU/mL, HBV DNA target not detected, normal ALT) of HBV infection[9, 12].

The REP 401 study (NCT02565719) investigated the safety and efficacy of TDF + pegIFN + NAPs in HBeAg negative chronic HBV infection. This study also examined the activity of REP 2165, a bioequivalent variant of REP 2139 (Figure 1A) with accelerated clearance not impacting HBV antiviral activity *in vivo*[10] potentially useful in improving HBsAg declines with high frequency dosing for the small proportion of patients who may experience slower rates of HBsAg clearance[12].

Materials and Methods

Study design

The REP 401 study is an open label, randomized, controlled, phase 2 study assessing the safety and efficacy of addition of either REP 2139-Mg or REP 2165-Mg (250mg IV infusion qW) to a backbone therapy of TDF (Viread®, 300mg PO qD) and pegIFN (Pegasys®, 180 μg SC qW). Calculation of statistical power in the REP 401 study was based on historical HBsAg response observed in previous trials with NAPs versus HBsAg response observed with pegIFN or pegIFN + NUCs in chronic HBV infection.

The complete trial design is illustrated in Figure 1B and consisted of two parts. In Part A (Figure 1C) all 40 participants received 24 weeks of TDF monotherapy prior to introduction of pegIFN. Randomization was conducted at the Week 25 visit (first dose of pegIFN) and employed three stratification criteria: HBsAg ≤ 5000 vs > 5000 IU/mL, HBV DNA ≤ $1.74x10^4$ IU/mL vs > $1.74x10^4$ IU/mL and age ≤ 40 vs > 40 years and was conducted via a remote interactive web response system using covariate adaptive algorithm[14]. Randomization assigned 20 participants to each of the control and experimental groups and within each of these groups, assignment of 10 participants to receive REP 2139-Mg and 10 participants to receive REP 2165-Mg (predetermined NAP to be administered in the case of crossover to the addition of NAPs in the control group).

Prior to the initiation of the REP 401 study, the low rates of HBsAg loss and functional cure with pegIFN or NUCs +pegIFN had been well described[15-17] as well as the ability to discern the futility of pegIFN therapy to achieve functional cure with a lack of HBsAg loss at 24 weeks of therapy[18-21]. Based on these known limitations with NUCs + pegIFN and the ability of NAPs in previous clinical studies to achieve high rates of HBsAg loss and improved control of infection persisting after therapy[9, 12], continued dosing of TDF + pegIFN beyond 24 weeks in the control group was not considered ethical in the absence of HBsAg loss..

In Part B of the study (Figure 1D), treatment in experimental group proceeded to complete 48 weeks of combination therapy. Based on the poor HBsAg response observed in the control group, all participants in this group were crossed over to 48 weeks of NAP-based combination therapy, with 10 participants each receiving either REP 2139-Mg or REP 2165-Mg (Figure 1B).

All participants received treatment-free follow up evaluations 4, 12, 24 and 48 weeks after completion of therapy (Week 73 in the Experimental group and Week 96 in the Control group). Dose reductions were not performed for transaminase elevations unless accompanied by abnormal total bilirubin, INR or other signs of liver dysfunction (please refer to the REP 401 protocol for complete dose reduction rules).

Primary outcomes were the safety and tolerability of REP 2139-Mg or REP 2165-Mg when combined with TDF + pegIFN compared to TDF + pegIFN alone through the first 48 weeks of therapy and subsequently throughout 48 weeks of NAP-based combination therapy (treatment weeks 24-72 in the experimental group and weeks 48-96 in the control group). Secondary outcomes were quantitative HBsAg decline and HBsAg seroconversion in control and experimental groups over the first 48 weeks of the study and throughout 48 weeks of combination therapy (weeks 24-72 in the experimental group and 48-96 in the control group) and the establishment of virologic control or functional cure after removal of all therapy in both groups.

The REP 401 study was conducted at three sites in Chișinău, Republic of Moldova; two sites at the Toma Ciorbă Infectious Diseases Hospital and a third site at the Republican Clinical Hospital. The study protocol was compliant with current guidelines on Good Clinical Practice, with the Declaration of Helsinki and was approved by the National Ethics Committee and National Medicines Agency of the Republic of Moldova.

Participants

Participants were all Caucasian, 18 - 55 years old with chronic HBV infection not receiving antiviral therapy or immunotherapy for at least 6 months prior to enrollment. Except in one case (see Table 1), all participants were treatment naïve. Chronicity of infection could be confirmed by documented presence of HBV infection > 6 months in duration in 36/40 participants (see Table 1) and was confirmed in the remaining 4 participants by verification of HBcAg IgG positive and IgM negative status at baseline. HBV infection in all participants at baseline was HBeAg negative / anti-HBe positive with HBsAg ≥ 1000 IU/mL, HBV DNA > 2000 IU/mL and ALT < 10x ULN. Patients were excluded with co-existent HDV, HCV, HIV or active CMV infection or with evidence of cirrhosis or other liver disease. Determination of cirrhosis was based upon ultrasound, hematologic and hepatic function according to accepted practice at the

trial sites. Complete enrolment criteria are provided in the REP 401 study protocol (see http://replicor.com/wp-content/uploads/xxxx/xx/REP 401protocol.pdf). All patients provided written informed consent at enrollment. Protocol deviations allowed 1) the enrollment of one participant with well controlled Gilbert's syndrome and 2) return to follow-up assessment for two participants who did not complete therapy and had protracted absence from the study for personal reasons.

Study drugs

Both REP 2139 and REP 2165 are phosphorothioate oligonucleotides (PS-ONs) previously described with similar antiviral effects in pre-clinical studies[10]. REP 2165 is a analog of REP 2139 designed to be more rapidly cleared by substitution of 2'OMe adenosine with 2'OH adenosine at positions 11, 21 and 31 (Figure 1A), which increases susceptibility to nuclease attack at these positions[10]. Both NAPs are formulated for use as magnesium chelate complexes, a formulation previously shown to improve the administration tolerability of NAPs[12]. The magnesium chelate complex formulation was designed to be more effective in neutralizing infusion reactivity than the calcium chelate complex formulation used in previous studies[9, 12]. REP 2139-Mg and REP 2165-Mg were provided in 5cc glass vials containing 4.2cc of either REP 2139 or REP 2165 at 62.5 mg/mL (based on total sodium salt mass) and administered by IV infusion over 2 hours in 250 mL of normal saline All patients received 250mg qW of NAP, regardless of body weight. TDF and pegIFN were administered according to their package inserts (except for transaminase elevations – see above).

Safety and efficacy assessments

Weekly physical evaluation and periodic ECG, ophthalmic, and liver ultrasound were accompanied by weekly or biweekly biochemical and hematological assessments and Fibroscan at baseline, end of treatment and 24 and 48 weeks of follow-up performed at GCLP (2011) and ISO (15189:2007) certified test labs used in the previous REP 301 study[9]. Virologic response was assessed every 4 weeks and included HBsAg (LLOQ = 0.05 IU/mL) and anti-HBs using the quantitative Abbott Architect® platform, HBeAg,anti-HBe, HBcAg IgG and HBcAg IgM using the qualitative Abbott Architect® platform and HBV DNA using the Abbott Realtime PCR assay (LLOQ = 10 IU/mL). Sample dilution and retesting was performed when HBsAg was > 250 IU/mL and anti-HBs > 1250 mIU/mL. All virologic assessments were performed under the

supervision of UD and AK. Safety and efficacy data not presented in the article is available in the supplementary appendix.

Statistical analysis

Previous studies of NUC + pegIFN in chronic HBV infection reported overall HBsAg declines from baseline of 0.71 $\log_{10}$ [6] with pegIFN and 0.7 $\log_{10}$ (SD ~1..0)[16] with NUCs + pegIFN. The previous REP 301 study with REP 2139-Ca + pegIFN reported an overall 4.15 $\log_{10}$ HBsAg decline from baseline (SD 2.24) in HBeAg negative HBV / HDV co-infection[9]. As such, the difference in HBsAg decline from baseline between TDF + pegIFN (control group from weeks 1-48) and TDF + pegIFN + NAPs (experimental group from weeks 1-48) was expected to be approximately 3 $\log_{10}$. Based on these historical data, enrollment of 20 participants in control and experimental groups yielded a 98% probability to detect a 3 $\log_{10}$ difference in HBsAg responses between groups and would be adequately powered (80% probability) to detect differences as low as 2.05 $\log_{10}$ between groups (α=0.05, two tailed).

Due to the crossover of the control arm at Week 49, statistical analyses of primary and secondary outcomes were performed between groups based on data prior to crossover at Week 48 (Part A, Figure 1C) or between participants receiving 48 weeks of REP 2139-Mg or REP 2165-Mg in the experimental (Weeks 25-73) and control (Week 49-97) groups (Part B, Figure 1D). Statistical analysis of primary and secondary outcome measures was conducted on an intent to treat basis. Primary outcomes with continuous variables were evaluated by T-test and included comparison of mean minima of platelet and WBC counts and mean maxima of transaminase elevations between groups in Part A and between REP 2139-Mg and REP 2165-Mg in Part B. Primary outcomes with discrete variables were analyzed by X^2 test. Statistical analysis of secondary outcomes included comparison of mean HBsAg and HBV DNA declines, and anti-HBs elevations between groups in Part A and between REP 2139-Mg and REP 2165-Mg in Part B.

For the purposes of plotting data and statistical analysis, HBsAg values of 0.00 IU/mL were right censored to 0.01 IU/mL, anti-HBs values of < 0.1 were right censored to 0.1, HBV DNA < LLOQ was right censored to 10 IU/mL and HBV DNA TND was right censored to 1 IU/mL. The AUC for transaminase elevations in all participants during pegIFN exposure was estimated using the iterative trapezoid method. Statistical significance was considered met with $P < 0.05$.

Role of the funding source

AV and MB designed the study. AV performed the analysis of the data and in concert with all authors, the interpretation of the data, the writing of this report and the decision to submit the paper for publication. All authors had access to the study data and reviewed and approved the final manuscript.

Results

Participants

Forty non-cirrhotic participants with chronic HBeAg negative HBV infection (HBsAg > 1000 IU/mL and HBV DNA > 2000 IU/mL) were enrolled between September 2015 and June 2016 with complete results available in April 2019. Group characteristics at baseline and at randomization are shown in Table 1 and disposition of participants from recruitment to the end of follow-up is presented in Figure S1.

Adverse events during the conduct of the study

Unrelated serious AEs included one case of pneumonia which resolved within two weeks with supportive therapy with no interruption of study drugs, one case of appendicitis (during TDF monotherapy) requiring appendectomy, one death due to polytrauma 24 weeks into the follow up (from an accidental woodcutting accident) and one case of autoimmune thrombocytopenia. This latter case of thrombocytopenia occurred 4 weeks into the follow-up in the absence of viral rebound or any other clinically significant finding, rapidly responded to therapy with steroids, etamsylate and eltrombopag and fully resolved by the 12-week follow-up visit. PegIFN-related serious AEs included one case of depression (requiring early withdrawal from treatment with completion of follow-up) and nausea and weakness which responded to supportive therapy without dose alteration. No serious AEs attributable to REP 2139-Mg or REP 2165-Mg were observed. Two participants withdrew (one control and one experimental) for personal reasons not related to safety or efficacy after 24 weeks of TDF + pegIFN + REP 2165-Mg therapy. There was one case viral rebound after FW12 which was accompanied by hepatic decompensation. This decompensation (potentially exacerbated by chronic solvent exposure to solvents at work and repeated overexertion during therapy and follow-up) improved following supportive therapy but further follow-up was not possible due to withdrawn consent.

The administration of REP 2139-Mg and REP 2165-Mg was asymptomatic throughout the entire course of therapy in 37/40 participants (see Table S1 for additional details).

Part A safety and efficacy: TDF + pegIFN versus TDF + pegIFN + NAPs

TDF monotherapy was well tolerated and was accompanied by transaminase normalization in many participants (Figure 2 G-H, J-K). The addition of either pegIFN or pegIFN + NAPs at

week 25 was accompanied by similar grade 1-2 declines in platelet and WBC counts (Figure 2A-C, D-F). Grade 1 fever and myalgia were more prevalent ($P < 0.05$) in patients receiving NAPs (Table 2). Transaminase elevations accompanied the introduction of pegIFN in both groups (Figure 2 G, H, J, K, S2) but occurred in significantly more participants (Table 2, $P < 0.001$) and were significantly stronger (Figure 2 I, L) in the presence of NAPs. Transaminase flares > 3x ULN were observed during TDF + pegIFN in 6/20 patients in the control group and in 18/20 patients in the experimental group. Throughout all transaminase elevations, no evidence of altered liver function (bilirubin, albumin and INR) was observed (Figure S4, S5).

During TDF monotherapy (Weeks 1-24), neither HBsAg decline nor HBsAg seroconversion was observed (Figure 3A, B, E, F). HBV DNA universally declined and was < LLOQ (10 IU/mL) in 28/40 participants by week 24 (Figure 3H, I). Following the addition of pegIFN from weeks 25-48 in the control group, HBsAg declines > 1 $\log_{10}$ IU/ml occurred in 3/20 patients (Figure 3A) but were otherwise < 0.5 $\log_{10}$ from baseline. No HBsAg seroconversion was observed (Table 3, Figure 3E). Following the introduction of pegIFN + NAPs from weeks 25-48 in the experimental group, immediate and rapid 4-6 $\log_{10}$ declines of HBsAg occurred in 15/20 patients as early as 10 weeks with moderate declines in an additional 4/20 patients (Figure 3B). HBsAg became < 1 IU/mL in 14/20 patients and TND (0.00 IU/mL) in 10/20 participants (Table 3, Figure 3B) with HBsAg seroconversion in 11/20 patients (Table 3, Figure 3F), all with HBsAg < 1 IU/mL. HBsAg reduction from baseline was significantly improved (Figure 3C, D) and seroconversion was significantly more prevalent with significantly higher levels of anti-HBs (Figure 3G) in the presence of NAPs compared to TDF + pegIFN alone. HBV DNA decline continued similarly in both groups and was < LLOQ in 18/40 participants by week 48 (Figure 3J).

Part B safety and efficacy: 48 weeks of REP 2139-Mg versus REP 2165-Mg with TDF + pegIFN

At week 49, all participants in the experimental group continued existing therapy to complete 48 weeks of combination therapy. In the control group, all participants crossed over at week 49 to TDF + pegIFN + REP 2139-Mg or REP 2165-Mg for 48 weeks due to poor HBsAg decline (Figure 3A). Safety and antiviral responses between REP 2139-Mg and REP 2165-Mg were compared over the course of 48 weeks of TDF + pegIFN + NAP therapy in control and experimental groups according to Figure 1D.

AEs during 48 weeks of NAP combination therapy were similar between REP 2139-Mg and REP 2165-Mg (Table S2). Management of thrombocytopenia with pegIFN dose reduction and or eltrombopag in 26 patients (Figure S3) maintained ≤ grade 2 thrombocytopenia except in 4 patients (Table S2). Neutropenia and leucopenia were stable and self-regulating to ≤ grade 2 in the absence of G-CSF.

Throughout 48 weeks of TDF + pegIFN + NAPs, transaminase elevations above baseline occurred in 38/40 participants and were otherwise asymptomatic. Flares were most pronounced in both groups during the initial declines in HBsAg (Figure S6, S7) and declined or self-resolved (n=13) during therapy in 37/40 patients (Table 3, Figure S8). Liver synthetic function (bilirubin, albumin or INR) remained normal throughout (Figure S4, S5). Following the introduction of NAPs, flares were generally stronger in the experimental group than in the control group (Figure S9, S10).

Following the crossover in the control group to TDF + pegIFN + NAPs, HBsAg declines and HBsAg seroconversion were observed in 15/20 and 12/20 participants respectively (Figure 4A left; Figure 4C, left). Milder HBsAg declines were observed in the remaining 5 control participants. HBsAg declines and HBsAg seroconversion in the experimental group were maintained throughout therapy (Figure 4A, middle; Figure 4C, middle). Mild (≤ 1 $\log_{10}$) rebounds in HBsAg levels during therapy occurred in 3/40 patients (Figure 4A, B open arrows) which were followed by decline to 0.08 IU/mL (n=1) or a new stable setpoint lower than baseline (n=2). At the end of therapy in the experimental group, HBsAg reduction was > 1 $\log_{10}$ from baseline in 17/20 participants, < 1 IU/mL in 14/20 participants and ≤ 0.05 IU/mL in 13/20 participants. In the control group, HBsAg reduction was > 1 $\log_{10}$ from baseline in 19/20 participants, < 1 IU/mL in 14/20 participants and ≤ 0.05 IU/mL in 11/20 participants (Table 3, Figure 4A, right; Figure 4C, right; Figure S11).

At the end of therapy, combined HBsAg reductions from both groups were > 1 log10 from baseline in 35/40 participants, < 1 IU/mL in 27/40 patients and ≤ 0.05 IU/mL in 24/40 patients (Figure S12). Anti-HBs titers increased over the course of therapy, reaching ≥ 10 mIU/mL (n=24), > 100 mIU/mL (n=19), > 1000 mIU/mL (n=12), > 10,000 mIU/mL (n=10), > 50,000 mIU/mL (n=4) with a maximum of 233,055 mIU/mL in one participant by the end of therapy (Figure 4C, D; Figure S12). HBV DNA remained well controlled and was < 100 IU/mL in all

patients and < LLOQ in 32/40 participants (Figure 4H, I, Table 3, Figure S0). No significant difference in HBsAg declines, anti-HBs levels or HBV DNA declines were observed following addition of NAPs between control and experimental groups or with REP 2139-Mg or REP 2165-Mg (Figure 4).

Follow-up

Platelet and WBC counts returned to baseline by 24 weeks of follow-up (Figure S13, S14). Transaminases normalized and remained stable at 48 weeks of follow-up in 31/34 participants (Table 3, Figure S6, S7). Viral rebound (HBV DNA > 2000 IU/mL) occurred in 5 control participants and 4 experimental participants (Tables S3-S6). Transaminase flares > 3x ULN were associated with viral rebound in four patients (Figure S6, S7). LMS increased during therapy (Figure S12) but steadily declined during follow-up, becoming lower than baseline measurements in 19 patients and normal in 20 patients at the end of 48 weeks of follow-up (Table 3, Figure S12).

HBsAg reduction from baseline persisted in 24/34 participants completing 48 weeks of follow-up, with HBsAg < 1 IU/mL in 17/34, and < LLOQ in 14/34 participants (Table 3, Figure S12). HBsAg seroconversion persisted in 17/34 patients and HBV DNA ≤ 2000 IU/mL persisted in 27/34 patients, with HBV DNA < LLOQ in 20/34 and TND in 17/34 participants. With completion of scheduled therapy and ≥ 24 weeks of follow-up, 28/36 participants experienced functional control with 14 of these further maintaining functional cure and HBsAg seroconversion (Table 3, Tables S3-S6). Viral rebound observed in three participants at the FW12 visit (03-023, 01-008 and 02-023) self resolved to virologic control in the absence of therapy (Tables S3, S4, S5). Virologic control / functional cure rates in participants completing scheduled therapy and ≥ 24 weeks of follow-up were 6/18 (33%) and 6/18 (33%) respectively in the control group and 8/18 (44%) and 8/18 (44%) respectively in the experimental groups (Tables S3-S6). These rates were not statistically different between groups (P = 0.789).

DISCUSSION

Administration tolerability of REP 2139-Mg and REP 2165-Mg in the REP 401 study was improved over REP 2139-Ca from previous studies[9, 12], indicating that SC administration of REP 2139-Mg or REP 2165-Mg will also be well tolerated. Delivery of NAPs to the liver occurs with SC administration *in vivo*[22] and the safety and efficacy of SC administration of REP 2139-Mg will be examined in future studies.

All PS-ONs are accompanied by mild but stable depression of platelet and WBC counts and have little hepatoxicity in humans[23]. In non-human primates , 6-month exposure at 10x the clinical dose of REP 2139 used in the REP 401 study achieved marked accumulation of REP 2139 in the liver but no detectable alteration in liver function or histopathology [10]. To date, all 32 participants receiving NAP monotherapy (REP 2055 and REP 2139-Ca) for 15 – 56 weeks had mild but stable depression of platelet and WBC counts at the 500mg dose[9, 12]. Reduction of REP 2139-Ca to 250mg did not alter hematological response following the addition of pegIFN in the previous REP 301 study[9] and 250mg of REP 2139-Mg or REP 2165-Mg did not impact the hematological response to pegIFN in the REP 401 study, which was effectively managed with pegIFN dose reduction and eltrombopag without affecting antiviral response.

During NAP monotherapy in previous studies, transaminase flares only occurred in participants experiencing HBsAg decline to < 1 IU/mL, self resolved to ≤ baseline levels during therapy, and were associated with the establishment of functional control and functional cure during follow-up[9, 12]. Additionally, strong transaminase flares were observed following addition of pegIFN to REP 2139-Ca therapy only when HBsAg became < 1 IU/mL during REP 2139-Ca monotherapy[9]. All transaminase flares emerging during NAP monotherapy in previous studies were otherwise asymptomatic, with normal liver function observed throughout and when functional control or functional cure was achieved, normal liver function has persisted for at least 5 years of treatment-free follow-up[12].

Transaminase flares occurred in 95% of participants in the REP 401 study and were also correlated with initial HBsAg declines, self resolved or declined during continuing NAP therapy and normalized in 94% of participants completing 48 weeks of follow-up. These flares were not accompanied by alterations in liver function and otherwise asymptomatic. The attenuation of flares in the control group did not appear be the result of weaker declines in serum viremia or

increases in anti-HBs. Although transaminase flares may have a beneficial effect on HBsAg clearance, they occurred in 6 participants where HBsAg remained > 1000 IU/mL throughout therapy. Additional studies examining the immunological components of these flares will be required to better understand the etiology of and the role these flares are playing during therapy.

The relatively poor HBsAg response observed in control participants during Part B of the study receiving only TDF + pegIFN is similar to that observed in the most recent study examining TDF + pegIFN[7] which, while reporting HBsAg loss in 6% of all participants with 48 weeks of therapy[7] , did not observe any HBsAg loss in the 24 genotype D participants enrolled[7] (the predominant genotype in the REP 401 study, see Table 1).

The addition of REP 2139-Mg or REP 2165-Mg to a backbone therapy of TDF + pegIFN did not affect the control of HBV DNA by TDF and resulted in combined reduction of HBsAg to < LLOQ and HBsAg seroconversion (up to 233,055 mIU/mL) in 60% of participants during therapy. In participants completing therapy and 24-48 weeks of follow-up, 28/36 (78%) established virologic control with 14/36 (39%) of participants further establishing functional cure with persistent HBsAg seroconversion. Transaminases normalized in 32/34 (94%) of participants completing 48 weeks of follow-up (32/36 [89%] completing therapy and ≥ 24 weeks of follow-up) 17 of whom had elevated transaminases at baseline (Table 1). LMS continually declined during follow-up in participants with functional control / cure and normalized in 20 participants by the end of follow-up, 9 of these with elevated LMS at baseline. Previous studies have confirmed the stability of virologic control of functional cure and normal liver function and declines in LMS following NAP therapy with 2-5 year follow-ups[9, 12, 13] and additional follow-up is planned to confirm the long-term stability of the therapeutic outcomes in this study. During follow-up, viral rebound occurred in participants where HBsAg was still detectable at the end of 48 weeks of combination therapy (≥ 57.9 IU/mL), who did not complete therapy or where HBsAg clearance occurred very late in therapy (Tables S3-S6). These observations suggest that persistent exposure to pegIFN while HBsAg is cleared may be important for the establishment of virologic control and functional cure.

The presence of normal baseline ALT observed in the REP 401 study is consistent with the elevated rates of normal ALT previously described in chronic HBeAg HBV infection in Moldova[24, 25]. This may be due the higher upper limit of normal of ALT (50 U/L) in the

Moldovan population in general and / or to genetic differences in the ethnic subgroups present in this patient population. In participants in the REP 401 study with normal ALT at baseline, average baseline HBV DNA was 110,056 IU/mL (range 2130 – 779600 IU/mL) indicating that inactive HBV infection was not present in these participants.

The REP 401 study is limited by its restricted genotype (mostly D), having mostly male participants and the lack of cirrhotic patients. and inclusion of a NUC + REP 2139-Mg control in future studies will be required to examine the virologic response in the absence of pegIFN. Additionally, future studies should include NUC-experienced participants with well controlled HBV DNA. However, the results in the current REP 401 study significantly extend previous clinical study results achieving higher rates of HBsAg loss, HBsAg seroconversion, incidence of transaminase flares and rates of functional cure with persistent HBsAg seroconversion compared to previous studies with NAPs or with outcomes observed with TDF + pegIFN. These results suggest that the addition of NAPs to NUCs + pegIFN can increase the likelihood of establishing control of infection and normalization of liver function and reversal of liver inflammation and or fibrosis with finite therapy.

Although not statistically different, HBsAg levels were elevated in the control group relative to the experimental group (see Table 1). This is likely due to a HBsAg randomization threshold for NAPs lower than optimal for this patient population. HBsAg response to therapy has not been dependent on baseline HBsAg in previous studies (where HBsAg loss with NAP monotherapy has been observed with baseline HBsAg levels as high as 158,180 IU/mL)[12]. Additionally, in the current study, HBsAg, anti-HBs and ALT responses during therapy and therapeutic outcomes were not correlated with baseline HBsAg ($P \geq 0.256$, Figure S15).

Exclusion of subjects for elevated heavy metals was based on early studies in Dhaka, Bangladesh (which has one of the worlds highest concentrations of heavy metals) where dysphagia, dysgeusia and alopecia were observed during REP 2139-Ca therapy in all participants[12]. Mineral mobilization from bones (driven by mineral elimination accompanying chronic PS-ON exposure[26]) likely drove these effects but since these early studies, several PS-ON drugs with stability similar to REP 2139 have been approved for use following extensive clinical development worldwide[27], including third world regions where heavy metal exposure is greater than in the developed world. The absence of the above symptoms with these PS-ONs and with

NAPs in the REP 301 and REP 401 studies supports abandoning the heavy metal exclusion requirement in future studies.

The poor HBsAg response in a small proportion of patients in the REP 401 study has been observed in previous clinical trials with NAPs and currently has no correlation with baseline virologic characteristics. Clearance of HBsAg from the blood during NAP therapy is dependent on the inhibition of SVP secretion by NAPs and the host immune function to clear virus and SVP which may explain the improvement in HBsAg loss following the addition of pegIFN or thymosin α1[12]. Increased frequency NAP dosing has rescued HBsAg response in the REP 101 study[12] suggesting that more frequent NAP dosing, perhaps with REP 2165-Mg, can be evaluated in future studies to improve HBsAg declines in the small proportion of patients who experience slower HBsAg during REP 2139-Mg-based therapy.. Finally, a backbone therapy of NUC + REP 2139-Mg needs to be evaluated with other immunotherapies such as thymosin α1, therapeutic vaccines or pattern recognition receptor agonists.

CONTRIBUTORS

AV and MB designed the study, VP, GP, IM, VC, LC, PJ, LI, VS, TM and AJ collected data and AK and UD supervised virologic assessments. AV performed the data analysis and wrote the manuscript with assistance from all authors.

DECLARATION OF INTERESTS

MB and AV are employees and shareholders of Replicor and inventors of patents assigned to Replicor. The Institute for Virology received compensation from Replicor for performing virologic testing. All other authors have no interests to declare.

ACKNOWLEDGEMENTS

The conduct of the study was supported by Replicor Inc. The authors thank Dr. Sergiu Merioara for his assistance during the study.

REFERENCES

1. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 2004;350:1118-29.

2. Polaris Observatory C. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 2018;3:383-403.

3. Organization WH. Global Hepatitis Report. 2017.

4. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381:468-75.

5. Chevaliez S, Hezode C, Bahrami S, et al. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol 2013;58:676-83.

6. Brunetto MR, Moriconi F, Bonino F, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 2009;49:1141-50.

7. Marcellin P, Ahn SH, Ma X, et al. Combination of Tenofovir Disoproxil Fumarate and Peginterferon alpha-2a Increases Loss of Hepatitis B Surface Antigen in Patients With Chronic Hepatitis B. Gastroenterology 2016;150:134-144 e10.

8. Blanchet M, Sinnathamby V, Vaillant A, et al. Inhibition of HBsAg secretion by nucleic acid polymers in HepG2.2.15cells. Antiviral Res 2019;164:97-105.

9. Bazinet M, Pantea V, Cebotarescu V, et al. Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and REP 301-LTF): a non-randomised, open-label, phase 2 trial. Lancet Gastroenterol Hepatol 2017;2:877-889.

10. Roehl I, Seiffert S, Brikh C, et al. Nucleic Acid Polymers with Accelerated Plasma and Tissue Clearance for Chronic Hepatitis B Therapy. Mol Ther Nucleic Acids 2017;8:1-12.

11. Quinet J, Jamard C, Burtin M, et al. Nucleic acid polymer REP 2139 and nucleos(T)ide analogues act synergistically against chronic hepadnaviral infection in vivo in Pekin ducks. Hepatology 2018;67:2127-2140.

12. Al-Mahtab M, Bazinet M, Vaillant A. Safety and Efficacy of Nucleic Acid Polymers in Monotherapy and Combined with Immunotherapy in Treatment-Naive Bangladeshi Patients with HBeAg+ Chronic Hepatitis B Infection. PLoS One 2016;11:e0156667.

13. Bazinet M, Pantea V, Cebotarescu V, et al. Establishment of persistent functional remission of HBV and HDV infection following REP 2139 and pegylated interferon alpha 2a therapy in patients with chronic HBV/HDV co-infection: 18 month follow-up results from the REP 301-LTF study. J Hepatol 2018;68:S509.

14. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975;31:103-15.

15. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123-9.

16. Wiegand J, Brosteanu O, Kullig U, et al. Quantification of HBsAg and HBV-DNA during therapy with peginterferon alpha-2b plus lamivudine and peginterferon alpha-2b alone in a German chronic hepatitis B cohort. Z Gastroenterol 2011;49:1463-9.

17. Xie Q, Zhou H, Bai X, et al. A randomized, open-label clinical study of combined pegylated interferon Alfa-2a (40KD) and entecavir treatment for hepatitis B "e" antigen-positive chronic hepatitis B. Clin Infect Dis 2014;59:1714-23.

18. Sonneveld MJ, Rijckborst V, Boucher CA, et al. Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline. Hepatology 2010;52:1251-7.

19. Rijckborst V, Hansen BE, Ferenci P, et al. Validation of a stopping rule at week 12 using HBsAg and HBV DNA for HBeAg-negative patients treated with peginterferon alfa-2a. J Hepatol 2012;56:1006-11.

20. Moucari R, Mackiewicz V, Lada O, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009;49:1151-7.

21. Sonneveld MJ, Hansen BE, Piratvisuth T, et al. Response-guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen levels. Hepatology 2013;58:872-80.

22. Vaillant A. REP 2139: Antiviral Mechanisms and Applications in Achieving Functional Control of HBV and HDV Infection. ACS Infect Dis 2019;5:675-687.

23. Chi X, Gatti P, Papoian T. Safety of antisense oligonucleotide and siRNA-based therapeutics. Drug Discov Today 2017;22:823-833.

24. Lupaşco L. Diagnosticul hepatitelor cronice şi altor forme de boli cronice difuze ale ficatului, determinate prin examen profilactic al populaţiei sănătoase. Sănătate publică, economie şi management în medicină 2014;5:56-63.

25. Lupaşco LD, V-L.; Ţurcan, S.; Moscalu, I.; Ghelimici, T.; Sofroni, A. Bolile cronice difuze ale ficatului şi sindromul de citoliză:O investigare profilactică a populaţiei RM. Curierul Medical 2012;3:230-233.

26. Mata JE, Bishop MR, Tarantolo SR, et al. Evidence of enhanced iron excretion during systemic phosphorothioate oligodeoxynucleotide treatment. J Toxicol Clin Toxicol 2000;38:383-7.

27. Bennett CF. Therapeutic Antisense Oligonucleotides Are Coming of Age. Annu Rev Med 2019;70:307-321.

28. Goulis I, Karatapanis S, Akriviadis E, et al. On-treatment prediction of sustained response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B patients. Liver Int 2015;35:1540-8.

FIGURE LEGENDS

Figure 1. (A) NAP sequence: REP 2139 and REP 2156 are 40mer, fully phosphorothioated oligonucleotides with the sequence (A, 5-methyl C)$_{20}$. In REP 2139, all sugars are O-methylated at the 2' position and in REP 2165, three riboadenosines with unmodified sugars (underlined), render these nucleotides more susceptible to nuclease attack. (B) REP 401 study design. Forty patients received 24 weeks of TDF monotherapy followed by randomization into experimental and control groups (to receive REP 2139-Mg or REP 2165-Mg) at Week 24. Crossover for futility to experimental therapy in the control group occurred following 24 weeks of TDF + pegIFN at Week 49. Visits during treatment-free follow-up occurred 4, 12, 24 and 48 weeks after cessation of therapy. Analysis paradigms between groups during Part A of the study (see Methods) in the presence and absence of NAPs (C) and during Part B between REP 2139-Mg and REP 2165-Mg (D) are presented.

Figure 2. Hematological and transaminase alterations during the first 48 weeks in the REP 401 study. Individual responses of WBC (A, B) and platelet (D, E) counts and elevations in ALT (G, H and AST (J, K) are presented. Control group participants (receiving TDF + pegIFN) are (A, D, G. J) and experimental group participants (receiving TDF + pegIFN + NAPs) are (B, E, H, K). Dosing regimen is indicated at the top of (A) and (B). Analysis of differences in average (± standard deviation) WBC and platelet minima (C, F) and ALT and AST maxima during the first 48 weeks of therapy between experimental and control groups are presented. Normal ranges are indicated by horizontal dashed lines.

Figure 3. Changes in serum HBsAg, anti-HBs and HBV DNA during the first 48 weeks of therapy (Part A) in the REP 401 study. Control group participants (receiving TDF + pegIFN) are indicated (A, E, H) and experimental group participants (receiving TDF + pegIFN + NAPs) are indicated (B, F, I). Dosing regimen is indicated at the top of (A) and (B). Analysis of HBsAg (A-D), anti-HBs (E-G) and HBV DNA (H-J) are provided. Analysis of differences in absolute declines (C) and $\log_{10}$ reduction from baseline (D) of HBsAg, anti-HBs increases (G) and HBV DNA declines (J) at Week 48 between experimental and control groups are presented with average ± standard deviation indicated. Black dashed lines indicate LLOQ (HBsAg and HBV DNA) and seroconversion (anti-HBs). Dotted black lines indicate target not detected (HBsAg and HBV DNA) or no meaningful detectable anti-HBs (< 0.1 mIU/mL). Dashed horizontal line

in (D) indicates threshold (< 1 log_{10} from baseline) for HBsAg reduction indicating futility of further pegIFN treatment[19, 28]. Individual participants experiencing HBsAg reduction > 0.5 log_{10} from baseline are indicated in A.

Figure 4. Changes in serum HBsAg, anti-HBs and HBV DNA during the 48 weeks of TDF + pegIFN + NAPs (Part B) in the REP 401 study. Individual patient responses during combination therapy with REP 2139-Mg (A, C, E) or REP 2165-Mg (B, D, F) are presented. Analysis of HBsAg (A, B), anti-HBs (C, D) and HBV DNA (E, F) are provided. Each panel contains participant responses in the control group (left), experimental group (middle) and analysis of response at the end of therapy (average ± standard deviation) between control and experimental groups (right). Black dashed lines indicate LLOQ (HBsAg and HBV DNA) and seroconversion (anti-HBs). Dotted black lines indicate target not detected (HBsAg and HBV DNA) or no meaningful detectable anti-HBs (< 0.1 mIU/mL). HBsAg rebound in 02-010 (B) occurred during withholding REP 2165-Mg dosing due to pegIFN-related depression prior to early entry into follow-up. Late declines in HBsAg in 01-017 and 01-019 (A) were associated with late rebound during follow-up (see discussion). Patients with HBsAg fluctuations during therapy in A and B are indicated by open arrows (see discussion).

Table 1. Baseline demographics of participants in the REP 401 study.

Parameter		Control	Experimental
Baseline (prior to Week 1)			
Participants enrolled		20	20
Documented history of infection prior to therapy [years, median (range)]		9 (0.1-19[a])	7 (0.25-26[b])
Previous therapy	NUCs	0	1[c]
	pegIFN	0	0
Age [median (range)]		40.5 (22-53)	40.5(23-52)
Males (percentage)		17 (85)	16 (80)
HBV genotype (percentage)	A	1 (5)	2 (10)
	D	19 (95)	18 (90)
Fibroscan[d] (percentage)	≤ 7 kPa	12 (60)	10 (50)
	>7-9 kPa	4 (20)	7 (35)
	>9-11 kPa	0 (0)	2 (10)
	> 11 kPa	4 (20)	1 (5)
Virologic baseline [median (range)]	HBV DNA (IU/mL)	$8.7x10^4$ ($2.2x10^3$ - $7.1x10^7$)	$4.8x10^4$ ($2.13x10^3$ - $7.1x10^7$)
	HBsAg (IU/mL)	9302.5 (1575-53073)	8743 (1147-31184)
	Anti-HBs (mIU/mL)	0 (0-5.11)	0 (0-35)
ALT [U/L, median (range)])		49 (21-302)	56.5 (20-276)
Normal ALT (< 50 U/L, percentage)		11 (55)	8 (40)
Platelets $x10^6$/mL, [median (range)]		175 (148-260)	168.5 (124-331)
Liver median stiffness [kPa, median (range)]		6.65 (4.3-14.1)	7.3 (4.4-19.6)
At randomization (Week 25 – start of pegIFN)			
Participants who received REP 2139-Mg		10 (starting at week 49)	10
Participants who received REP 2165-Mg		10 (starting at week 49)	10
Participants who received pegIFN		20	20
Virology [median (range)]	HBV DNA (IU/mL)	<10 (TND-1793)	<10 (TND – 675)
	HBsAg (IU/mL)	9213.5 (1466-34636)	7891 (779-31083)
	Anti-HBs (mIU/mL)	0.08 (0-2.37)	0 (0-18)
HBV DNA TND (percentage)		6 (30)	4 (20)
ALT [U/L, median (range)]		36 (21-94)	39.5 (17-100)
Normal ALT (< 50 U/L, percentage)		16 (80)	17 (85)
Platelets $x10^6$/mL, [median (range)]		185.5 (125-305)	189 (133-320)

No significant differences in baseline parameters was observed between groups ($p > 0.05$). Liver stiffness was not evaluated at randomization. TND, target not detected. Documentation of infection at least 6 months prior to treatment could not be obtained in three[a] and one[b] participants. Chronicity of infection in these 4 participants was subsequently confirmed by HBcAg IgG positivity and IgM negativity. [c] this participant was diagnosed with HBV infection in 1988 and several years prior to enrollment, had received lamivudine for 1y 3m (halted due to resistance) and telbuvidine for 3-4 months (halted due to poor tolerability). [d] Metavir correlates to Fibroscan ranges are as follows: F0 (normal): ≤ 7 kPa, F1-F2: >7-9 kPa, F2-F3 >9-11 kPa, F3-F4: > 11 kPa.

Table 2

Participants experiencing adverse events during weeks 1 – 48 in the REP 401 study.

Adverse event		TDF (Weeks 1-24) (n=40)	TDF + pegIFN (Weeks 25-48) (n=20)	TDF + pegIFN + NAPs (Weeks 25-48) (n=20)
ALT elevation	Grade ≥2	0	8	19
	Grade ≥3	0	3	12
	Grade 4	0	1	6
Neutropenia grade 3[a]		0	0	0
Thrombocytopenia grade 3[a]		0	0	1
Fever		0	7	17
Fatigue / weakness		3	5	3
Leukopenia		0	5	4
Headache		3	4	4
Myalgia		1	4	10
Abdominal pain / discomfort		2	3	0
Nausea / vomiting		2	0	6
Arthralgia		1	2	3
Chills		1	2	1
Dizziness / vertigo		2	2	1
Flatulence		3	2	0
Anemia		0	1	1
Arthritis		0	1	0
Back pain		1	1	3
Bilateral calf pain / lower extremities		1	1	2
Bone pain		0	1	0
Erectile dysfunction		0	1	0
Hyperglycemia		0	1	0
Hypertriglyceridemia		0	1	1
Hypoglycemia		0	1	0
Hypophosphatemia		0	1	0
Insomnia		2	1	2
Pulpitis		0	1	0
Upper respiratory infection		4	1	0
Apnea		0	0	0
Appendicitis		1	0	0
Asthenia		0	0	2
Autoimmune hypertrophic thyroiditis		0	0	0
Chest pain		1	0	1
Dermatitis		1	0	0
Diarrhea		1	0	0
Dysfunctional metrorrhagia		0	0	0
Elevated 25OH-vitamin D		0	0	3
Hematuria		0	0	2
Hypernatremia		0	0	1
Hypotension		0	0	1
Hypothyroidism		0	0	0
Nephritis		0	0	0
Numbness or itching		1	0	3
Orchitis		1	0	1
Pain under right rib		0	0	0
Photosensitivity		0	0	0
Portal hypertension		0	0	0
Rash		0	0	1
Sacroiliitis		0	0	0
Sleepiness		1	0	0
Spondylopathy		0	0	1
Sweating		0	0	1
Tachycardia		0	0	0
Dry mouth		0	0	0

a: all patients experienced ≤ grade 2 platelet and WBC count reduction with the introduction of interferon

Table 3. Virologic and biochemical outcomes in the REP 401 trial.

Parameter	Baseline	Part A						Part B						CTL + EXP
		Week 24			Week 48			EOT			Follow-up week 48[c]			Completed therapy and 24-48 weeks of follow-up (n=36)[a-c]
		CTL	EXP	*P*	CTL	EXP	*P*	CTL[a]	EXP[b]	*P*	CTL (n=16)	EXP (n=18)	*P*	
HBsAg[d]														
> 1 log_{10} reduction from baseline	0	0	0		3 (15)	18 (90)		19 (95)	16 (85)	0.117	11 (68.7)	14 (77.8)	0.808	27 (75)
< 1000 IU/mL	0	0	0		3 (15)	17 (85)		18 (90)	17 (85)	0.674	11 (68.7)	14 (77.8)	0.808	26 (72.2)
< 100 IU/mL	0	0	0		1 (5)	15 (75)		16 (80)	15 (75)	0.714	10 (62.5)	13 (72.2)	0.805	23 (63.9)
< 1 IU/mL	0	0	0	0.781	0	14 (70)	< 0.01	14 (70)	14 (70)	0.781	8 (50)	9 (50)	0.956	18 (50)
≤ LLOQ (0.05 IU/mL)	0	0	0		0	10 (50)		11 (55)	13 (65)	0.602	6 (37.4)	8 (44.4)	0.879	15 (41.7)
TND (0.00 IU/mL)	0	0	0		0	10 (50)		10 (50)	13 (65)	0.436	4 (25)	8 (44.4)	0.483	13 (36.1)
Anti-HBs														
Seroconversion (≥ 10 mIU/mL)	2 (5)[e]	0	1 (5)[f]	0.701	0	11 (55)	< 0.01	11 (55)	13 (65)	0.602	8 (50)	9 (50)	0.948	19 (52.8)
HBV DNA														
≤ 2000 IU/mL	0	20 (100)	20 (100)	0.781	20 (100)	20 (100)	0.781	20 (100)	20 (100)	0.781	11 (68.7)	16 (88.9)	0.313	28 (77.8)
< LLOQ (10 IU/mL)	0	13 (65)	15 (75)	0.578	8 (40)	10 (50)	0.607	17 (85)	12 (60)	0.08	9 (56.2)	11 (61.1)	0.914	21 (58.3)
TND	0	6 (30)	4 (20)	0.556	8 (40)	10 (50)	0.607	12 (60)	11 (55)	0.733	7 (43.7)	10 (55.6)	0.766	17 (47.2)
ALT														
Normal (≤ 50 U/L)	20 (50)	16 (80)	17 (85)	0.701	1 (5)	0 (0)	0.701	8 (40)	5 (25)	0.404	15 (93.7)	17 (94.4)	0.946	32 (88.9)
Liver median stiffness[g]														
< baseline	NA	ND	ND	ND	NA	NA	ND	4 (20)	7 (35)	0.373	7 (43.7)	11 (61.1)	0.796	19 (52.8)
Normal (≤ 7 kPa)	21 (52.5)	ND	ND	ND	NA	NA	ND	2 (10)	6 (30)	0.11	7 (43.7)	14 (77.8)	0.113	20 (55.5)
Virologic outcomes														
Virologic control[h]	NA	NA	NA	ND	NA	NA	ND	NA	NA	ND	5 (31.2)	8 (50)	0.712	14 (38.9)
Functional cure[i]	NA	NA	NA	ND	NA	NA	ND	NA	NA	ND	6 (37.5)	8 (50)	0.880	14 (38.9)

Numbers in parenthesis are percentages. NA, not applicable, ND = not determined, TND = target not detected

a) 2 participants did not complete therapy (see Figure S1, Tables S5 and S6), EOT LMS was not assessed in these participants.
b) 1 participant did not complete therapy (see Figure S1 and Table S4), EOT LMS was not assessed.
c) communication was lost with 4 participants after follow-up week 24. One participant withdrew consent after FW24, 1 participant death after FW24 unrelated to study (Figure S1, Table S4 and see Results)
d) Qualitative HBsAg data only available for one patient at FW48 (see Figure S1 and Table S3)
e) Baseline HBsAg in these patients was 9595 and 10736 IU/mL.
f) This participant with HBsAg seroconversion at the end of TDF monotherapy was seroconverted at baseline.
g) Liver median stiffness was not assessed at Week 24 or 48.
h) HBsAg positive, HBV DNA ≤ 2000 IU/mL, normal ALT.
i) HBsAg < 0.05 IU/mL, HBV DNA TND, normal ALT.

Figure 1

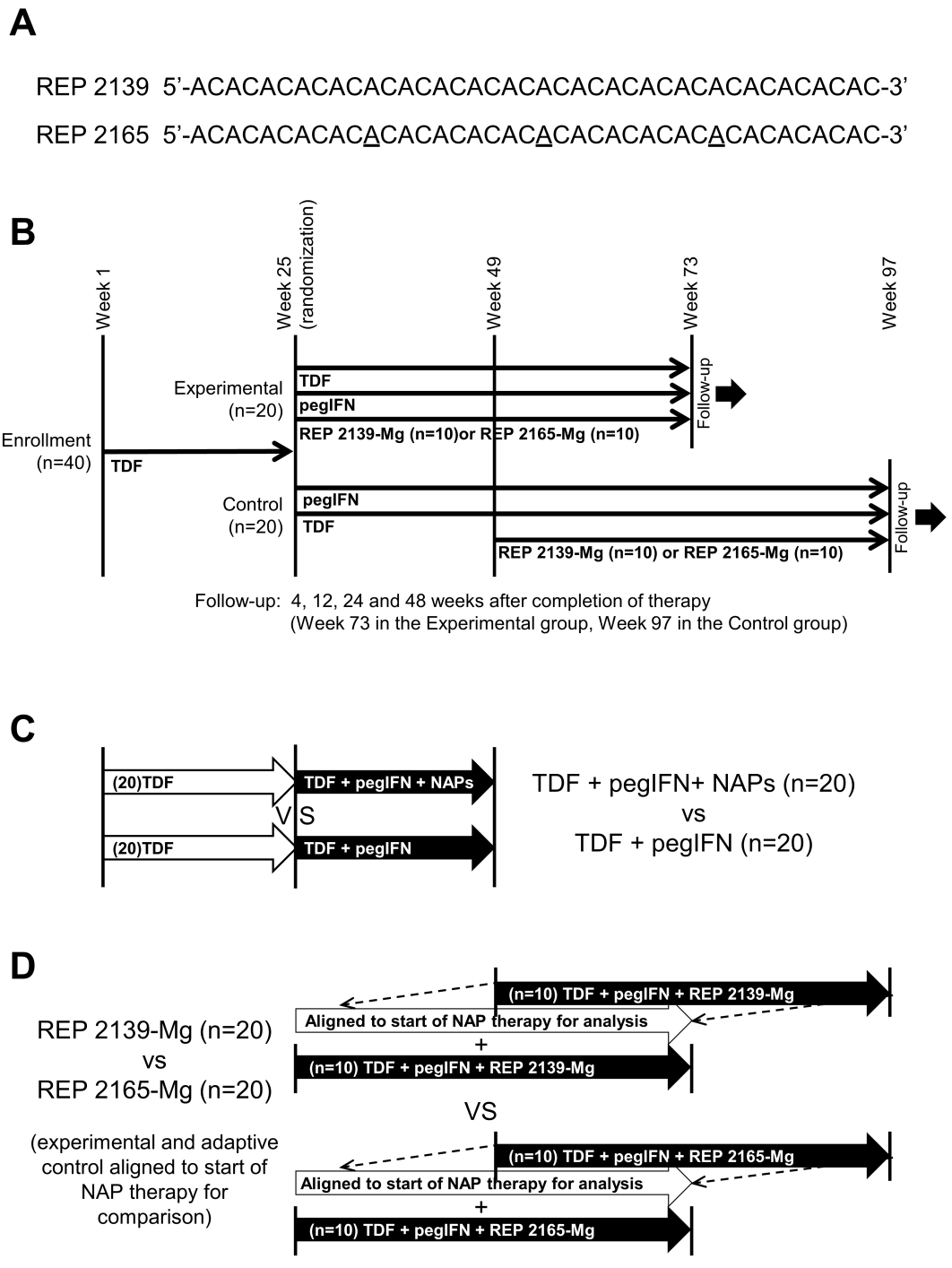

A
REP 2139 5'-ACACACACACACACACACACACACACACACACACACACAC-3'
REP 2165 5'-ACACACACACACACACACACACACACACACACACACAC-3'
B
Week 1
Week 25 (randomization)
Week 49
Week 73
Week 97
Experimental (n=20)
TDF
pegIFN
REP 2139-Mg (n=10)or REP 2165-Mg (n=10)
Follow-up
Enrollment (n=40)
TDF
Control (n=20)
pegIFN
TDF
REP 2139-Mg (n=10) or REP 2165-Mg (n=10)
Follow-up
Follow-up: 4, 12, 24 and 48 weeks after completion of therapy
(Week 73 in the Experimental group, Week 97 in the Control group)
C
(20)TDF
TDF + pegIFN + NAPs
VS
(20)TDF
TDF + pegIFN
TDF + pegIFN+ NAPs (n=20)
vs
TDF + pegIFN (n=20)
D
REP 2139-Mg (n=20)
vs
REP 2165-Mg (n=20)
(experimental and adaptive control aligned to start of NAP therapy for comparison)
(n=10) TDF + pegIFN + REP 2139-Mg
Aligned to start of NAP therapy for analysis
+
(n=10) TDF + pegIFN + REP 2139-Mg
VS
(n=10) TDF + pegIFN + REP 2165-Mg
Aligned to start of NAP therapy for analysis
+
(n=10) TDF + pegIFN + REP 2165-Mg

Figure 2

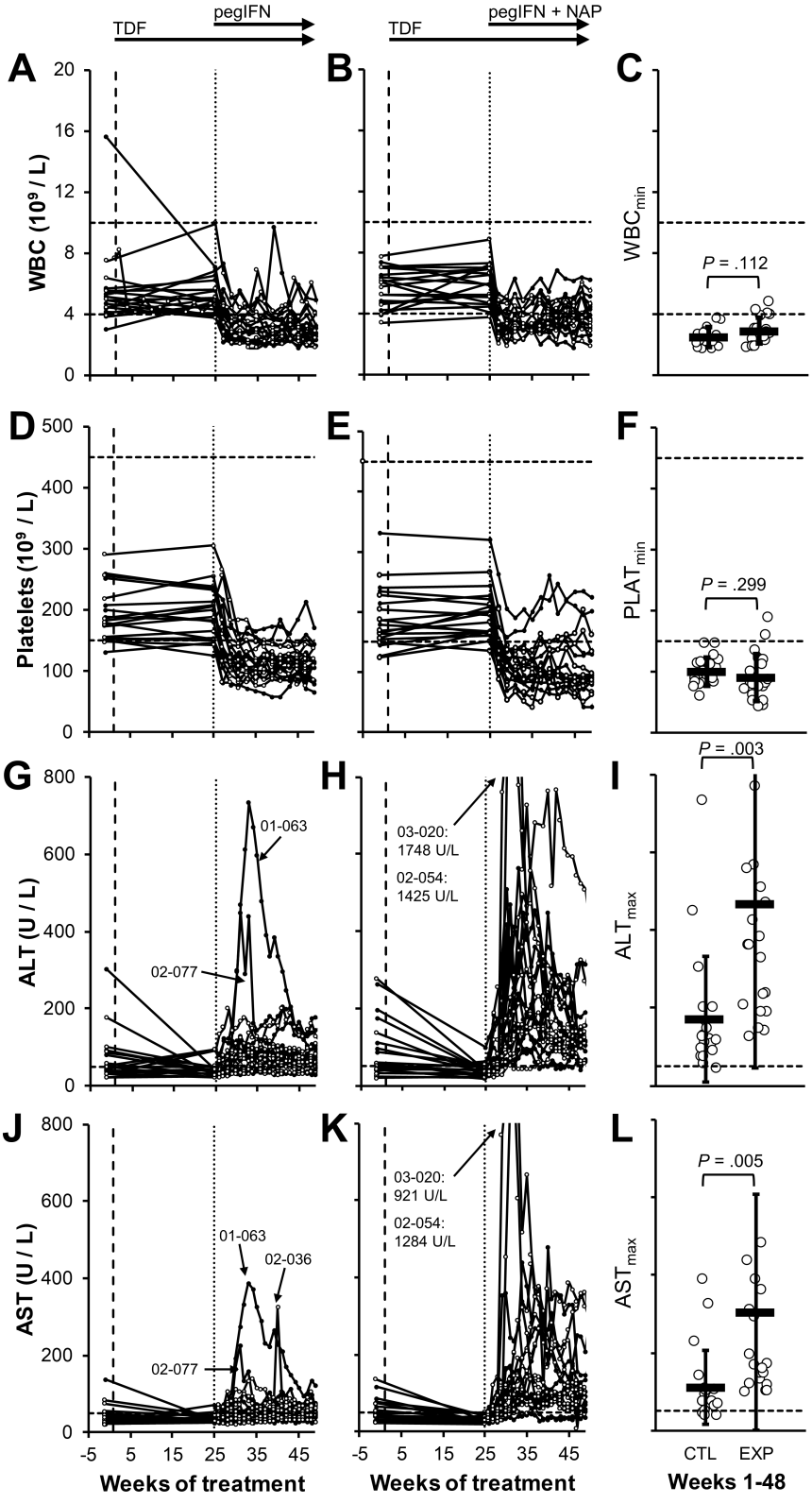

TDF
pegIFN
TDF
pegIFN + NAP
A
B
C
WBC (10⁹ / L)
WBC_min
P = .112
D
E
F
Platelets (10⁹ / L)
PLAT_min
P = .299
G
H
I
ALT (U / L)
01-063
02-077
03-020:
1748 U/L
02-054:
1425 U/L
ALT_max
P = .003
J
K
L
AST (U / L)
01-063
02-036
02-077
03-020:
921 U/L
02-054:
1284 U/L
AST_max
P = .005
Weeks of treatment
Weeks of treatment
CTL
EXP
Weeks 1-48

Figure 3

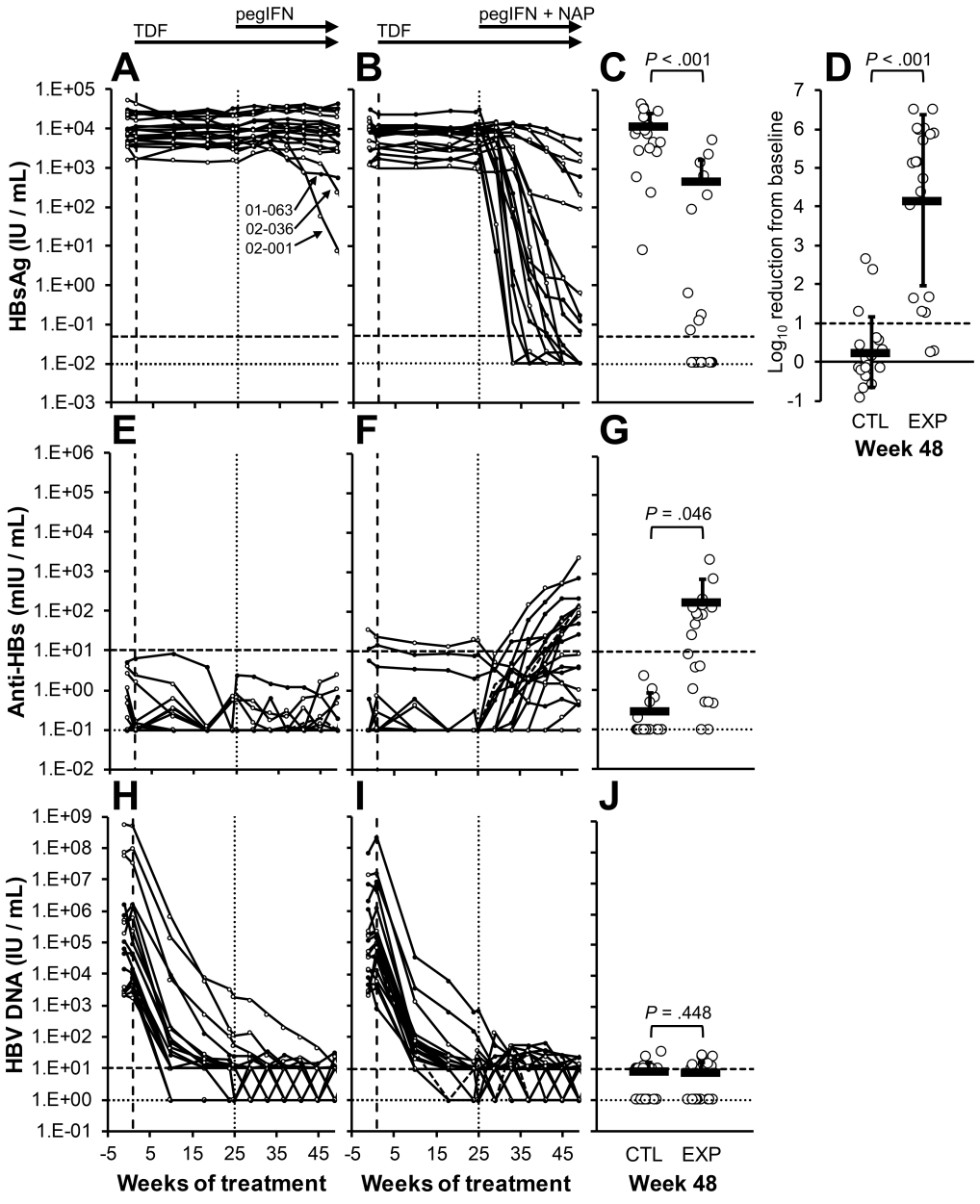
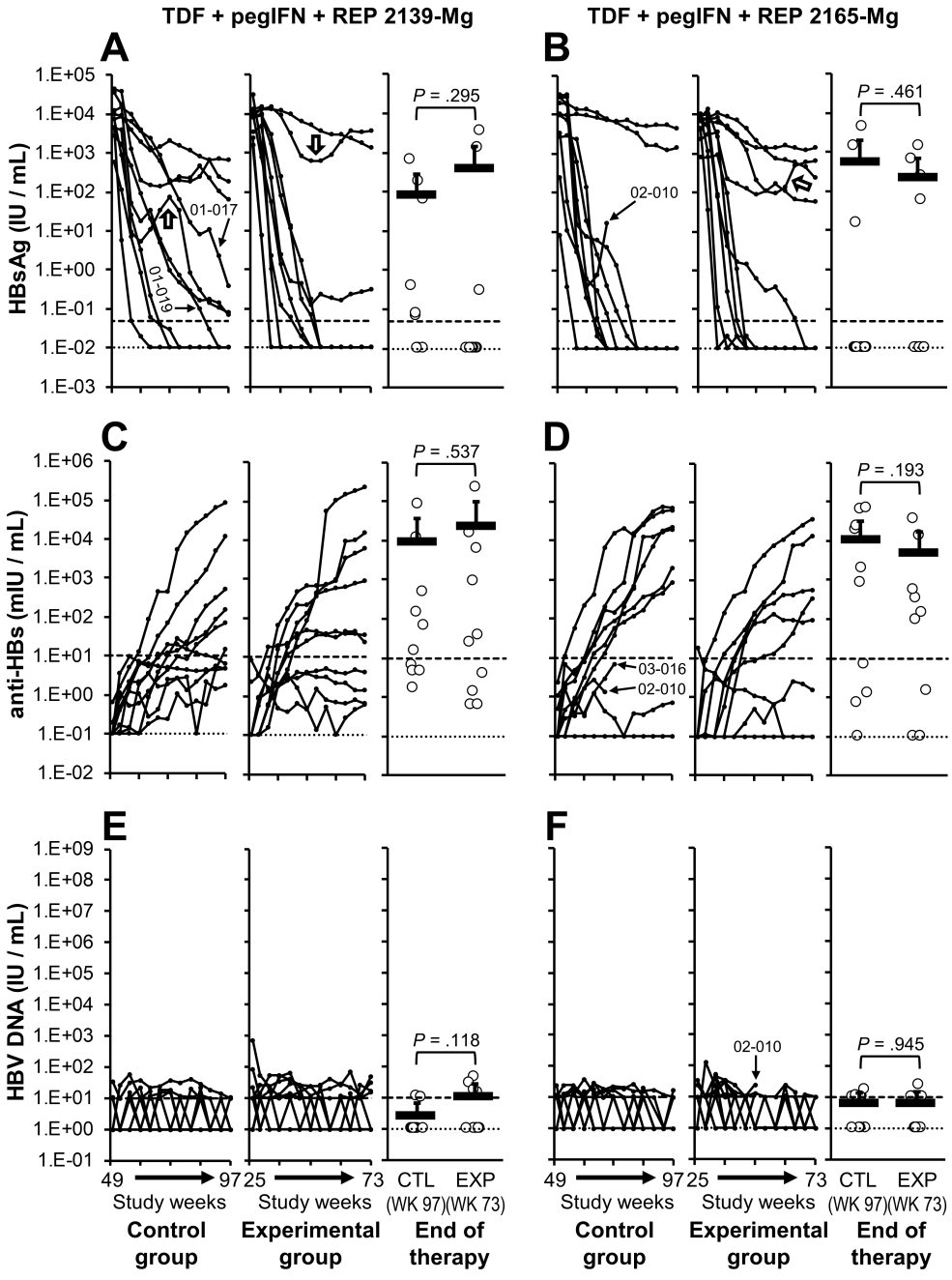

TDF
pegIFN
TDF
pegIFN + NAP
A
B
C
D
P < .001
P < .001
HBsAg (IU / mL)
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
1.E-02
1.E-03
01-063
02-036
02-001
Log10 reduction from baseline
7
6
5
4
3
2
1
0
-1
CTL
EXP
Week 48
E
F
G
P = .046
Anti-HBs (mIU / mL)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
1.E-02
H
I
J
P = .448
HBV DNA (IU / mL)
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
-5
5
15
25
35
45
Weeks of treatment
Weeks of treatment
CTL
EXP
Week 48

Figure 4


TDF + pegIFN + REP 2139-Mg
TDF + pegIFN + REP 2165-Mg
A
B
C
D
E
F
HBsAg (IU / mL)
anti-HBs (mIU / mL)
HBV DNA (IU / mL)
P = .295
P = .461
P = .537
P = .193
P = .118
P = .945
01-017
01-019
02-010
03-016
49
97
25
73
CTL
EXP
(WK 97)
(WK 73)
Study weeks
Control group
Experimental group
End of therapy

What you need to know:

Background and Context: Nucleic acid polymers (NAPs) inhibit assembly and secretion of hepatitis B virus (HBV) subviral particles.

New Findings: In an open-label, phase 2 study of the safety and efficacy of the NAPs REP 2139 or REP 2165 combined with tenofovir disoproxil fumarate (TDF) and pegylated interferon alfa-2a (pegIFN) in patients with negative chronic HBV infection who were negative for HB e antigen (HBeAg), addition of NAPs to TDF + pegIFN did not alter tolerability and significantly increased rates of functional cure during and after therapy.

Limitations: This was an open-label phase 2 study–further studies are needed.

Impact: The NAPs REP 2139 or REP 2165 can be added to TDF and pegIFN therapy to improve outcomes of patients with chronic HBV infection.

Lay Summary: The authors tested the efficacy and safety of a new class of anti-HBV drugs called NAPs in patients with chronic infections. The drugs are safe and increase the efficacy of other treatments for chronic HBV infection.