Update on Safety and Efficacy in the REP 401 Protocol: REP 2139-Mg or REP 2165-Mg Used in Combination with Tenofovir Disoproxil Fumarate and Pegylated Interferon Alpha-2A in Treatment Naïve Caucasian Patients with Chronic HBeAg Negative HBV Infection

M. Bazinet[1], V. Pantea[2], G. Placinta[2], I. Moscalu[3], V. Cebotarescu[2], L. Cojuhari[2], P. Jimbei[4], L. Iarovoi[2], V. Smesnoi[4], T. Musteata[4], A. Jucov[2,3], A. Krawczyk[5] A. Vaillant[1]

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

EASL The Home of Hepatology
THE INTERNATIONAL LIVER CONGRESS™ 2017
19-23 APRIL, AMSTERDAM, THE NETHERLANDS

INTRODUCTION

The nucleic acid polymer (NAP) REP 2139 clears serum HBsAg and improves the efficacy of immunotherapy to establish functional control of chronic HBV infection and HBV / HDV co-infection. The REP 401 protocol (NCT02565719) is a randomized, controlled trial assessing the safety and efficacy of REP 2139 and a REP 2139 derivative with improved tissue clearance (REP 2165) in combination with tenofovir disoproxil fumarate (TDF) and pegylated interferon alpha-2a (peg-IFN) in treatment naïve patients with chronic HBeAg negative HBV infection.

METHODS

Twenty four weeks of lead-in TDF (300mg PO qD) is followed by randomization (1:1) into experimental and control groups (Table 1). The experimental group will receive 48 weeks of TDF (300mg PO qD), peg-IFN (180ug SC qW) and REP 2139-Mg or REP 2165-Mg (1:1, 250 mg IV infusion qW) (Table 2). The control group will receive 48 weeks of TDF + peg-IFN but will crossover to 48 weeks of experimental therapy in the absence of a 3 log drop in HBsAg after 24 weeks of peg-IFN (Figure 1). Viremia is monitored on the Architect platform. HBV RNA and HBcrAg (Fujirebio Lumipulse®) were determined from frozen serum samples at DDL Diagnostic Diagnostic Laboratory (Rijswijk, The Netherlands)

Table 1. Pre-treatment demographics in the REP 401 protocol

Parameter		Adaptive comparator control (TDF + peg-IFN)	Experimental (TDF + peg-IFN + NAPs)
Age (average / median)		36.9 / 36	38.6 / 39.5
Sex		27M / 3F	26M / 4F
HBV genotype	A	1	2
	D	19	18
Metavir score (based on Fibroscan)	F0-F1	12	10
	F2	3	6
	F2-F3	0	3
	F3-F4	3	1
Virologic baseline (average / median)	HBV DNA (IU/mL)	$3.6x10^7$ / $8.7x10^4$	$4.8x10^6$ / $4.8x10^4$
	HBsAg (IU/mL)	14775.7 / 9302.5	9018.1 / 8743
	Anti-HBs (mIU/mL)	0.78 / 0.1	2.778 / 0.1
ALT (U/L, average / median)		71.65 / 49	91.95 / 56.5

Table 2. REP 2139 versus REP 2165

NAP	Sequence 5' - 3'	Stability in human plasma 7 days @ 37°C (% of untreated standard)	
		Neutral	Acidified
REP 2139	ACACACACACACACACACACACACACACACACACAC	93	86
REP 2165	ACACACACACACACACACACACACACACACACAC	89	36

A = 2' O-methyl ribose modification in REP 2139 is 2'OH in REP 2165, allowing greater susceptibility to endonuclease attack.

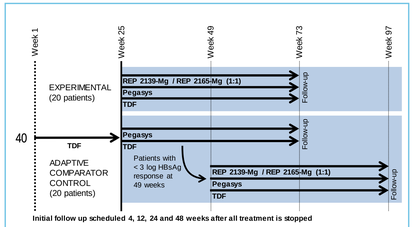

Figure 1. REP 401 protocol design

RESULTS

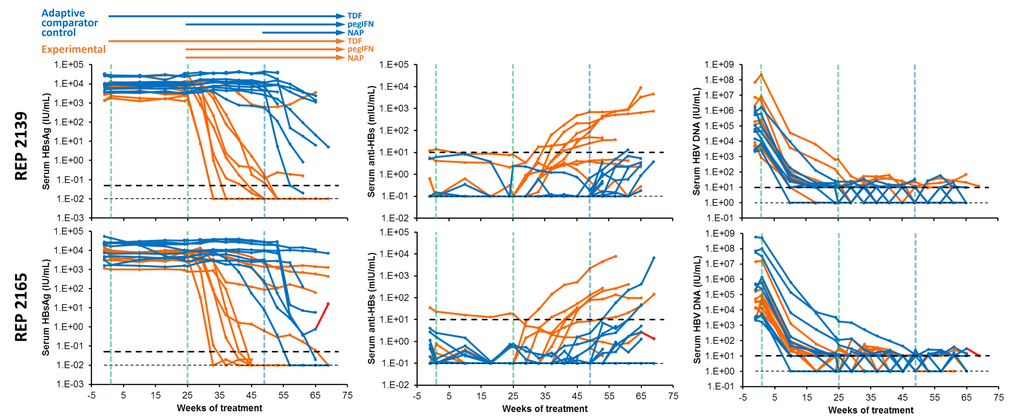

Figure 2. Serum HBsAg, anti-HBs and HBV dynamics in the REP 401 protocol.
Individual tracings during treatment in the adaptive control (blue) and experimental (orange) groups are provided for patients receiving REP 2139 (top) and REP 2165 (bottom). Bold dashed lines indicate LLOQ (HBsAg, HBV DNA) or 10mIU/mL (anti-HBs). Fine dashed lines indicate target not detected (HBsAg and HBV DNA) or no significant anti-HBs present (< 0.1 mIU/mL). Red segment indicates off-treatment rebound in a single patient who withdrew from the trial for depression attributed to Pegasys exposure.

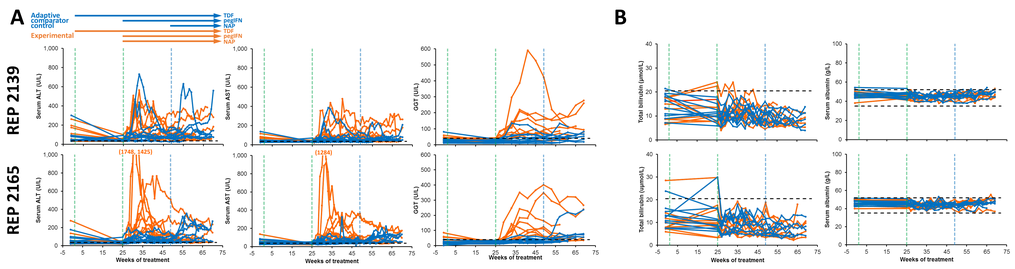

Figure 3. Serum transaminase flares (A) and liver synthetic function (B) in the REP 401 protocol.
Individual tracings during treatment in the adaptive control (blue) and experimental (orange) groups are provided for patients receiving REP 2139 (top) and REP 2165 (bottom). Dashed lines indicate the upper limit of normal (ALT, AST, GGT and total bilirubin) or the normal range (albumin). INR levels over time are not affected in any patient (data not shown).

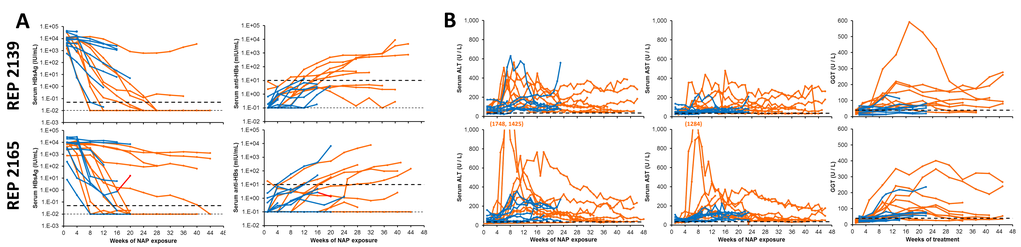

Figure 4. Pre-exposure to pegIFN supresses evolution of transaminase flares with NAP-mediated HBsAg reduction.
Results are synchronized to the start of NAP therapy in the control and experimental groups. Individual tracings of HBsAg and anti-HBs (A) or liver transaminases (B) in patients receiving NAPs at the start of peg-IFN (experimental group, orange) or patients receiving NAPs after 24 weeks of pegIFN exposure (adaptive control group, blue) (see figure 1). HBsAg and anti-HBs response (A) is similar with NAP exposure either at the start (orange) or after 24 weeks of pegIFN (blue). Liver transaminase elevations (B) are substantially depressed in patients receiving NAPs after 24 weeks of pegIFN (blue) compared to patients receiving NAPs at the start of peg-IFN (orange). Bold dashed lines in (A) indicate LLOQ (HBsAg) or 10mIU/mL (anti-HBs). Fine dashed lines in (A) indicate target not detected (HBsAg) or no significant anti-HBs present (< 0.1 mIU/mL). Dashed lines in (B) indicate the upper limit of normal.

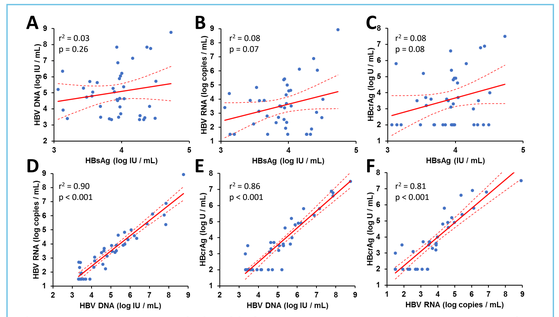

Figure 5. Pre-treatment relationship between HBsAg, HBV DNA, HBV RNA and HBcrAg in HBeAg negative patients.
Regression analysis was preformed for all 40 patients in the REP 401 protocol. No relationship existed between serum levels of HBsAg and HBV DNA (A), HBV RNA (B) or HBcrAg (C). HBV DNA, HBV RNA or HBcrAg levels were all highly related to each other (D, E, F) but not below 10^4 IU/mL HBV DNA or 10^3 copies /mL HBV RNA. Regression lines (red) are flanked by 95% prediction intervals.

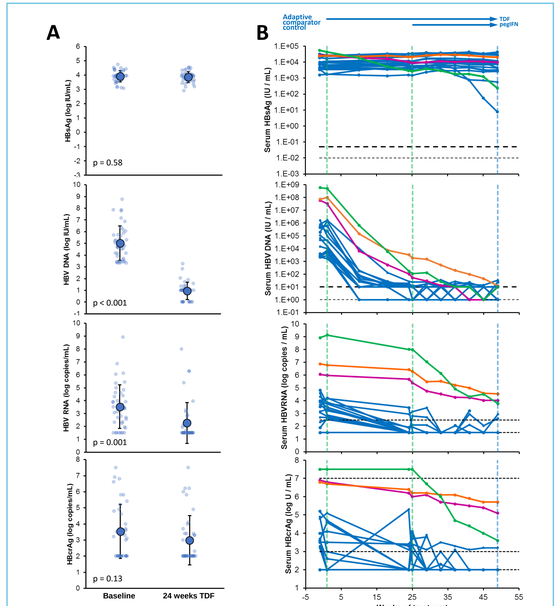

Figure 6. HBV RNA and HBcrAg response during TDF and pegIFN in HBeAg negative patients.
Analysis of changes in HBV RNA and HBcrAg were performed during (A) 24 weeks of TDF monotherapy (all 40 patients) and (B) during TDF and peg-IFN in the control group (20 patients). 24 weeks of TDF (A) caused no changes in HBsAg however significant changes were observed not only in HBV DNA but also HBV RNA. HBcrAg dropped with TDF in many patients but was not statistically significant different in the population. HBV RNA and HBcrAg levels continually declined during TDF and peg-IFN in the absence of NAP therapy (B) but no significant changes in serum HBsAg were observed even for those patients with high pre-treatment HBcrAg with continuous declines during treatment (green, pink and orange lines). Declines in HBV RNA and HBcrAg were similar in NAP treated patients in the experimental group (data not shown).

CONCLUSIONS

- **REP 2139 and REP 2165 continue to demonstrate well tolerated elimination of HBsAg in most patients.**
- **PegIFN therapy is associated with dramatic elevations in anti-HBs and transaminase flares only in the absence of HBsAg.**
- **Transaminase flares occur in the absence of altered liver synthetic function, suggesting these flares are therapeutic in nature and reflect elimination of infected hepatocytes and/or hepatocytes harbouring integrated HBsAg.**
- **Pre-exposure to pegIFN does not affect clearance of HBsAg by NAPs or evolution of anti-HBs but transaminase flares are substantially reduced, suggesting that suppression of the immune response in the liver may become more pronounced with continued pegIFN exposure.**
- **Pre-treatment levels of HBsAg are not related to HBV DNA, HBV RNA or HBcrAg levels. Moreover HBsAg levels do not change during TDF/pegIFN treatment when significant reductions in HBV RNA and HBcrAg do occur, suggesting the bulk of circulating HBsAg is derived from integrated HBsAg in HBeAg negative patients.**
- **TDF may also suppress cccDNA activity, either by preventing replenishment of cccDNA or via a direct immunostimulatory activity.**

ACKNOWLEDGEMENTS

This work was supported by Replicor Inc.

REFERENCES

Noordeen, F et al. (2013). Antimicrob. Agents Chemother. 57: 5299-5306.
Noordeen, F. et al.(2015). PLOS ONE 10: e0140909Al-Mahtab et al., 2016
Al-Mahtab, M. et al. (2016). PLOS ONE 11: e0156667
Quinet, J., et al. (2016). J. Hepatol. 64: S385
Bazinet, M., et al. (2016). Hepatol. 64: 1122A
Vaillant, A. (2016). Antiviral Res. 133: 32-40

DISCLOSURES

MB and AV are employees of and shareholders in Replicor Inc. The other authors have nothing to disclose.

CONTACT INFORMATION

Andrew Vaillant: availlant@replicor.com