Achieving functional cure of HBV and HBV / HDV co-infection with REP 2139: Completed follow-up in the REP 401 and REP 301-LTF studies

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INTRODUCTION

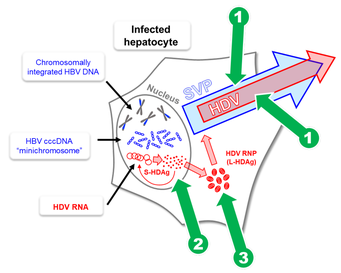
Nucleic acid polymers (NAPs) inhibit the assembly and secretion of HBV subviral particles and interact with small and large forms of the hepatitis delta antigen. The REP 401 study (NCT02565719) examined the safety and efficacy of tenofovir disoproxil fumarate (TDF), pegylated interferon alfa-2a (pegIFN) and two NAPs (REP 2139 and REP 2165) in HBeAg negative chronic HBV infection. The REP 301-LTF study (NCT02876419) is a long-term follow-up study of previous treatment of HBeAg negative chronic HBV / HDV co-infection with pegIFN and REP 2139 (REP 301: NCT02233075).

Figure 1. Antiviral effects of REP 2139:

(1) Inhibition of HBV SVP assembly / secretion and HDV envelopment.

(2) Potential inhibition of HDV RNA synthesis via interaction with S-HDAg.

(3) Potential inhibition of HDV RNP formation via interaction with L-HDAg.



MATERIAL & METHODS

Participants in both studies were treatment naïve, HBeAg negative with HBsAg > 1000 IU/mL prior to treatment. In REP 401 study, 40 participants received 48 weeks of TDF + pegIFN + REP 2139 or REP 2165, 20 of whom were crossed over to this therapy after demonstrating poor HBsAg response to 24 weeks of TDF + pegIFN (Figure 3). In the REP 301 study, 12 participants received 15 weeks of REP 2139, followed by 15 weeks of REP 2139 + pegIFN, followed by 33 weeks of pegIFN. Completed treatment-free follow-up is 48 weeks in the REP 401 study and 3.5 years in the REP 301-LTF study. HDV RNA, HBV DNA, HBsAg and anti-HBs are followed every 6 months using standard assays (Robogene MK II RT-PCR, Abbott RealTime HBV, Abbott Architect). HBV RNA was followed by qRTPCR (DDL Diagnostic) and HBcrAg was followed by Fujirebio Lumipulse. Median hepatic stiffness was evaluated by Fibroscan.

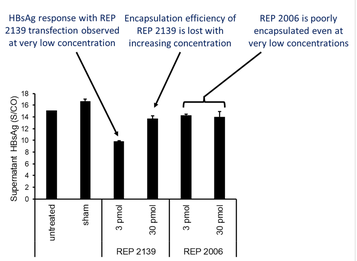
TRANSFECTION ARTIFACTS IN *IN VITRO* EVALUATION OF NAPS

Table 1.
Transfection *in vitro* does not predict activity *in vivo* or in human subjects

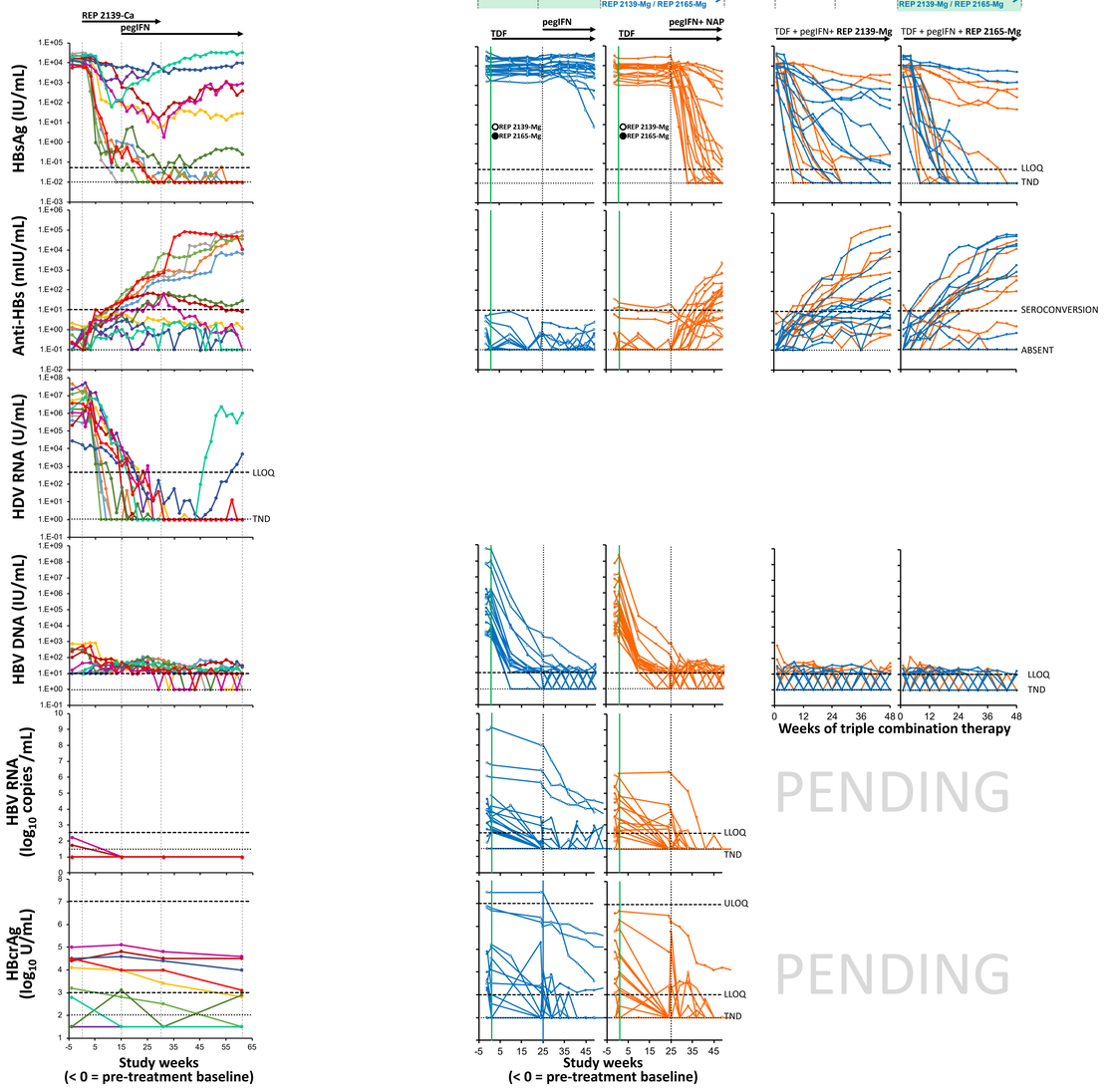
Phosphorothioate oligonucleotides			DNA NAPs			modified RNA NAPs		
			REP 2055	REP 2031	REP 2006	REP 2139	REP 2165	REP 2107
Sequence / structure			$(dAdC)_{20}$	$(dC)_{40}$	$(dN)_{40}$	$(2'OMeA\text{-}2'OMe\text{-}5MeC)_{20}$	$(2'OMeA\text{-}2'OMe\text{-}5MeC)_{20}$*	$(2'OMeN)_{40}$
Properties			No 2° structure	Inactivated in ERGIC (tetramerization at acidic pH)	Mild 2° structure	No 2° structure	No 2° structure	Moderate 2° structure
Activity in vivo[1-4]			YES	NO	YES	YES	YES	YES
Activity in humans[5-7]			YES	ND	ND	YES	YES	ND
Activity in vitro	Correct uptake and trafficking	Primary duck liver co-culture[8]	YES	NO	YES	ND	ND	YES
		HepG2.2.15 + UNC7938[9]	YES	attenuated	ND	YES	ND	YES
	Incorrect uptake and trafficking	HepG2.2.15 + transfection	YES	YES	NO	NO	NO	NO

* A_{11}, A_{21} and A_{31} are 2'OH, ND = not determined

Figure 2. Transfection artifacts with RNAiMAX in HepG2.2.15 cells. Loss of encapsulation efficiency with RNAiMAX drives loss of activity with REP 2139 at increasing concentrations. Mildly stable secondary structure inherently present in REP 2006 blocks encapsulation. Transfection cannot evaluate activity of oligonucleotides with different modifications and / or sequences.



ANTIVIRAL RESPONSE DURING THERAPY



CONCLUSIONS

1. **Encapsulation efficiency with cationic lipids varies greatly for oligonucleotides with different modifications and sequences. Cationic lipid-based transfection (including RNAiMAX) does not predict NAP efficacy *in vivo*.**
2. **Combination therapy with REP 2139 and pegIFN achieves high rates of HBsAg loss and seroconversion accompanied by HBV RNA, HBcrAg and HDV RNA loss.**
3. **Functional cure of HDV infection (HDV RNA TND, normal ALT) off therapy is achieved in 64% of participants and persists for at least 3.5 years.**
4. **All participants with functional cure of HDV have either virologic control or functional cure of HBV.**
5. **Rates of positive therapeutic outcomes in HBV / HDV infection are expected to significantly improve with the triple combination regimen (48 weeks of TDF + pegIFN + REP 2139-Mg) used in the REP 401 study.**
6. **Triple combination regimen of TDF + pegIFN + REP 2139-Mg is safe, well tolerated and establishes high rates of functional cure in HBeAg negative chronic HBV infection.**

THERAPEUTIC OUTCOMES DURING TREATMENT-FREE FOLLOW-UP (HBV / HDV, REP 301-LTF)

Table 2. Maintenance of clinical, HBsAg and HDV RNA responses

Completed treatment and 3.5 years of follow-up		11
Clinical response	Normal ALT	8/11 (73%)
	Normal / declining liver median stiffness	7/11 (64%)
HBsAg response	< 1 IU/ml	6/11 (55%)
	≤ LLOQ (0.05 IU/mL)	5/11 (42%)
	Seroconversion	4/11 (36%)
HDV RNA response	**> 2 log_{10} reduction from baseline**	**9/11 (82%)***
	TND	**7/11 (64%)**

***2 participants maintaining 2.67 and 2.12 log_{10} reduction from baseline did not maintain normal liver function during follow-up.**

Table 3. HBV outcomes in participants with persistent HDV RNA negativity

Functional cure of HDV at 3.5 years of follow-up (HDV RNA TND, ALT normal)		7
HBV DNA response	≤ 2000 IU/mL	7/7 (100%)
	Target not detected (TND)	5/7 (71%)
HBV virologic response	Virologic control HBV (HBV DNA ≤ 2000 IU/mL, normal ALT)	3/7 (43%)
	Functional cure HBV (HBsAg < LLOQ, HBV DNA TND, normal ALT)	4/7 (57%)
	HBV clinical benefit, no therapy required (Low risk of progression, reduced risk of HCC)	**7/7 (100%)**

THERAPEUTIC OUTCOMES DURING TREATMENT-FREE FOLLOW-UP (HBV, REP 401)

Table 4. Maintenance of clinical and HBV and outcomes

Completed treatment and ≥ 24 weeks of follow-up		36 (32 completed 48 weeks of follow-up)
Clinical response	Normal ALT	89%
	Normal liver median stiffness	56%
HBsAg response	< 1000 IU/mL	72%
	< 1 IU/ml	50%
	≤ LLOQ (0.05 IU/mL)	42%
	Seroconversion	53%
HBV DNA response	≤ 2000 IU/mL	78%
	Target not detected (TND)	47%
Virologic response	**Virologic control (Inactive HBV) (HBV DNA ≤ 2000 IU/mL, normal ALT)**	**39%**
	Functional cure (HBsAg < LLOQ, HBV DNA TND, normal ALT)	**39%**
	Clinical benefit, no therapy required (Low risk of progression, reduced risk of HCC)	**78%**

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DISCLOSURES

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