Potent and durable off-treatment reduction of HBsAg levels and cccDNA-derived transcripts by the CAM-E ALG-001075 in cell-based experiments

Contact information
barbara.testoni@inserm.fr
ajekle@aligos.com

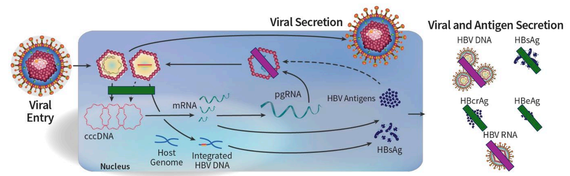
Audrey Diederichs[1,2], Maud Michelet[1,2], Yannick Debing[3], Jordi Verheyen[3], Hannah Vanrusselt[3], Julian A. Symons[4], Tse-I Lin[3], Lawrence Blatt[4], Fabien Zoulim[1,2,5], Andreas Jekle[4], Barbara Testoni[1,2]

[1]Université Lyon 1, Inserm, PaThLiv, UMR 1350, Lyon, France; [2]The Lyon Hepatology Institute, IHU EVEREST, Lyon, France; [3]Aligos Belgium BV, Leuven, Belgium; [4]Aligos Therapeutics, Inc., South San Francisco, CA; [5]Hepatology Department, Hospices Civils de Lyon, France

EASL CONGRESS Barcelona Spain 27–30 May 2026

Introduction & Aim

The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic hepatitis B. Capsid assembly modulators (CAMs) affect HBV core protein assembly into aberrant structures (CAM-A) or empty capsids (CAM-E)[1], inhibiting HBV RNA encapsidation (primary mechanism of action (MOA)). At higher concentrations, CAMs also interfere with the disassembly of viral particles, preventing establishment of covalently closed circular DNA (cccDNA, secondary MOA). ALG-001075, a novel, potent CAM-E, has shown in vitro profound inhibition of HBV DNA (primary MOA) and reductions in cccDNA and viral antigens (secondary MOA) [3,4]. Moreover, pevifoscorvir sodium (pevy), a prodrug of ALG-001075, has demonstrated potent reductions of HBV DNA, RNA, HBsAg, HBcrAg and HBeAg in subjects with chronic hepatitis B virus infection[2]. Here, we provide experimental evidence from cell-based experiments that ALG-001075 potently and durably inhibits HBsAg and HBeAg secretion and intracellular HBV RNA levels.

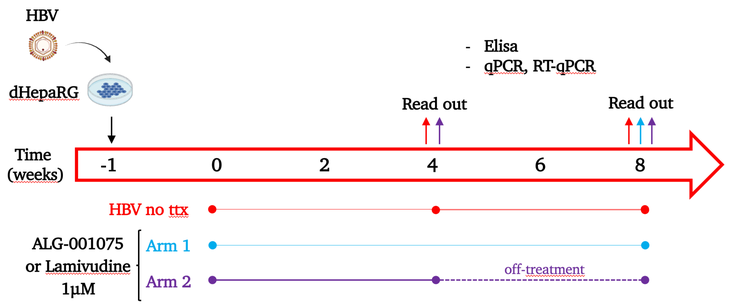


1st MOA causes the formation of empty capsids
— Reduces viral secretion (HBV DNA/RNA) at subnanomolar concentrations[3]

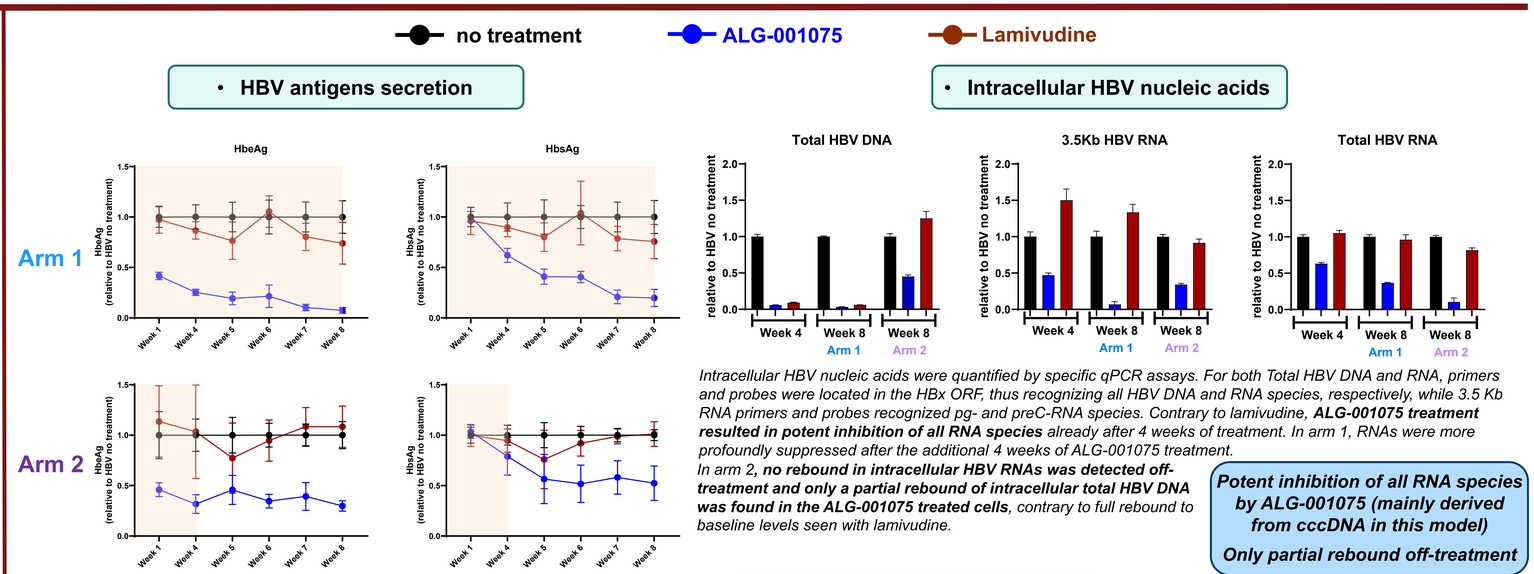
2nd MOA prevents capsid disassembly at higher concentrations of ALG-001075
— Prevents establishment/replenishment of cccDNA, which produces HBcrAg/HBeAg/HBsAg[4]

Methods

Differentiated HepaRG cells were treated from day 6 post-infection with 1 µM ALG-001075 or lamivudine (3TC) for 1 month, then ALG-001075 was either continued for an additional month (Arm 1) or stopped (Arm 2) and cells monitored off-treatment for 4 weeks. Harvested cells and supernatants were analyzed for HBeAg and HBsAg secretion by ELISA, intracellular HBV markers (total HBV DNA and RNA, 3.5Kb RNA) by qPCR, cell viability and hepatocyte differentiation markers.



Results



HBeAg and HBsAg were quantified by commercial Elisa assays (Autobio). Yellow shades indicate duration of treatment. ALG-001075 treatment resulted in ***potent inhibition of HBeAg secretion*** *and 38% inhibition of* ***secreted HBsAg*** *after 4 weeks of treatment, which was absent in lamivudine treated cells. During the additional 4 weeks of treatment* ***in arm 1, both HBeAg and HBsAg secretion continued to decrease and reached 90% and 80% inhibition****, respectively.*
In arm 2, HBeAg inhibition was maintained off-ALG-001075 treatment and HBsAg levels continued to decline *reaching 80% reduction with respect to untreated and lamivudine treatment.*

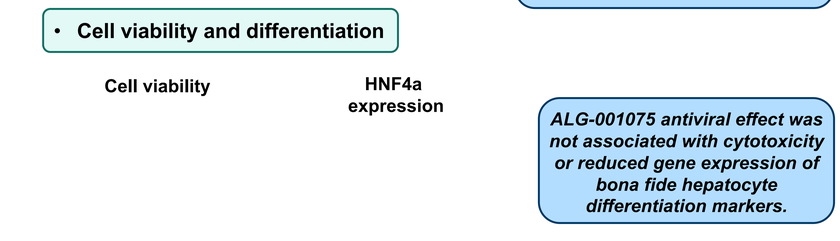
Continuous decrease in HBeAg and HBsAg under ALG-001075 treatment

No rebound off-treatment

Intracellular HBV nucleic acids were quantified by specific qPCR assays. For both Total HBV DNA and RNA, primers and probes were located in the HBx ORF, thus recognizing all HBV DNA and RNA species, respectively, while 3.5 Kb RNA primers and probes recognized pg- and preC-RNA species. Contrary to lamivudine, ***ALG-001075 treatment resulted in potent inhibition of all RNA species*** *already after 4 weeks of treatment. In arm 1, RNAs were more profoundly suppressed after the additional 4 weeks of ALG-001075 treatment.*
*In arm 2****, no rebound in intracellular HBV RNAs was detected off-treatment and only a partial rebound of intracellular total HBV DNA was found in the ALG-001075 treated cells****, contrary to full rebound to baseline levels seen with lamivudine.*

Potent inhibition of all RNA species by ALG-001075 (mainly derived from cccDNA in this model)

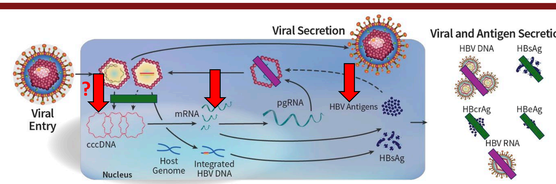
Only partial rebound off-treatment



ALG-001075 antiviral effect was not associated with cytotoxicity or reduced gene expression of bona fide hepatocyte differentiation markers.

ATP production was measured by CellTiter-Glo assay; HNF4a mRNA expression is representative of hepatocyte-specific differentiation markers.

Conclusions



Long-term ALG-001075 treatment resulted in **profound suppression of HBeAg, HBsAg and intracellular HBV RNAs which was durable after treatment withdrawal** in HBV-infected HepaRG cells, suggesting a **potential reduction in cccDNA level and/or transcriptional activity** under these experimental conditions. The sustained reductions in viral antigens observed here in a preclinical model were also seen in a long-term follow-up study where subjects with chronic HBV infection, after completion of 96 weeks of pevy monotherapy, were treated with entecavir for 24-48 weeks (Poster WED-588 by L Mak et al.)

References

[1] Zoulim F, Zlotnick A, Buchholz S, Donaldson E, Fry J, Gaggar A, Hu J, Kann M, Lenz O, Lin K, Mani N, Nassal M, Delaney W, Wang S, Westman G, Miller V, Janssen HLA. Nomenclature of HBV core protein-targeting antivirals. Nat Rev Gastroenterol Hepatol. 2022 Dec;19(12):748-750.

[2] Yuen M, Agarwal K, Jucov A et al. ALG-000184 (pevifoscorvir sodium) monotherapy in participants with chronic HBV infection: a phase 1, multicentre, randomised, dose escalation trial. The Lancet Gastroenterology & Hepatology, 2026; 11, 218-231

[3] Sandrine Vendeville et. al, The Discovery and Preclinical Profile of ALG-000184, a Prodrug of the Potent Hepatitis B Virus Capsid Assembly Modulator ALG-001075; Journal of Medicinal Chemistry Vol. 67:23 21126-21142;

[4] Jordi Verheyen et. al, AASLD 2025 Poster #1251;

Acknowledgements

This work was supported by grants from Aligos Therapeutics to FZ's and BT's institution and by the French ANR (IHU EVEREST: ANR-23-IAHU-0008)

YD, JV, HV, JAS, TL, LB and AJ are employee and stakeholders of Aligos Therapeutics and may own stock.