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Poster 1251

Capsid Assembly Modulator ALG-001075 Prevents cccDNA Formation and HBV DNA Integration In Vitro

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BACKGROUND

The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic hepatitis B (CHB).[1] Capsid assembly modulators (CAMs) are small molecules that bind the hepatitis B core protein (HBc) and prevent the encapsidation of pregenomic RNA (pgRNA), blocking HBV DNA production (primary mechanism of action). At higher concentrations, they also interfere with the disassembly of viral particles, preventing establishment of covalently closed circular DNA (cccDNA, secondary mechanism of action, Figure 1). CAMs may also prevent HBV DNA integration into the host genome by blocking the production of double-stranded linear DNA (dslDNA), the main precursor for integration (primary mechanism), and by preventing dslDNA-containing viral particles from delivering their payload into the host nucleus (secondary mechanism).

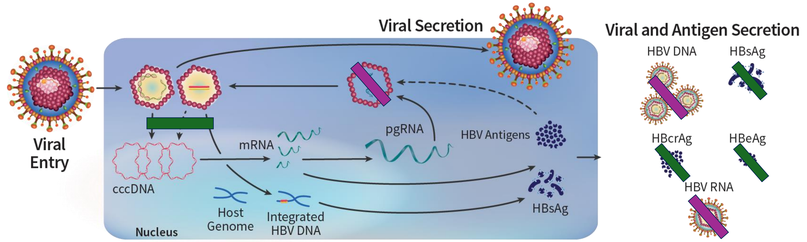

Figure 1 – HBV replication cycle with primary and secondary CAM mechanisms highlighted. HBV replication starts with viral entry of the hepatocyte through NTCP, followed by endosomal release, transfer to the nuclear pore complex and delivery of the rcDNA or dslDNA to the nucleus to form cccDNA or to be integrated into the host genome. Subsequent transcription from cccDNA yields viral antigens and pgRNA, which is packaged into new viral particles that are released from the cell. CAMs interfere with HBV replication by preventing pgRNA encapsidation, reducing HBV DNA and RNA (primary mechanism, purple boxes), and by blocking capsid disassembly and cccDNA establishment, reducing viral antigens (secondary mechanism, green boxes).

Pevifoscorvir sodium, also known as ALG-000184, is a prodrug of ALG-001075, a novel capsid assembly modulator leading to the formation of empty capsids (CAM-E).[2] ALG-000184 has demonstrated substantial reductions of HBV DNA, RNA, HBsAg, HBeAg and HBcrAg in subjects with chronic HBV infection.[3] Here, we investigated the effects of ALG-001075 on HBV cccDNA formation, dslDNA production and HBV DNA integration *in vitro*.

METHODS

HBV cccDNA was quantified in HBV-infected HepG2-NTCP cells using Southern blot and digital PCR (dPCR). Production of double-stranded linear DNA (dslDNA), which can lead to HBV DNA integration, from HBV-expressing HepG2.117 cells[4] was investigated using peptide nucleic acid (PNA) clamp-based qPCR.[5] The level of HBV DNA integration after HBV infection of HepG2-NTCP cells was quantified using genomic DNA isolation, HBV sequence enrichment, next-generation sequencing and subsequent identification of HBV-host junctions.[6]

RESULTS

ALG-001075 prevents HBV cccDNA establishment

When ALG-001075 was added together with the HBV inoculum to HepG2-NTCP cells, it resulted in dose-dependent declines of HBV rcDNA, dslDNA and cccDNA, as assessed through Southern blot (Figure 2). At 100 nM, this resulted in a complete visual disappearance of the cccDNA band. Similar results were obtained through quantification of total HBV DNA and cccDNA by dPCR, with the EC_{50} value for cccDNA prevention at approximately 25 nM, in line with values reported earlier for the secondary mechanism of ALG-001075.[2]

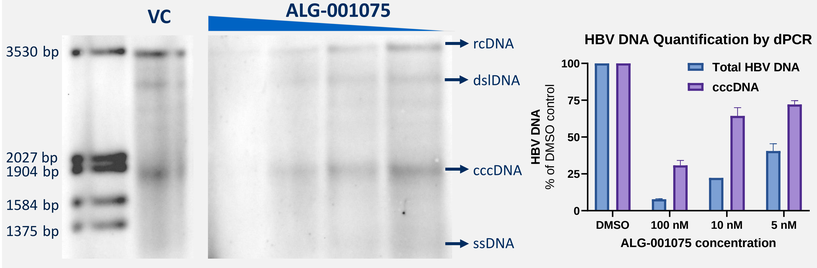

Figure 2 – ALG-001075 dose-dependently prevents cccDNA establishment. Left: Southern blot of HBV-infected HepG2-NTCP cells, treated with ALG-001075 at 100, 10, 5 and 1 nM (left to right) at the time of infection. **Right:** dPCR quantification of total HBV DNA and cccDNA of similarly generated samples. T5 exonuclease digestion was performed before cccDNA quantification. cccDNA, covalently closed circular DNA; dPCR, digital PCR; dslDNA, double-stranded linear DNA; rcDNA, relaxed circular DNA; ssDNA, single-stranded DNA; VC, virus control.

ALG-001075 efficiently blocks production of HBV dslDNA, a precursor to HBV DNA integration

Production of total HBV DNA and dslDNA from HepG2.117 cells was assessed through a previously published PNA clamp-based qPCR method.[5] As expected based on their primary mechanism, CAM-Es ALG-001075 and ALG-000111 (a close structural analog of ALG-001075)[7] resulted in pronounced reductions at clinically relevant micromolar concentrations (Figure 3), with all values below the lower limit of quantification of the assay. Tenofovir disoproxil fumarate (TDF) and entecavir (ETV) also reduced dslDNA levels. Testing of a broad concentration range of ALG-001075 confirmed potent inhibition of total HBV and dslDNA production with EC_{50} values below 10 nM.

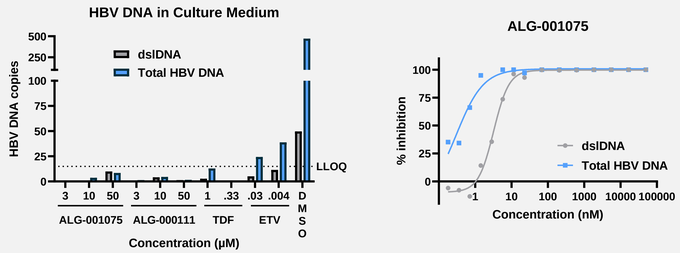

Figure 3 – CAM-Es and nucleos(t)ide analogs reduce total HBV DNA and dslDNA production in HepG2.117 cells. Left: dslDNA and total HBV DNA copies per condition. **Right:** Percentage inhibition of dslDNA and total HBV DNA in function of ALG-001075 concentration. dslDNA, double-stranded linear DNA; ETV, entecavir; LLOQ, lower limit of quantification; TDF, tenofovir disoproxil fumarate.

A close analog of ALG-001075 prevents HBV DNA integration in vitro

To assess the impact of nucleoside analogs and CAM-Es on HBV DNA integration, HepG2-NTCP cells were HBV-infected and subsequently cultured for 14 days in the presence or absence of ETV or ALG-000111. Total genomic DNA (> 5 kb) was extracted, enriched for HBV-containing sequences and subjected to next-generation sequencing. HBV-host DNA junctions were identified in each sample through a dedicated bio-informatics pipeline.[6] Treatment with both ETV (100 nM) and ALG-000111 at a low concentration (10 nM, engaging the primary but not the secondary mechanism of action of CAM-Es) resulted in a roughly 3-fold reduction in the number of detected junctions (Figure 4).

Increasing the concentration of ALG-000111 to 10,000 nM (within the physiologically relevant range for ALG-0001075 when dosed as prodrug pevifoscorvir sodium), resulted in an 85% reduction in the number of detected integrants. This suggests that the secondary mechanism of CAMs could provide a better protection against HBV DNA integration compared to nucleos(t)ide analogs for which such protection has already been demonstrated clinically.[8,9]

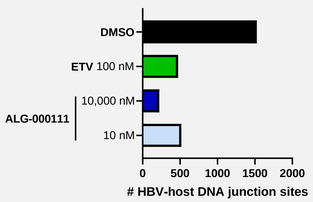

Figure 4 – CAM-E ALG-000111 prevents HBV DNA integration to a greater extent than ETV when both are added at physiologically relevant concentrations.

CONCLUSIONS

- **ALG-001075 demonstrated potent prevention of HBV cccDNA formation, confirming earlier in vitro findings of inhibition of cccDNA markers, such as HBsAg and HBV RNA.[2] This provides a potential explanation for the antigen declines observed in HBeAg-positive patients with chronic HBV infection.[3]**
- **Furthermore, ALG-001075 inhibited the production of dslDNA, the main precursor for HBV DNA integration, and ALG-000111, a close structural analog of ALG-001075, was shown to directly prevent HBV DNA integration.**
- **These results confirm the best-in-class properties of ALG-001075 and its superiority over commonly used nucleos(t)ide analogs.**

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