Background

Hepatitis B virus (HBV) capsid assembly is a promising target for the treatment of chronic HBV. Class II capsid assembly modulators (CAMS) can induce rapid HBV core protein (HBc) assembly into empty capsids, thus inhibiting HBV DNA encapsidation and subsequent formation of infectious HBV particles. CAMs can also block cccDNA establishment by blocking viral DNA release from the capsid after entry. As a part of our efforts to advance multiple structurally diverse CAMs, we report on ALG-000111, a novel Class II CAM with excellent antiviral activity and efficacy in the AAV-HBV mouse model.

Methods

Antiviral activity on HBV DNA was determined in HepG2.117 cells using quantitative PCR, with and without 0.1% human serum. Optimized assays were performed to assess inhibition over several log₁₀ viral titers and longevity of the antiviral effect after compound removal. Activity was also studied in HBV-infected primary hepatocytes both the primary effect on HBV DNA and the secondary effect on cccDNA establishment. Further characterization was performed using biochemical quenching assays, electron microscopy visualization, immunofluorescent HBc staining, and Western blotting. ADME properties were evaluated in vitro, and in vivo antiviral efficacy was assessed in the AAV-HBV mouse model.

Results

**ALG-000111 and prodrug ALG-000286 are potent HBV DNA inhibitors**

The HepG2.117 cell line contains a stably integrated genotype D HBV genome. ALG-000111 and its prodrug ALG-000286 were highly effective in reducing the amount of produced HBV DNA, with EC₅₀ values below 1 nM. Addition of 0.1% human serum to the culture medium resulted in a modest 7-fold shift of the antiviral activity of ALG-000111, indicating a moderate impact of plasma protein binding.

**ALG-000111 induces deep and sustained HBV DNA knock-down**

The HepG2.117-based DNA assay was optimized by DNsase digestion to remove background genomic/integrated HBV DNA and to exclusively detect encapsidated HBV DNA by qPCR. This allowed to determine accurate IC₅₀ and EC₅₀ values for ALG-000111 and ALG-000286, confirming their potential to reduce HBV DNA levels by several orders of magnitude.

**ALG-000111 induces rapid assembly of empty capsids in vitro**

To probe target engagement, ALG-000111 was incubated with recombinant HBc conjugated with fluorescent dye. Fluorescence is quenched when capsids are assembled. Binding of ALG-000111 to HBc induces rapid capsid assembly. Empty capsid formation with regular morphology, typically observed for Class II CAMs, was confirmed by electron microscopy.

**ALG-000111 treatment leads to cytoplasmic HBeAg accumulation**

Treatment of HBV-expressing cells with different classes of CAMs may lead to changes in cellular HBeAg levels. Here, an accumulation was observed when HepG2.117 cells were treated with ALG-000111, as shown by Western blot. In addition, immunofluorescent staining for HBeAg revealed that the increase in HBeAg levels mainly occurred in the cytoplasm.

Conclusions

ALG-000111 demonstrates sub-nanomolar activity and deep knockdown of HBV DNA in cell-based assays, combined with a modest serum shift, long-lasting antiviral activity and desirable PK properties. Its prodrug form, ALG-000286, improves formulation properties and leads to a strong decline in HBV DNA in the AAV-HBV mouse model, making it a promising Class II CAM candidate for further development.

References


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