Capsid Assembly Modulator ALG-000111 and its Prodrug ALG-000286 Display Excellent In Vitro and In Vivo Antiviral Activity

Abstract 1386

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Background

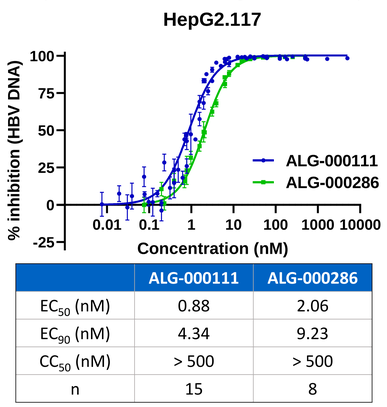
Hepatitis B virus (HBV) capsid assembly is a promising target for the treatment of chronic hepatitis B. Class II capsid assembly modulators (CAMs) induce rapid HBV core protein (HBc) assembly into empty capsids, thus inhibiting HBV RNA encapsidation and subsequent formation of infectious HBV particles.[1] CAMs can also block cccDNA establishment by blocking viral DNA release from the capsid after entry.[2] 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

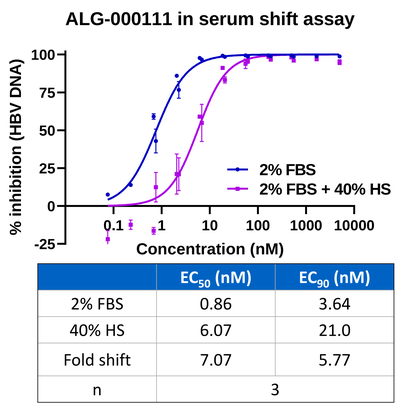
Antiviral activity on HBV DNA was determined in HepG2.117 cells using quantitative PCR, with and without 40% human serum. Optimized assays were performed to assess inhibition over several $\log_{10}$ viral titers and longevity of the antiviral effect after compound removal. Activity was also studied in HBV-infected primary human 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 HBc Western blotting. ADME properties were evaluated in vitro, and in vivo antiviral efficacy was assessed in the AAV-HBV mouse model.[3]

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 EC_{50} values below 1 nM. Addition of 40% 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	ALG-000286
EC_{50} (nM)	0.88	2.06
EC_{90} (nM)	4.34	9.23
CC_{50} (nM)	> 500	> 500
n	15	8

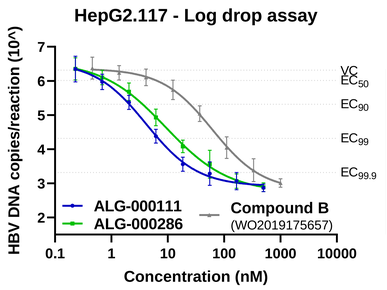


	EC_{50} (nM)	EC_{90} (nM)
2% FBS	0.86	3.64
40% HS	6.07	21.0
Fold shift	7.07	5.77
n	3	

Figure 1 – Left: *Dose-response curve for compound-induced inhibition of HBV DNA replication in HepG2.117 cells. Curves and values represent mean ± SEM from 15 or 8 independent experiments, respectively.* ***Right:*** *Dose-response curves for ALG-000111-induced inhibition of HBV DNA replication in HepG2.117 in the presence of 2% fetal bovine serum (FBS) or 2% FBS + 40% human serum (HS). Curves and values represent mean ± SEM from 3 independent experiments.*

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

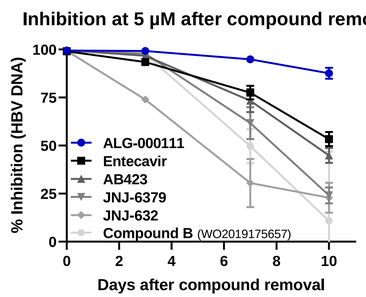
The HepG2.117-based DNA assay was optimized by including DNase digestion to remove background genomic/integrated HBV DNA and to exclusively detect encapsidated HBV DNA by qPCR. This allowed us to determine accurate EC_{90}, EC_{99} and $EC_{99.9}$ values for ALG-000111 and ALG-000286, confirming their potential to reduce HBV DNA levels by several orders of magnitude.

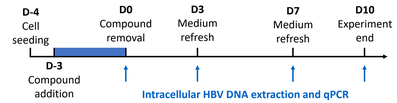


	ALG-000111	ALG-000286	Compound B
EC_{50} (nM)	0.66	0.92	6.22
EC_{90} (nM)	1.88	3.35	24.9
EC_{99} (nM)	4.81	10.4	83.6
$EC_{99.9}$ (nM)	15.3	39.7	377
n	3		2

Figure 2: *Dose-response curve for compound-induced inhibition of HBV DNA replication in HepG2.117 cells in the log drop assay. Curves and values represent mean ± SEM from 2 or 3 independent experiments. Compound B is a reference Class II CAM.*

In addition, a viral rebound assay was set up in which HepG2.117 cells were incubated with compound for 3 days, followed by compound removal, extensive washing and periodic assessment of intracellular HBV DNA. ALG-000111 behaved in a superior manner to entecavir and other CAMs, retaining 95% and 88% inhibition 7 and 10 days after compound removal, respectively.



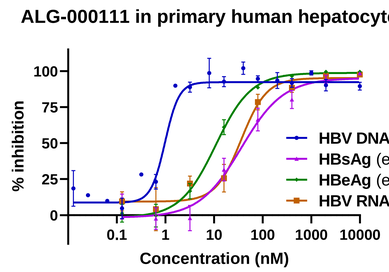


Inhib. (5µM)	Day 7	Day 10
ALG-000111	94.9%	87.6%
Entecavir	77.6%	53.4%
AB423	73.4%	44.9%
JNJ-6379	61.7%	24.1%
JNJ-632	30.5%	22.9%
Compound B	49.7%	10.9%

Figure 3: *Percentage inhibition of HBV DNA replication in HepG2.117 cells for each listed compound at 5 µM at different times after compound removal. Curves and values represent mean ± SEM from 2 or 3 independent experiments.*

ALG-000111 is a potent inhibitor of RNA encapsidation and cccDNA establishment in HBV-infected primary human hepatocytes

When ALG-000111 was added to an established HBV infection in primary human hepatocytes (5 days post infection), HBV DNA production was potently inhibited. In addition, cccDNA formation was strongly inhibited when compound was added at the time of infection, as evidenced by reductions in extracellular HBsAg and intracellular HBV RNA.



	EC_{50} (nM)	EC_{90} (nM)
HBV DNA*	0.98	3.22
HBsAg#	41.7	651
HBeAg#	11.4	78.1
HBV RNA#	34.0	189
n	5	

Figure 4: *Dose-response curves for ALG-000111-induced inhibition of HBV DNA, RNA, HBsAg and HBeAg production in HBV-infected primary human hepatocytes. Values represent mean ± SEM from 5 independent experiments. extra = extracellular; intra = intracellular. * Compound added 5 days after infection; # compound added at time of infection.*

ALG-000111 induces rapid assembly of empty capsids in vitro

To prove target engagement, ALG-000111 was incubated with recombinant HBc conjugated with fluorescent dye. Fluorescence is quenched when capsids are assembled.[4] Binding of ALG-000111 to HBc induced rapid capsid assembly. Empty capsid formation with regular morphology, typically observed for Class II CAMs, was confirmed by electron microscopy.

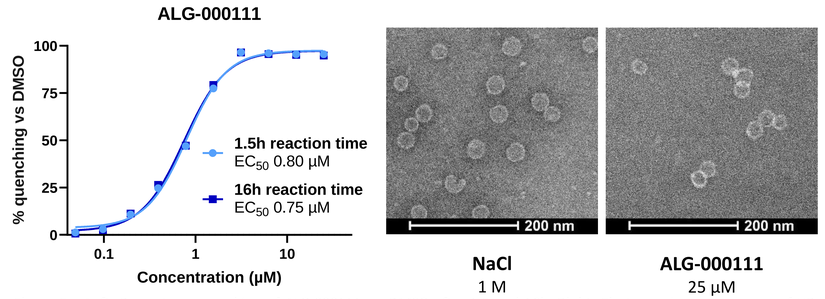
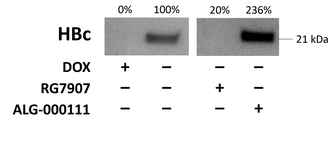


Figure 5 – Left: *Percentage quenching of ALG-000111 vs. DMSO after 1.5 and 16h.* ***Right:*** *Electron microscopy image of HBc incubated with NaCl (1M) or ALG-000111 (25 µM). Empty capsids with normal morphology can clearly be observed.*

ALG-000111 treatment leads to cytoplasmic HBc accumulation

Treatment of HBc-expressing cells with different classes of CAMs may lead to changes in cellular HBc 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 HBc revealed that the increase in HBc levels mainly occurred in the cytoplasm.



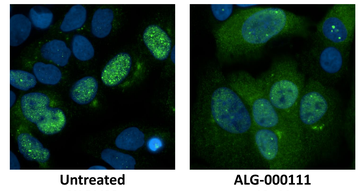


Figure 6 – Left: *Western blot for HBc on HepG2.117 lysates; Doxycycline (DOX) and RG7907 (a Class I CAM) treatment reduce HBc levels, whereas ALG-000111 increases HBc levels.* ***Right:*** *Immunofluorescent staining of HepG2.117 cells for HBc; ALG-000111 induces cytoplasmic HBc accumulation compared to the untreated control.*

Prodrug ALG-000286 is a potent inhibitor of HBV DNA production in vivo

The AAV-HBV model was used to assess the efficacy of prodrug ALG-000286 in vivo.[3] A biphasic reduction in HBV DNA was observed, with 3.7 $\log_{10}$ IU/mL reduction after 28 days of treatment. No decreases in HBsAg or HBeAg were observed.

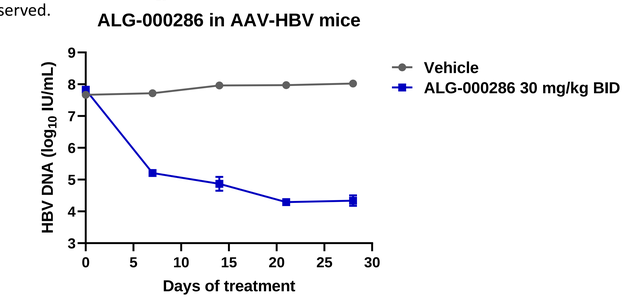


Figure 7: *HBV DNA levels over time in AAV-HBV mouse serum. Curves and values represent means ± SEM from 4 mice per group.*

ALG-000111 has good in vitro ADME properties

ALG-000111 has good drug-like properties such as stability in SGF/SIF, liver microsomes and hepatocytes across species, high permeability across Caco-2 monolayers, and low CYP450-mediated drug-drug interaction potential. ALG-000111 and its prodrug ALG-000286 achieved similar plasma exposures at equivalent oral doses in mice, although the prodrug form can considerably improve formulation properties.

Parameter	ALG-000111
SGF/SIF $t_{1/2}$	>480 / >480 min
Mouse/Rat/Human liver microsomes $t_{1/2}$	>60 / >60 / >60 min
Mouse/Rat/Dog/Cyno/Human Hepatocyte $t_{1/2}$	>360 / 194 / >360 / 84 / >360 min
GSH adducts	None
%ppb Mouse/Rat/Dog/Cyno/Human	94.8/92.6/93.7/92.9/95.5%
P_{app} (A-B)x10^{-6} cm/s, B-A/A-B	3.67/3.2
CYP Inhibition on 1A2/2B6/2C8/2C9/ 2C19/2D6/3A4-M/3A4-T	IC_{50} > 10 µM

Table 1: *Summary of in vitro ADME properties for ALG-000111.*

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

[1] Yang et al 2019 ACS Infect Dis, 5: 713-24. [2] Berke et al 2017 Antimicrob Agents Chemother, 61: e00560-17. [3] Yang et al 2014 Cell Mol Immunol, 11: 71-8. [4] Stray et al 2006 Nat Biotechnol, 24: 358-62.

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