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Abstract 818

ALG-005398 is a Potent Non-HAP Class I HBV Capsid Assembly Modulator that Strongly Reduces HBsAg Levels In Vivo

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Background

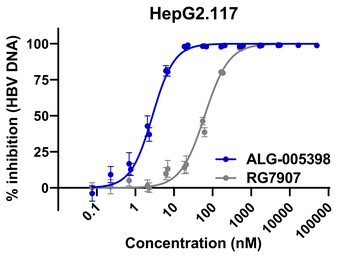
Hepatitis B virus (HBV) capsid assembly is an attractive target for the treatment of chronic hepatitis B. In addition to inhibiting HBV RNA encapsidation and formation of infectious HBV particles, Class I capsid assembly modulators (CAMs) induce HBV core protein (HBc) aggregation and sustained HBsAg reduction in AAV-HBV mice.[1,2] All known Class I CAMs are heteroaryldihydropyrimidines (HAPs), such as RG7907 and GLS4.[3,4] As a part of our efforts to advance multiple structurally diverse CAMs, we report on ALG-005398, a first representative of a series of non-HAP Class I CAMs, identified using structure-based drug design and scaffold hopping.

Methods

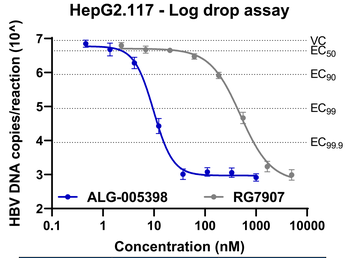
Antiviral activity on HBV DNA was determined in HepG2.117 cells using quantitative PCR. Inhibition over several orders of magnitude was assessed in an optimized log drop assay. Both the primary effect on HBV DNA and the secondary effect on cccDNA establishment were studied in HBV-infected primary human hepatocytes (PHH). Further characterization was performed using biochemical quenching assays, electron microscopy visualization, immunofluorescent HBc staining, and HBc Western blotting. In vivo antiviral efficacy was assessed in the AAV-HBV mouse model.[5]

ALG-005398 is a potent HBV DNA inhibitor

The HepG2.117 cell line contains a stably integrated genotype D HBV genome. ALG-005398 was highly effective in reducing the amount of produced HBV DNA (EC_{50} = 3 nM). Including additional DNase digestion steps[6] allowed to determine accurate EC_{90}, EC_{99} and $EC_{99.9}$ values for ALG-005398, confirming its potential to reduce HBV DNA levels by several orders of magnitude, with an impressive 3 log_{10} reduction at a concentration of only 14 nM.



	ALG-005398	RG7907
EC_{50} (nM)	3.41	62.2
EC_{90} (nM)	10.6	304
CC_{50} (nM)	> 500	>500
n	20	

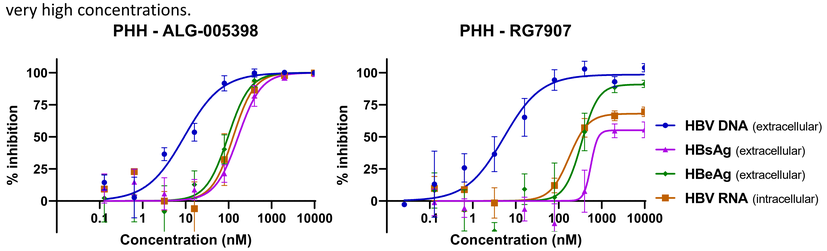


	ALG-005398	RG7907
EC_{50} (nM)	1.70	59.6
EC_{90} (nM)	4.80	204
EC_{99} (nM)	8.12	458
$EC_{99.9}$ (nM)	13.7	989
n	3	2

Figure 1 – Left: *Dose-response curves for inhibition of HBV DNA replication in HepG2.117 cells. Curves and values represent mean ± SEM from 20 independent experiments.* ***Right:*** *Dose-response curves for 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. VC = virus control.*

ALG-005398 is a potent inhibitor of RNA encapsidation and cccDNA establishment in HBV-infected primary human hepatocytes

When ALG-005398 was added to an established HBV infection in PHH (5 days post infection), HBV DNA production was blocked efficiently. 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. In contrast, RG7907 was much less effective in preventing cccDNA formation, not reaching full inhibition, even at very high concentrations.

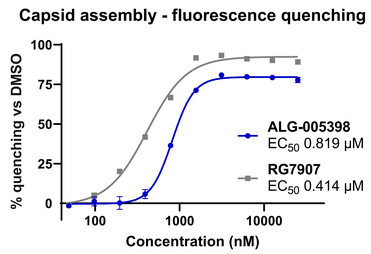


EC_{50} / EC_{90} (nM)	ALG-005398	RG7907
HBV DNA*	9.29 / 83.6	5.41 / 55.3
HBsAg#	167 / 650	848 / >10,000
HBeAg#	97.3 / 353	372 / 2730
HBV RNA#	128 /446	294 / >10,000
n	3	2

Figure 2: *Dose-response curves for ALG-005398- and RG7907-induced inhibition of HBV DNA, RNA, HBsAg and HBeAg production in HBV-infected primary human hepatocytes. Values represent mean ± SEM from 2 or 3 independent experiments.* ** Compound added 5 days after infection;* *# compound added at time of infection.*

ALG-005398 induces rapid formation of small aberrant capsids in vitro

To prove target engagement, ALG-005398 was incubated with recombinant HBc conjugated with a fluorescent dye. Fluorescence is quenched when capsids are assembled.[7] Binding of ALG-005398 to HBc induced rapid oligomerization, with slightly lower maximum quenching but a steeper slope compared to RG7907 (3.4 vs 1.9). Note that a steeper slope was also observed for ALG-005398 in the log drop assay (fig 1; slope of 2.3 vs 1.5). Electron microscopy showed the formation of aberrant capsids, typically observed for Class I CAMs. Interestingly, the irregular particles induced by ALG-005398 are smaller (40-50 nm) than the typical HAP-induced aberrant capsids observed for RG7907 (60-70 nm), but still larger than the empty capsids formed in NaCl (30-35 nm).



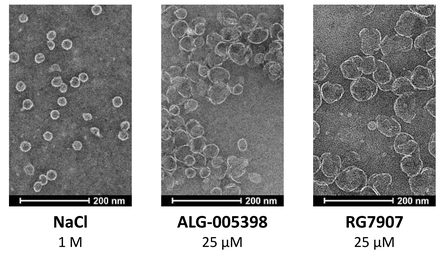
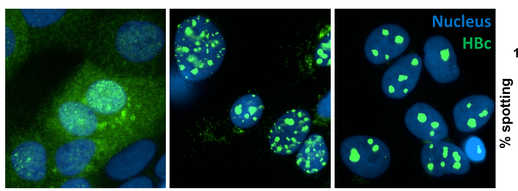
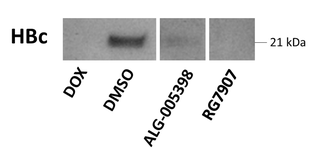


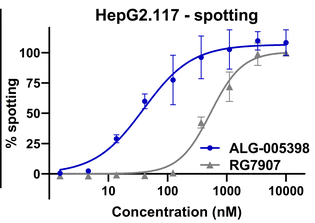
Figure 3 – Left: *Percentage quenching of ALG-005398 and RG7907 vs DMSO after 1.5h of incubation.* ***Right:*** *Electron microscopy images of HBc incubated with NaCl (1 M), ALG-005398 or RG7907 (25 µM). Empty capsids with normal morphology (NaCl) and aberrant particles (ALG-005398 and RG7907) can clearly be distinguished.*

ALG-005398 induces formation of small nuclear HBc aggregates in cellulo

HAP Class I CAMs have been reported to induce the formation of nuclear HBc spots, representing compound-induced HBc aggregates.[8] Such spots were also observed when HepG2.117 cells were treated with ALG-005398, although a different phenotype was observed compared to RG7907: spots were more numerous but smaller in size, in line with electron microscopy results. Quantification of the total spot area normalized to cell number and relative to RG7907 at 10 µM allowed the determination of EC_{50} and EC_{90} values, showing that ALG-005398 is approximately 10x more potent than RG7907 in inducing HBc spots. In Western blot, both RG7907 and ALG-005398 strongly reduced the amount of detectable HBc; possibly aggregated HBc is not solubilized under the tested conditions or does not migrate during SDS-PAGE due to its large molecular size.





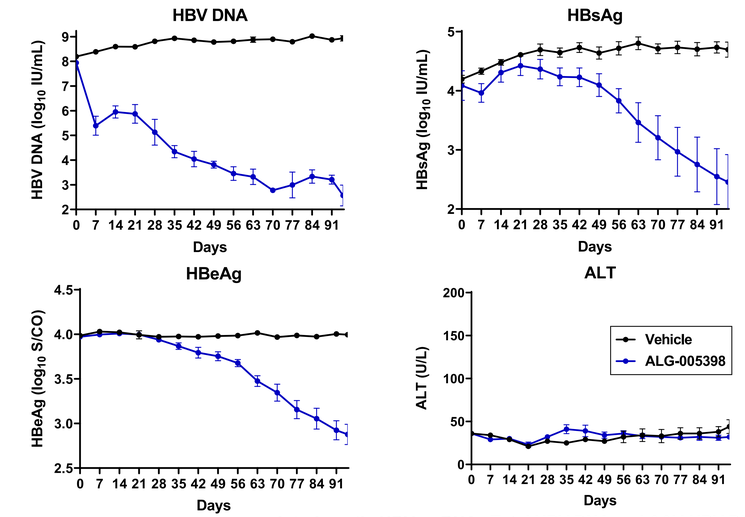


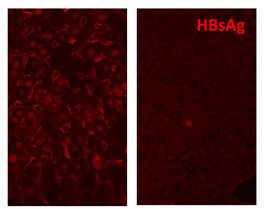
	ALG-005398	RG7907
EC_{50} (nM)	34.7	529
EC_{90} (nM)	192	1970
n	4	4

Figure 4 – Top left: *Immunofluorescent staining of HepG2.117 cells for HBc; Class I CAMs induce loss of cytoplasmic HBc and formation of nuclear HBc aggregates with different phenotypes for RG7907 and ALG-005398.* ***Right:*** *Quantification of spotting by automated image analysis (total spot area normalized to cell number and relative to RG7907 at 10 µM). Curves and values represent mean ± SEM from 4 independent experiments.* ***Bottom left:*** *Western blot for HBc in HepG2.117 lysates; Class I CAMs (both at 2 µM) and doxycycline (DOX) reduce HBc levels.*

ALG-005398 reduces HBsAg levels in vivo with minimal ALT increase

The AAV-HBV model was used to assess the efficacy of ALG-005398 in vivo.[5] ALG-005398 at 30 mg/kg/dose BID resulted in a multiphasic reduction in HBV DNA, with a 6.4 log_{10} IU/mL reduction after 95 days of treatment. A significant decrease in serum HBsAg levels was observed with a 2.24 log_{10} IU/mL reduction with paced kinetics, the main decline manifesting between day 49 and 95 of ALG-005398 treatment with only a minimal change in ALT. This is in contrast to RG7907 for which a pronounced ALT flare was reported.[1,2] Significant decreases in HBeAg levels were also noted but with a slight delay compared to HBsAg decline. Immunohistochemical staining of liver sections at day 95 showed a dramatic disappearance of intrahepatic HBsAg. In line with serum HBV DNA, liver pgRNA levels were also significantly reduced. Interestingly, quantification of liver total HBV and AAV-HBV levels demonstrated a 10-fold reduction in the amount of AAV-HBV episome in the livers of Class I CAM-treated animals, explaining the reported sustained HBsAg reduction after stopping treatment with a HAP Class I CAM.[1,2]





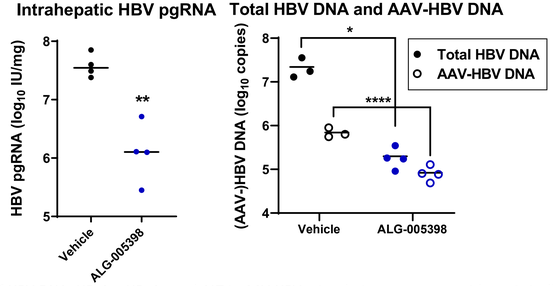


Figure 5 – Top: *Evolution of serum levels of HBV DNA, HBsAg, HBeAg and ALT in AAV-HBV mice during treatment, with statistically significant declines for HBV DNA, HBsAg and HBeAg. Values represent mean ± SEM for 4 animals per group.* ***Bottom left:*** *Immunofluorescent HBsAg staining in AAV-HBV mouse livers at end of treatment.* ***Bottom right:*** *Quantification of intrahepatic pgRNA, total HBV DNA and AAV-HBV episomal DNA after end of treatment. Lines represent group means.*

Conclusions

ALG-005398 is the first non-HAP Class I CAM reported to date. It showed excellent in vitro and in vivo antiviral activity, including a pronounced HBsAg reduction in vivo. The minimal ALT elevation and paced antiviral kinetics shown for this lead compound confirm that this is an attractive chemotype for further development. Class I CAMs from other chemical series are also advancing in the Aligos pipeline.

References

[1] Zhou et al 2018 J Hepatol, 68: S770. [2] Zhou et al 2019 J Hepatol, 70: e98. [3] Yuen et al 2021 Lancet Gastroenterol Hepatol, 6: 723-32. [4] Zhang et al 2021 Clin Infect Dis, 73: 175-82. [5] Yang et al 2014 Cell Mol Immunol, 11: 71-8. [6] Debing et al 2021 J Hepatol, 75 S2: PO-1386. [7] Stray et al 2006 Nat Biotechnol, 24: 358-62. [8] Corcuera et al 2018 Antiviral Res, 158: 135-42.

Financial disclosures

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