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Preclinical Assessment of a Novel Capsid Assembly Modulator, ALG-001075, Demonstrates Best-in-Class In Vitro Potency and In Vivo Antiviral Efficacy

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Background

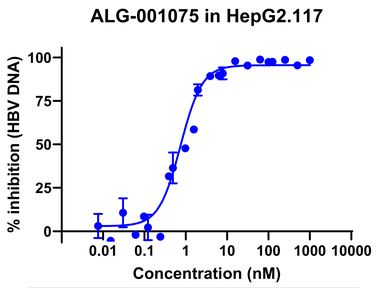
The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic hepatitis B. Capsid assembly modulators (CAMs) affect HBV core protein assembly into aberrant structures (class-I) or empty capsids (class-II), inhibiting HBV RNA encapsidation.[1] As a part of our efforts to advance multiple structurally diverse CAMs from both classes, we report on ALG-001075, a novel class-II CAM with excellent antiviral potency, pharmacokinetic properties and efficacy in the mouse adeno-associated virus (AAV)-HBV model.

Methods

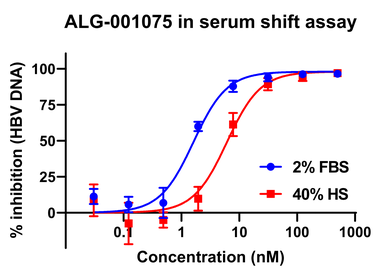
Antiviral activity on HBV DNA was determined in HepG2.2.15 and HepG2.117 cells using qPCR, with and without 40% human serum. ALG-001075 was tested against a broad range of HBV genotypes and constructs with 3 known CAM resistance mutations in a transient antiviral assay. Activity was also assessed in primary human hepatocytes infected with HBV, both with compound either included in the inoculum or added after establishment of infection. HBV DNA, HBsAg and HBeAg were quantified in culture medium by qPCR and αLISA. Intracellular HBV RNA was quantified by RT-qPCR. A biochemical quenching assay was used to confirm the mechanism of action of the compound.[2] Pharmacokinetics were evaluated following oral dosing in mouse, rat, dog and monkey. In vivo antiviral efficacy was assessed in the AAV-HBV mouse model.[3]

ALG-001075 is a potent sub-nanomolar inhibitor of HBV DNA

The HepG2.2.15 and HepG2.117 cell lines contain a stably integrated genotype D HBV genome. ALG-001075 proved 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 results in 4-fold shift of the antiviral efficacy of ALG-001075, indicating a moderate impact of plasma protein binding.



	HepG2.117	HepG2.2.15
EC_{50} (nM)	0.57	0.53
EC_{90} (nM)	2.96	1.84
CC_{50} (nM)	> 500	> 500
n	10	3



	EC_{50} (nM)	EC_{90} (nM)
2% FBS	1.49	7.28
40% HS	6.29	30.8
Fold shift	4.22	4.22
n	3	

Figure 1 – Left: *Dose-response curve for ALG-001075-induced inhibition of HBV DNA in HepG2.117. Values represent mean ± SEM from 10 independent experiments.* ***Right:*** *Dose-response curves for ALG-001075-induced inhibition of HBV DNA in HepG2.117 in the presence of 2% fetal bovine serum (FBS) or 2% FBS + 40% human serum (HS). Values represent mean ± SEM from 3 independent experiments.*

ALG-001075 inhibits a broad panel of HBV genotypes and retains some activity to known CAM resistance mutations

A broad panel of HBV strains from genotypes A to J were tested in a transient HBV assay, showing overall good activity against all genotypes. Genotype E strain HE974384 showed a particularly lower sensitivity (EC_{50} 33.7 nM, n=3). This harbors a I105F mutation, a position associated with CAM resistance mutations.[4-5] ALG-001075 was also tested in constructs harboring the known resistance mutations T33N, Y118F and T128I.[4,6] Although ALG-001075 was affected by T33N, it only resulted in a 37-fold shift, compared to a 225-fold shift for GLS4.

The impact of Y118F was also more limited for ALG-001075. Interestingly, slightly increased sensitivities were observed for T128I, despite reported resistance for other class-II CAMs with this mutation.[6]

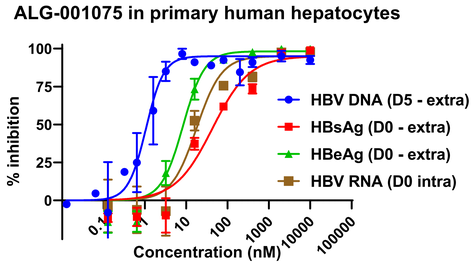
Genotype	Mean EC_{50} (nM)	Number of strains
A	3.36	4
B	2.43	5
C	2.00	4
D	5.47	4
E	16.4	3
F	4.03	5
G	5.54	4
H	8.14	4
I	2.5	2
J	1.7	1

	GLS4		ALG-001075	
	EC_{50} (nM)	Fold shift	EC_{50} (nM)	Fold shift
WT	14.7	1	4.58	1
T33N	3,310	225	170	37.2
Y118F	45.9	3.11	7.67	1.67
T128I	8.71	0.59	2.63	0.57
n	≥3			

Table 1 – Left: *Average activity of ALG-001075 per genotype (n≥1 for each strain).* ***Right:*** *Overview of the activity of GLS4 and ALG-001075 against constructs carrying the indicated mutations.*

ALG-001075 is a potent inhibitor of RNA encapsidation and cccDNA establishment in HBV-infected primary human hepatocytes

When ALG-001075 was added to an established HBV infection in primary human hepatocytes (5 days post infection), HBV DNA production was potently inhibited. In addition, ALG-001075, when added at the time of infection, strongly inhibited cccDNA formation, as shown by reductions in extracellular HBsAg and intracellular HBV RNA.

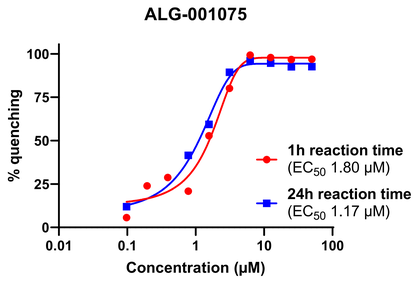


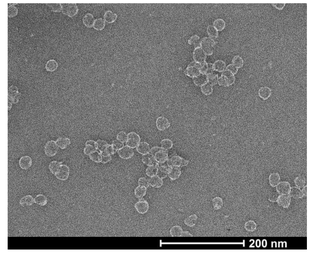
	EC_{50} (nM)	EC_{90} (nM)
HBV DNA	1.12	4.46
HBsAg	48.2	837
HBeAg	8.55	35.1
HBV RNA	19.1	133
n	5	

Figure 2: *Dose-response curves for ALG-001075-induced inhibition of HBV DNA, RNA, HBsAg and HBeAg in primary human hepatocytes. Values represent mean ± SEM from 5 independent experiments. D0/5 = day of compound addition after infection; extra = extracellular; intra = intracellular.*

ALG-001075 induces rapid assembly of empty capsids in vitro

To prove target engagement, ALG-001075 was incubated with recombinant HBV core protein conjugated with fluorescent dye. Fluorescence is quenched when capsids are assembled.[2] Binding of ALG-001075 to HBV core induced capsid assembly in less than 3 minutes. Class-II empty capsid formation was confirmed by electron microscopy.





ALG-001075 (25 µM)

Figure 3 – Left: *Percentage quenching of ALG-001075 vs. DMSO after 1 and 24h.* ***Right:*** *Electron microscopy image of HBV core incubated with ALG-001075. Empty capsids can clearly be distinguished.*

ALG-001075 is a potent inhibitor of HBV DNA production in vivo

The AAV-HBV model was used to assess the efficacy of ALG-001075 in vivo.[3] Up to 5 log_{10} IU/ml reduction in HBV DNA was observed, with several animals in the 15 mg/kg BID group being below the limit of quantification. No decreases in HBsAg or HBeAg were observed.

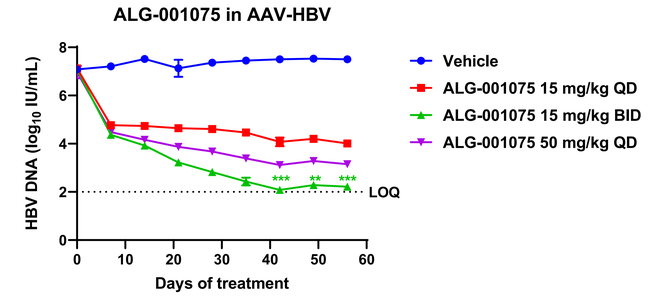
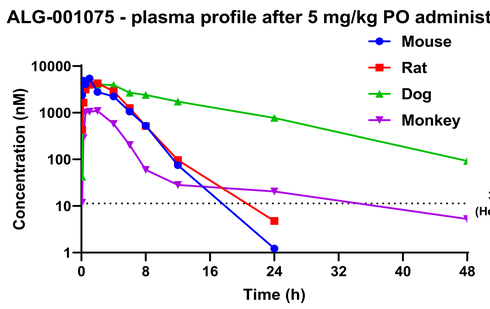


Figure 4: *HBV DNA levels over time in the AAV-HBV mouse. Values represent means ± SEM from 6 animals per group. Number of stars represents the number of mice with undetectable HBV DNA at that time point.*

ALG-001075 has good ADME properties

Liver microsome half-life of ALG-001075 was above 60 minutes in all tested species, including human. Plasma protein binding was 88.8% for human. $P_{app\ A->B}$ was 1.8 x 10^{-6} cm/s with an efflux ratio of 7.8. ALG-001075 has no in vitro reactive metabolites or CYP inhibition or induction potential, or off-target kinase and receptor liabilities. ALG-001075 has low clearance and high oral bioavailability with liver exposure being 4-fold higher than plasma in rodents. ALG-001075 is predicted to have QD dosing in humans.



Species	Plasma C_{max} (nM)	Plasma AUC_{inf} (nM.h)
Mouse	5,413	18,907
Rat	4,340	20,649
Dog	4,506	57,984
Monkey	1,159	5,182

Figure 5 – Left: *ALG-001075 plasma profiles after 5 mg/kg PO administration.* ***Right:*** *Plasma C_{max} and AUC_{inf} for the different species. Values represent means from at least 3 animals per species.*

Conclusions

With sub-nanomolar activity in cell-based assays, ALG-001075 is among the most potent class-II CAMs reported to date, the compound efficiently blocks both HBV genome encapsidation and de novo cccDNA formation. It demonstrates excellent preclinical efficacy and pharmacokinetic properties, predicting QD dosing in humans. ALG-001075 warrants further development as a potential best-in-class CAM.

References

[1] Yang et al 2019 ACS Infect Dis, 5: 713-24. [2] Stray et al 2006 Nat Biotechnol, 24: 358-62. [3] Yang et al 2014 Cell Mol Immunol, 11: 71-8. [4] Berke et al 2017 Antiviral Res, 144: 205-15. [5] Zhang et al 2019 EASL-AASLD HBV endpoints, 8-9 March 2019, London, UK, OP-01. [6] Berke et al 2017 AASLD conference, 2-24 October 2017, Washington, DC , 940.

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