AASLD 2019 #703

Preclinical Assessment of Potency and Efficacy of a Novel Class-II Capsid Assembly Modulator, ALG-001024

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Background

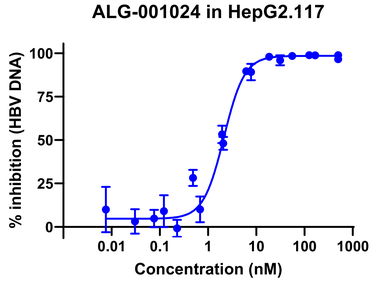
The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic hepatitis B. Capsid assembly modulators (CAMs) affect HBV core protein assembly into aberrant structures (class-I) or empty capsids (class-II), inhibiting HBV RNA encapsidation.[1] As a part of our efforts to advance multiple structurally diverse CAMs from both classes, we report on ALG-001024, a novel class-II CAM with excellent antiviral potency, pharmacokinetic properties and efficacy in the mouse adeno-associated virus (AAV)-HBV model.

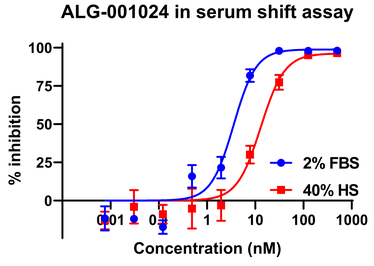
Methods

Antiviral activity on HBV DNA was determined in HepG2.2.15 and HepG2.117 cells using qPCR, with and without 40% human serum. ALG-001024 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 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-001024 is a potent nanomolar inhibitor of HBV DNA

The HepG2.2.15 and HepG2.117 cell lines contain a stably integrated genotype D HBV genome. ALG-001024 proved highly effective in reducing the amount of produced HBV DNA, with EC_{50} values below 10 nM. Addition of 40% human serum to the culture medium results in 4-fold shift of the antiviral efficacy of ALG-001024, indicating a moderate impact of plasma protein binding.





	HepG2.117	HepG2.2.15
EC_{50} (nM)	2.07	4.59
EC_{90} (nM)	6.64	14.9
CC_{50} (nM)	> 500	> 500
n	7	3

	EC_{50} (nM)	EC_{90} (nM)
2% FBS	3.61	12.4
40% HS	13.7	60.0
Fold shift	3.78	4.83
n	4	

Figure 1 – Left: *Dose-response curve for ALG-001024-induced inhibition of HBV DNA in HepG2.117. Values represent mean ± SEM from 7 independent experiments.* ***Right:*** *Dose-response curves for ALG-001024-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 4 independent experiments.*

ALG-001024 inhibits a broad panel of HBV genotypes and shows a typical class-II CAM resistance profile

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 demonstrated a particularly lower sensitivity (EC_{50} 42.8 nM, n=3). This harbors a I105F mutation, a position associated with CAM resistance mutations.[4-5] ALG-001024 was also tested in constructs harboring the known resistance mutations T33N, Y118F and T128I.[4,6] ALG-001024 was affected by T33N and Y118F similarly to GLS4.

A 6-fold shift was observed for mutation T128I for ALG-001024, which is different from GLS4, but in line with reports describing resistance for other class-II CAMs with this mutation.[4,6]

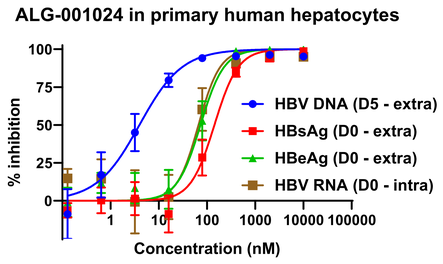
Genotype	Mean EC_{50} (nM)	Number of strains
A	2.97	4
B	2.28	5
C	1.74	4
D	7.37	4
E	25.2	3
F	5.92	5
G	6.27	4
H	8.51	4
I	2.68	2
J	2.04	1

	GLS4		ALG-001024	
	EC_{50} (nM)	Fold shift	EC_{50} (nM)	Fold shift
WT	14.7	1	7.63	1
T33N	3310	225	1682	220
Y118F	45.9	3.11	20.9	2.73
T128I	8.71	0.59	45.7	5.99
n	≥3			

Table 1 – Left: *Average activity of ALG-001024 per genotype (n≥1 for each strain).* ***Right:*** *Overview of the activity of GLS4 and ALG-001024 against construct carrying the indicated mutations.*

ALG-001024 is a potent inhibitor of RNA encapsidation and cccDNA establishment in HBV-infected primary human hepatocytes

When ALG-001024 was added to an established HBV infection in primary human hepatocytes (5 days post infection), HBV DNA production was potently inhibited. In addition, ALG-001024, when added at the time of infection, strongly inhibited cccDNA formation, as shown by reductions in extracellular HBsAg and intracellular HBV RNA.

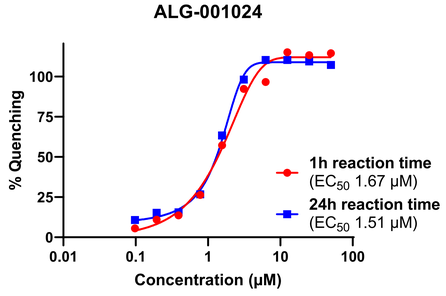


	EC_{50} (nM)	EC_{90} (nM)
HBV DNA	3.97	37.2
HBsAg	143	497
HBeAg	73.5	252
HBV RNA	66.7	207
n	5	

Figure 2: *Dose-response curve for ALG-001024-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-001024 induces rapid assembly of empty capsids in vitro

To prove target engagement, ALG-001024 was incubated with recombinant HBV core protein conjugated with a fluorescent dye. Fluorescence is quenched when capsids are assembled.[2] Binding of ALG-001024 to HBV core induced capsid assembly in less than 3 minutes. Class-II empty capsid formation was confirmed by electron microscopy.



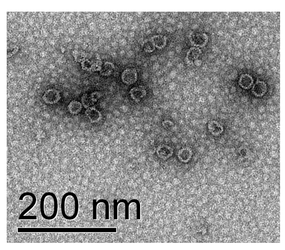


Figure 3 – Left: *Percentage quenching of ALG-001024 vs. DMSO after 1 and 24h.* ***Right:*** *Electron microscopy image of HBV core incubated with ALG-001024. Empty capsids can clearly be distinguished.*

ALG-001024 is a potent inhibitor of HBV DNA production in vivo

The AAV-HBV model was used to assess the efficacy of ALG-001024 in vivo under different dosing regimens.[3] Up to 3 log_{10} IU/ml reduction in HBV DNA was observed after 14 days treatment in the 15 mg/kg BID. No reductions were observed in HBsAg and HBeAg.

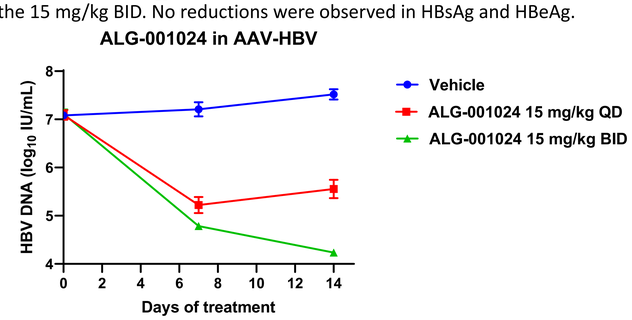
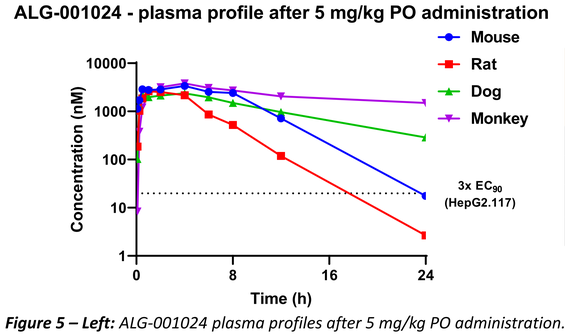


Figure 4: *HBV DNA levels over time in the AAV-HBV mouse. Values represent means ± SEM from 6 animals per group.*

ALG-001024 has good ADME properties

Liver microsome half-life of ALG-001024 was above 60 minutes in all tested species, including human. Plasma protein binding was 95.0% for human. $P_{app\ A->B}$ was 9.4 x 10^{-6} cm/s with an efflux ratio of 0.8. ALG-001024 has no in vitro reactive metabolites, CYP inhibition or induction potential, or off-target kinase and receptor liabilities. ALG-001024 has low clearance and high oral bioavailability with liver exposure being 4- to 8-fold higher than plasma in rodents. ALG-001024 is predicted to have the potential for human QD dosing.



Species	Plasma C_{max} (nM)	Plasma AUC_{inf} (nM.h)
Mouse	3,377	30,063
Rat	2,679	14,484
Dog	2,547	30,640
Monkey	3,808	94,397

Figure 5 – Left: *ALG-001024 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.*

Conclusion

ALG-001024 is a novel class-II CAM demonstrating potent inhibition of both HBV capsid formation and cccDNA formation. ALG-001024 demonstrated potent antiviral activity in the mouse AAV-HBV model. The compound has excellent pharmacokinetic properties consistent with the potential for once-daily dosing in humans.

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.

Financial disclosures

All authors are Aligos employees.