ALG-000184, a prodrug of capsid assembly modulator ALG-001075, demonstrates best-in-class preclinical characteristics for the treatment of chronic hepatitis B

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ALIGOS THERAPEUTICS

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Background

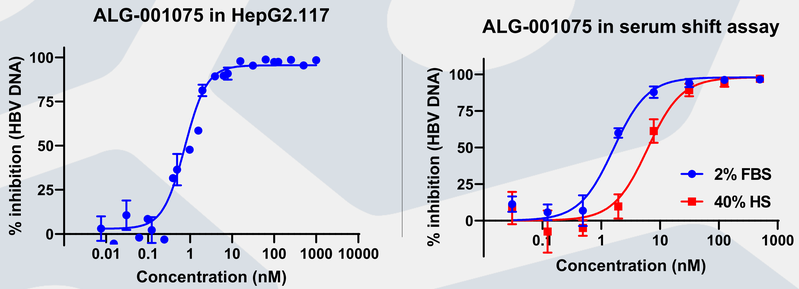
Capsid assembly modulators (CAMs) represent a clinically validated strategy for inhibiting hepatitis B virus (HBV) RNA encapsidation, leading to reductions in circulating HBV DNA and RNA in infected patients. We recently reported on ALG-001075, a novel class-II (normal, empty capsid formed) CAM with excellent antiviral activity and in vivo efficacy in a mouse adeno-associated virus-HBV model (Debing et. al., AASLD, 2019, poster 699). We now advance ALG-000184, a prodrug of ALG-001075, which demonstrates superior pharmacokinetic properties relative to ALG-001075.

Methods

Antiviral activity on HBV DNA was determined in HepG2.117 and HepG2.2.15 cells using quantitative PCR, with and without 40% human serum. Activity was also assessed in primary human hepatocytes (PHH) infected with HBV. Solubility, stability and permeability of ALG-001075 and ALG-000184 were evaluated in vitro. Pharmacokinetic properties of ALG-001075 were evaluated across species following oral dosing of ALG-001075 or ALG-000184 administered as aqueous formulations.

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 11.9-fold shift of the antiviral efficacy of ALG-001075, indicating a moderate impact of plasma protein binding.



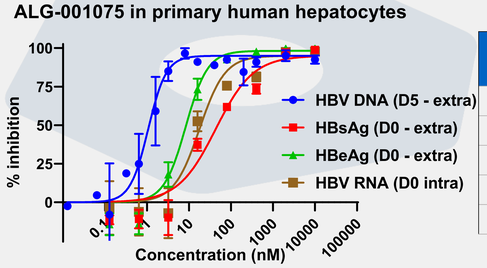
	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)	n
HepG2.117 2% FBS	0.63	3.17	>500	12*
HepG2.117 40% HS	7.48	36.6	n.d.	3
HepG2.2.15 10 % FBS	0.53	1.84	>500	3

(n=10 for cytotoxicity)

Figure 1 – Left: *Representative dose-response curve for ALG-001075-induced inhibition of HBV DNA in HepG2.117.* ***Right:*** *Representative dose-response curves for ALG-001075-induced inhibition of HBV DNA in HepG2.117 in the presence of 2% fetal bovine serum (FBS, blue) or 2% FBS + 40% human serum (HS, red).*

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 synthesis 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 (D5)	1.98	8.56
HBV DNA (D0)	2.68	11.6
HBsAg (D0)	70.0	630
HBeAg (D0)	10.5	45.3
HBV RNA (D0)	54.7	237

Figure 2: *Dose-response curve 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-000184 was developed to achieve high exposure of ALG-001075

Plasma exposure following oral administration of ALG-001075 is limited by its inherent low aqueous solubility. We investigated multiple prodrugs of ALG-001075 and discovered ALG-000184, which retained the superior preclinical properties of ALG-001075 and resulted in significantly greater aqueous solubility delivering enhanced ALG-001075 exposure and excellent dose proportionality.

Compound	SIF Stability	SGF Stability	Caco-2 $P_{app\ A->B}$, ER	Solubility (PBS)
ALG-001075	>12 hours	>12 hours	1.8 x 10^{-6} cm/s, 7.8	<1 mg/mL
ALG-000184	>12 hours	>12 hours	4.6 x 10^{-6} cm/s, 1.9	>120 mg/mL

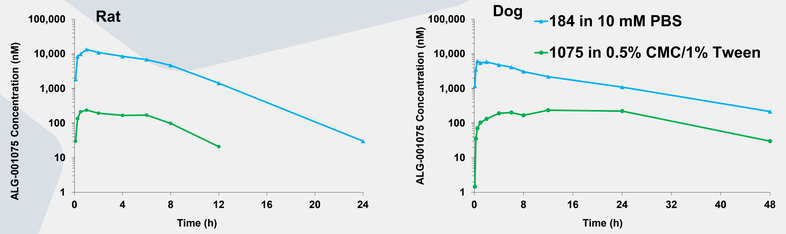


Figure 4 – *ALG-001075 plasma profiles after PO administration of ALG-001075 or ALG-000184 in the indicated species.* ***Left:*** *Rat (30 mg/kg)* ***Right:*** *Dog (5 mg/kg)*

ALG-000184 conversion to ALG-001075 was efficient across species when dosed in aqueous vehicle, achieving linear PK in rats and dogs

Following oral administration of ALG-000184 in aqueous solution, high exposures to ALG-001075 were obtained in preclinical species. The conversion to the parent compound was efficient with ALG-000184 exposure typically <0.2% of ALG-001075 exposure. In rats and dogs, systemic exposures to ALG-001075 increased linearly with dose.

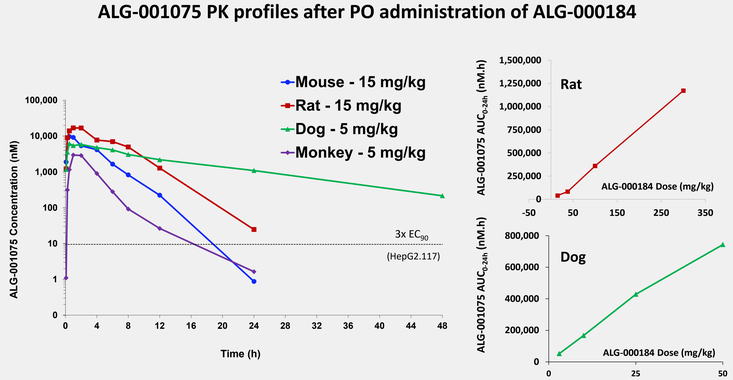


Figure 3 – Left: *ALG-001075 time concentration blood profiles after ALG-000184 PO administration.* ***Right:*** *Dose-linear increase in ALG-001075 exposure in rat (top) and dog (bottom).*

Conclusions

- ALG-001075 is among the most potent class-II CAMs reported to date
- ALG-001075 efficiently blocks both HBV genome encapsidation and de novo cccDNA formation
- Solubility of ALG-001075 was greatly improved by prodrug ALG-000184
- ALG-000184 is a highly soluble prodrug that rapidly and efficiently delivers ALG-001075 following oral dosing
- ALG-000184 is on-track to enter clinical development in Q4 2020

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