Poster # PB-007

Preclinical Antiviral, Pharmacological and Toxicological Characteristics of ALG-000184, a Prodrug of the Novel HBV Capsid Assembly Modulator ALG-001075

Andreas Jekle[1], Sucheta Mukherjee[1], Yannick Debing[2], Dieudonne Buh Kum[1], Hannah Vanrusselt[2], Qingling Zhang[1], Dinah Misner[1], Kha Le[1], Julian Symons[1], Sushmita Chanda[1]

[1]Aligos Therapeutics, Inc., South San Francisco, CA; [2]Aligos Belgium BV, Leuven, Belgium **** contact email: ajekle@aligos.com***

Background: ALG-000184 is a prodrug of ALG-001075, a novel class E capsid assembly modulator (CAM-E). ALG-000184 demonstrated best-in class reductions in hepatitis B virus (HBV) DNA and RNA in chronic hepatitis B (CHB) patients. Importantly, ALG-000184 reduced HBsAg levels in a subset of patients after only 28 days of dosing. Here, we describe the preclinical characteristics of ALG-000184/ALG-001075 including antiviral activity, pharmacological and toxicological assessments.

Materials and Methods: Cell-based antiviral activity was measured using HepG2.117 cells and primary human hepatocytes (PHH). In vivo activity was determined in the AAV-HBV mouse infection model. PK parameters were assessed in multiple preclinical species upon PO (oral) administration. Repeat dose toxicology studies with ALG-000184 were conducted in rats (4-, 13- and 26-weeks) and dogs (4-, 13- and 39-weeks) following daily PO doses and embryo fetal development studies were conducted in rats and rabbits and fertility studies were conducted in rats.

Results 1 - Cell-Based Antiviral Activity of ALG-001075

ALG-001075 and ALG-000184 potently reduced HBV DNA in HepG2.117 cells with EC_{50} values of 0.63 and 1.45 nM, respectively, without causing cytotoxicity up to the highest concentration tested (**Table 1**). ALG-001075 is at least 20-fold more active than other CAM-E (JNJ-632, AB-423) or CAM-A (GLS-4, RO7049389) compounds [1]. In PHH, ALG-001075 reduced HBV-DNA, HBV-RNA, HBsAg and HBeAg, indicating that ALG-001075 engages both the primary (encapsidation of pgRNA) and secondary (cccDNA formation) mechanism of action of CAMs (**Table 2**).

Table 1: Antiviral activity of ALG-001075 and ALG-000184 HepG2.117 cells

Compound	CAM Type	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)
ALG-001075	**E**	**0.63±0.39**	**3.17±3.44**	**>500**
ALG-000184	**E**	**1.45±0.64**	**4.75±1.35**	**>500**
GLS4	A	13.4±6.18	48.7±32.3	>10,000
RO7049389	A	61.8±22.1	249±105	>500
JNJ-632	E	87.0±25.9	219±57.8	>50,000
AB-423	E	54.8±13.5	258±147	46,038

Table 2: Antiviral activity of ALG-001075 in HBV infected primary human hepatocytes

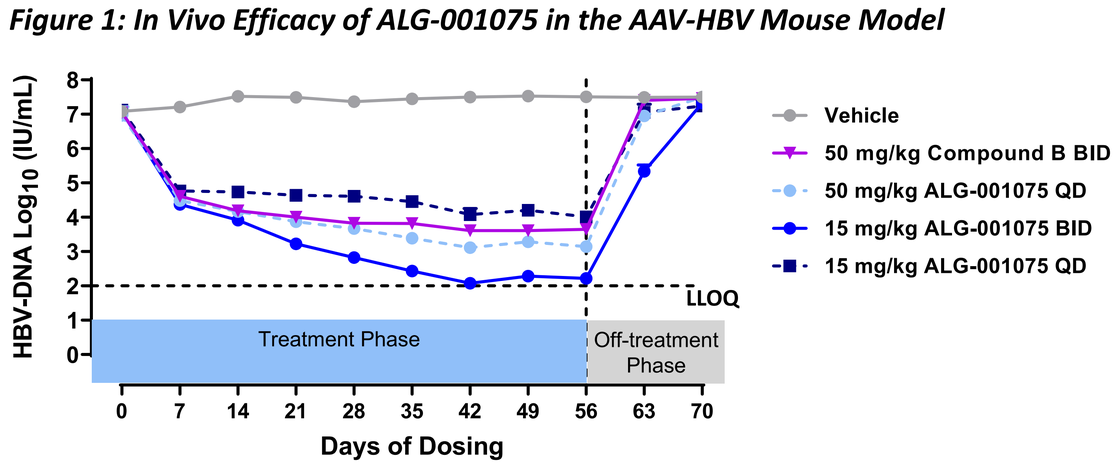
Read-Out	Days post-infection when ALG-001075 was added	EC_{50} (nM)
HBV-DNA[1]	5	1.98±3.10
HBV-DNA[1]	0	2.68±0.61
HBsAg[1]	0	70.0±27.8
HBeAg[1]	0	10.5±5.18
Intracellular HBV-RNA	0	54.7±52.6

1: Analyte measured in cell culture medium

Results 2 - In Vivo Antiviral Activity in the Mouse AAV-HBV Model

The AAV-HBV model was used to assess the efficacy of ALG-001075 in vivo. A dose-dependent reduction in plasma HBV-DNA levels was observed, with a maximum reduction of > 5 log_{10} IU/mL HBV DNA when ALG-001075 was dosed at 15 mg/kg BID for 56 days, with several animals reaching the limit of quantification. HBV-DNA levels returned to baseline levels within 2 weeks after the end of treatment (**Figure 1**). The reduction of plasma HBV-DNA concentrations in the 15 mg/kg ALG-001075 BID group exceeded the class E CAM comparator, Compound B[2] dosed at 50 mg/kg BID. No or only minimal decreases in HBsAg or HBeAg were observed. All compounds were dosed by oral gavage.

Figure 1: In Vivo Efficacy of ALG-001075 in the AAV-HBV Mouse Model



Results 3 - Physicochemical and DMPK Properties of ALG-000184

ALG-000184 demonstrated excellent physicochemical properties with high aqueous solubility, good oral bioavailability, and efficient conversion to ALG-001075 in all species studied to date. High ALG-001075 exposures with liver partitioning (3- to 4-fold in uninfected mice and ≥57-fold in HBV-AAV-infected mice) were achieved after oral dosing of ALG-000184 in aqueous solution.

Table 3: ALG-000184 Demonstrated Good Oral Bioavailability of ALG-001075 in All Nonclinical Species

Species	Mouse	Rat	Rabbit	Dog	Monkey
ALG-00184 dose (mg/kg)	**37.8**	**37.8**	**6.3**	**6.3**	**6.3**
ALG-001075 T_{max} (hr)	0.5	1.0	1.7	1.2	1.3
ALG-001075 C_{max} (ng/mL)	8,214	5,673	703	2,717	1,387
ALG-001075 AUC_{last} (ng.h/mL)	28,984	34,649	4,291	33,385	3,904
ALG-001075 $t_{1/2}$ (hr)	2.08	2.19	5.38	10.4	2.03
%F	70.7	41.6	NA	81.7	35.0

Results 4 - Toxicological Assessment of ALG-000184

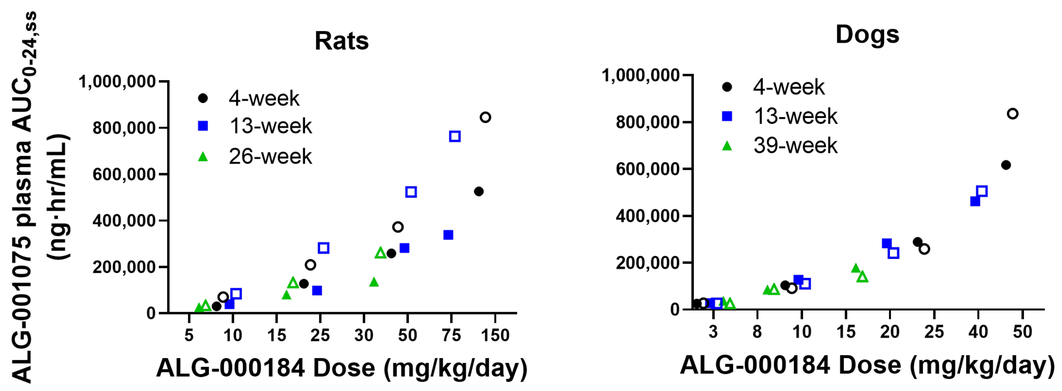
- ALG-000184 demonstrated a favorable safety profile in rats and dogs with monitorable and reversible effects and had no genotoxic potential nor adverse CNS or cardiovascular effects in safety pharmacology studies.
- In repeat dose studies, ALG-000184 was generally well tolerated. Histopathological findings were noted in kidneys (both rats and dogs) and systemic inflammation (reactive histiocytosis) in dogs at multiples of clinically efficacious plasma exposures where significant inhibition of HBV DNA was noted in CHB patients.
- High and linear increases in ALG-001075 plasma exposure were noted over the toxicology dose ranges studied, providing a significant exposure margin to clinical doses.
- ALG-000184 had no effects on embryofetal development in rats or rabbits or on fertility in either male or female rats up to the highest doses tested

Table 4: ALG-000184 Demonstrated a Favorable Toxicology Profile

Species	Duration	ALG-000184 Doses (mg/kg/day)	NOAEL Steady-State ALG-001075 AUC_{0-24} (ng·hr/mL)
Rat	26-week	0 (vehicle), 5, 15, **30**	199,000
Dog	39-week	0 (vehicle), 3, 7.5, **15**	160,000

NOAEL: No Observed Adverse Effect Level (underlined)

Figure 2: Dose Proportional Increases in ALG-001075 Plasma Exposures with Little to No Accumulation in Repeat Dose Toxicity Studies



Males represented in solid symbols and females in open symbols for both species

Conclusions:

- **ALG-001075 demonstrated potent antiviral activity in cell-based assays and the AAV-HBV mouse model.**
- **ALG-001075 engages both the primary (encapsidation of pgRNA) and secondary (cccDNA formation) MoA of CAMs in PHH.**
- **ALG-000184 has favorable physicochemical and DMPK properties.**
- **Based on the potent antiviral activity of ALG-001075 in vitro and in vivo, the high projected human C_{trough} levels of ALG-001075 and the favorable safety profile in preclinical species, ALG-000184 is currently advancing through Phase I clinical testing in CHB patients.**

Reference: (1): Zoulim F. et al., Nat rev Gastroenterol & Hepatol , 2022; (2) Lenz O. et al., WO2019175657A1, 2019

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