AASLD November 13-16, 2020
The Liver Meeting® Digital Experience

Poster #0823

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

Andreas Jekle[1], Qingling Zhang[1], Vladimir Serebryany[1], Michael Welch[1], Jyanwei Liu[1], Sandrine Vendeville[2], Yannick Debing[2], Dieudonne Buh Kum[2], Suping Ren[1], Cheng Liu[1], Jerome Deval[1], Dinah Misner[1], Sushmita Chanda[1], Pierre Raboisson[2], Julian A. Symons[1], Lawrence M. Blatt[1], Leonid Beigelman[1] and Dave B. Smith[1]

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

ALIGOS THERAPEUTICS

Background

Capsid assembly modulators (CAMs) represent a clinically validated strategy for the treatment of chronic hepatitis B. We recently reported on ALG-001075, a novel class-II CAM with excellent antiviral potency and in vivo efficacy in a mouse AAV-HBV model. Here, we describe the in vitro antiviral profile and ADME characteristics of ALG-000184, a prodrug of ALG-001075.

Methods

Antiviral activity on HBV DNA was determined using qPCR. The biochemical characteristics were studied using electron microscopy, size-exclusion chromatography and a fluorescence quenching assay. PK properties of ALG-001075 were evaluated in dogs following oral dosing of ALG-000184 administered as solutions in organic or aqueous vehicles or as tablets.

ALG-001075 has nanomolar antiviral activity in cell-based assays

In cell-based assays using HepG2.2.15 and HepG2.117 cells, ALG-001075 and ALG-000184 HBV viral replication with (sub) nanomolar activity (***Table 1***). HBV-DNA quantification was used to determine antiviral activity. Both compounds did not induce cytotoxicity at the highest concentration tested (500 nM). In side-by-side comparisons, ALG-001075 was substantially more active than other class I and II CAM reference compounds such as GLS4, RO7049389, JNJ-632 and AB-423. The potent inhibition of HBV replication by the prodrug ALG-000184 indicates that it is efficiently metabolized to the parent ALG-001075 inside the cells. Subsequent experiments were therefore performed only with ALG-001075.

Table 1: Antiviral activity and cytotoxicity of ALG-001075 and its prodrug ALG-000184 compared with reference CAMs in HepG2.2.15 and HepG2.117 cells

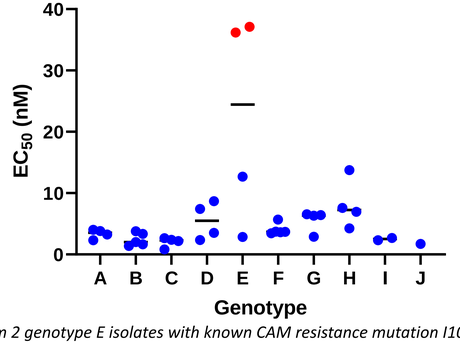
Compound	HepG2.2.15			HepG2.117		
	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)
ALG-001075	0.53 ± 037	1.84 ± 1.39	> 500	0.63 ± 0.39	3.17 ± 3.44	> 500
ALG-000184	ND	ND	ND	1.37 ± 0.73	4.98 ± 1.61	> 500
GLS4	3.52 ± 0.61	11.6 ± 5.30	> 1000	13.4 ± 6.18	48.7 ± 32.3	> 10,000
RO7049389	4.17 ± 0.08	16.5 ± 2.50	> 50,000	61.8 ± 22.1	249 ± 105	> 500
JNJ-632	ND	ND	ND	87.0 ± 25.9	219 ± 57.8	> 50,000
AB-423	ND	ND	ND	54.8 ± 13.5	258 ± 147	46,035

ND: not determined

ALG-001075 displays broad-spectrum antiviral activity against HBV genotypes A to J

ALG-001075 demonstrated broad antiviral activity when tested against 37 clinical isolates covering the HBV genotypes A to J. The mean EC_{50} against all 37 isolates was 6.11±7.94 nM (range; 0.80 nM to 37.12 nM). Exclusion of 2 genotype E isolates with the known CAM resistance mutation I105T resulted in a mean EC_{50} against the remaining 35 isolates of 4.44±2.95 nM (range: 0.80 nM to 13.76 nM) and a mean EC_{50} of <10 nM against each of the ten genotypes (***Figure 1***). These results indicate that ALG-001075 has broad antiviral activity against all HBV genotypes.

Figure 1: Broad-spectrum antiviral activity of ALG-001075 against 37 clinical isolates from HBV genotypes A to J. HBV sequences were and cloned into the pcDNA3.1 vector as a 1.1mer and transiently transfected into HepG2 cells. Intracellular HBV-DNA was quantified to calculate antiviral activity.

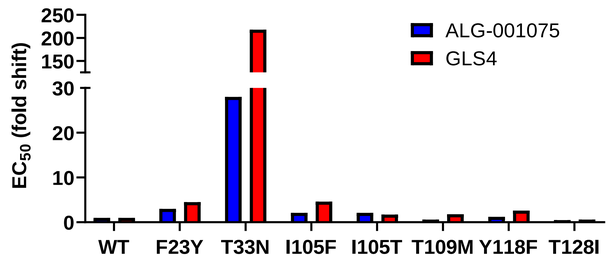


** Results from 2 genotype E isolates with known CAM resistance mutation I105T highlighted in red*

Resistance profile of ALG-001075

ALG-001075 retained antiviral activity when tested against the CAM resistance mutations F23Y, I105F, I105T, T109M, Y118F and T128I (0.5- to 3.0-fold shifts). In contrast, T33N reduced the antiviral activity of ALG-001075 28-fold (***Figure 2***). The CAM I reference compound GLS4 showed a similar resistance profile.

Figure 2: Antiviral activity of ALG-001075 and GLS4 against known CAM resistance mutations. Resistance mutations were cloned in the genotype D U95551 background (WT), and transiently transfected into HepG2 cells. Intracellular HBV-DNA was quantified to determine the antiviral activity. The fold shift was calculated by dividing the EC_{50} of a given mutation by the EC_{50} of the wildtype.



ALG-001075 retains activity against Nucleos(t)ide resistance mutations

ALG-001075 retains antiviral activity against mutations in the HBV polymerase conferring resistance to nucleos(t)ide inhibitors such as rtN236T and rtM204I or mutation combinations such as rtL180M+M204V+M250V+I169T and rtL180M+M204V+T184G+S202I.

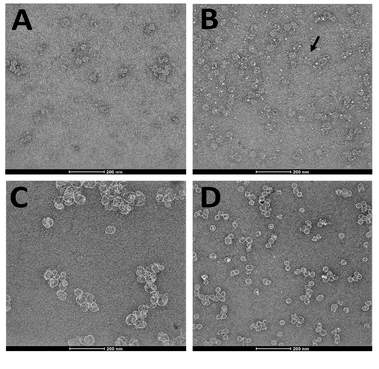
Table 2: Antiviral activity of ALG-001075 and nucleos(t)ide inhibitors tenofovir disoproxil fumarate (TDF), entecavir (ETV), and lamivudine (3TC) against known nucleos(t)ide resistance mutations. Resistance testing was performed as described in Figure 2. Green shading indicates no or minimal shift (<3X), orange moderate shift (5 to 20x) and red substantial shift (> 20x).

	ALG-001075	TDF	ETV	3TC
	EC50 (fold shift)			
Wildtype	1.0	1.0	1.0	1.0
rtN236T	1.3	2.9	ND	ND
rtM204I	1.3	5.8	ND	ND
rtL180M+M204V+M250V+I169T	2.3	ND	247	368
rtL180M+M204V+T184G+S202I	2.1	1.1	>450	>45

ALG-001075 induces a class II CAM phenotype

CAMs fall into two functional classes: Class I CAMs provoke the formation of aberrant high-order structures, while Class II CAMs induce assembly of morphologically intact, but empty, capsids (Berke et al. 2017; Zhang et al. 2019). Transmission electron microscopy (***Figure 3***) showed that ALG-001075 induces the formation of small (~30 nm) and evenly shaped spherical particles consistent with a CAM I phenotype.

Figure 3: Electron microscopy performed on recombinant core protein incubated alone (A) or in presence of Class II CAM JNJ-632 (B), Class I CAM GLS4 (C) or ALG-001075 (D). In the absence of a CAM, core protein formed few empty capsids with a diameter of ~ 30 nM (***A***). The Class II CAM JNJ-632 induced a mixture of small and evenly shaped spherical HBV capsids (~30 nm diameter) and other heterogenous particles of partially assembled capsids as indicated with arrow (***B***). In the presence of the Class I CAM GLS4, large aggregates with a diameter of ~50-60 nm are formed (***C***). ALG-001075 in the presence of Cp150 induced the formation of empty capsids with a dimeter of ~30 nm, indicative of the Class II CAM phenotype (***D***).



High aqueous solubility enabled good oral absorption of ALG-000184 in various formulations

ALG-000184 had an aqueous solubility >120 mg/mL. This prodrug was stable in simulated gastric and intestinal fluids (SGF and SIF). It also demonstrated overall high permeability and low efflux ratio across Caco-2 cells. Following oral administrations, ALG-000184 was converted to ALG-001075 efficiently (exposure typically <0.2% of ALG-001075) and resulted in high exposures to ALG-001075 in preclinical species.

In dogs following oral dose of ALG-000184, ALG-001075 exposure increased dose proportionally from 1 to 12.6 mg/kg regardless of the formulation as organic or aqueous solutions or as a tablet. Further increase of dose resulted in a greater than dose-proportional increase of the exposure. When dosed as tablets, oral bioavailability values in terms of ALG-001075 exposure in comparison to that following IV dose of ALG-001075 ranged between 93.3% to 108%.

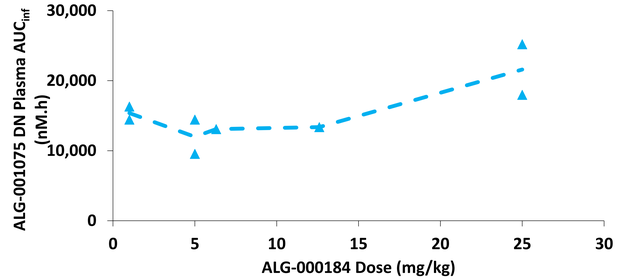


Figure 4: ALG-001075 plasma exposure (dose-normalized AUC_{inf}) in dogs vs. ALG-000184 dose. ALG-000184 was formulated in high organic solution, aqueous solution, or tablets.

Food intake had minimal effect on systemic ALG-001075 exposure in dogs following ALG-000184 oral administration in an aqueous solution

The high aqueous solubility of ALG-000184 reduced food effect. High fat diet delayed oral absorption of ALG-000184, as shown by ALG-001075 a longer T_{max} (from 0.83 to 2.67 hr). However, it only reduced ALG-001075 C_{max} and AUC_{inf} by 23% and 16%, respectively.

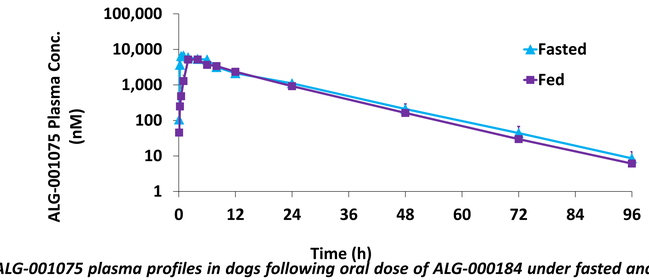


Figure 5: ALG-001075 plasma profiles in dogs following oral dose of ALG-000184 under fasted and fed conditions. Dogs were given ALG-000184 at 5 mg/kg in aqueous solution under fasted or fed with high fat diet conditions. This was a cross-over design with >7 days of washout period between dosing phases.

Conclusions:

ALG-001075 is a Class II CAM with potent and broad antiviral activity covering all genotypes A to J. ALG-001075 has a moderate 28-fold loss of activity against the known CAM resistance mutation T33N but retained activity against other CAM as well as nucleos/tide resistance mutations.
There was no marked food effect following oral administration of ALG-000184 in dogs. ALG-000184 in a tablet formulation resulted in complete oral absorption and high exposure to ALG-001075 in dogs. ALG-000184 is currently advancing in development as a potential best-in-class CAM.

References: Berke JM, et al. Antimicrob Agents Chemother 2017;61(8):e00560-17; Zhang X et al., ACS Infect Dis 2019;5(5):759–68

Financial Disclosure: All authors are employees or officers of Aligos Therapeutics, Inc.