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Preclinical Resistance Profile and Antiviral Activity of the Best-in-Class CAM-E ALG-001075, the Parent Compound of ALG-000184

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Background

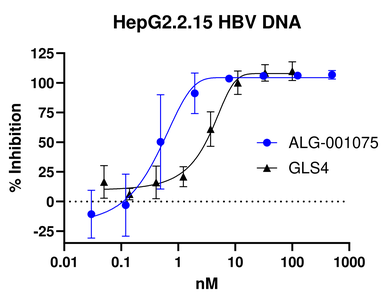
The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic hepatitis B (CHB). ALG-000184 is a prodrug of ALG-001075, a novel capsid assembly modulator leading to the formation of empty capsids (CAM-E). ALG-000184 has demonstrated best-in-class reduction of HBV-DNA, HBV-RNA and HBsAg in CHB patients. Here, we provide an update on the preclinical antiviral activity and viral resistance profile of ALG-001075.

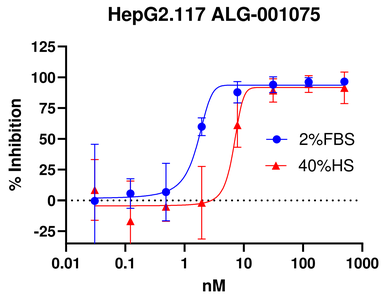
Methods

Antiviral activity on HBV DNA was determined in HepG2.2.15 and HepG2.117 cells using quantitative PCR, with and without 40% human serum. HepG2 cells were transiently transfected with plasmids encoding an overlength HBV genome from genotypes A-J, or containing amino acid mutations known to confer viral resistance to CAMs and nucleos(t)ide analogs.

ALG-001075 is a potent inhibitor of HBV DNA synthesis

The HepG2.2.15 and HepG2.117 cell lines contain a stably integrated genotype D HBV genome. ALG-001075 was highly effective in reducing the amount of HBV DNA produced, with sub-nanomolar EC_{50} values, and EC_{90} values of 1.84 and 3.17 nM in the respective cell lines. Addition of 40% human serum to the culture medium resulted in a ~5-fold shift of the antiviral activity of ALG-001075, indicating a moderate impact of plasma protein binding. In the HepG2.117 cell line, ALG-000184, the prodrug of ALG-001075, inhibits HBV replication with EC_{50} and EC_{90} values of 1.45 and 4.75 nM, respectively. The CC_{50} was > 500 nM for both ALG-001075 and ALG-000184.





HepG2.2.15	EC_{50} (nM)[1]	EC_{90} (nM)[1]
ALG-001075	0.53±0.37	1.84±1.39
GLS4	3.52±0.61	11.62±5.30
RG7909	4.17±0.08	16.5±2.50

[1] mean ± SD, n≥3

HepG2.117	EC_{50} (nM)[1]	EC_{90} (nM)[1]
ALG-001075	0.63±0.39	3.17±3.44
ALG-000184	1.45±0.64	4.75±1.35
GLS4	13.4±6.18	48.7±32.3
RG7909	61.8±22.1	249±105
JNJ-632	87.0±25.9	219±57.8
JNJ-6379	34.4±5.52	119±36.0
AB-423	54.8±13.5	258±147

[1] mean ± SD, n≥3

Figure 1 – Left: *ALG-001075 (blue) and GLS4 (black) dose-response curves for inhibition of HBV DNA in HepG2.2.15 cells; Values represent mean ± SD from at least 3 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, blue) or 2% FBS + 40% human serum (HS, red). Values represent mean ± SD from 3 independent experiments.*

ALG-001075 has broad antiviral activity against HBV genotypes A-J

The breadth of antiviral activity of ALG-001075 was tested in HepG2 cells transiently transfected with a vector encoding a 1.1mer of the HBV genome from clinical isolates covering genotypes A-J.

ALG-001075 inhibited 37 clinical isolates from genotypes A-J with an EC_{50} of 6.11±7.94nM (mean±SD). Excluding the two genotype E isolates LP797739 and HE974384, which harbor the known CAM resistance mutation I105T in core, the EC_{50} of ALG-001075 against the remaining 35 isolates was 4.44±2.95 nM, ranging from 0.80 to 13.76 nM.

Genotype	Number of isolates	EC_{50} (nM) Mean ± SD	
		ALG-001075	Entecavir
A	4	3.35±0.77	2.70±1.46
B	5	2.43±1.07	2.59±1.88
C	4	2.00±0.83	1.82±1.01
D	4	5.49±3.03	3.40±2.91
E	4	22.21±17.16	6.35±1.16
E*	2	7.77±6.95	5.49±0.80
F	5	4.03±0.94	1.52±0.52
G	4	5.54±1.78	2.45±0.80
H	4	8.13±4.02	3.94±2.22
I	2	2.50±0.24	2.98±0.07
J	1	1.70	5.83
Total	**37**	**6.11±7.94**	**3.11±2.02**
Total*	**35**	**4.44±2.95**	**2.87±1.80**

* Excluding two isolates harboring the I105T mutation in core

ALG-001075 retains antiviral activity against known nucleos(t)ide resistance mutants

The antiviral activity of ALG-001075 was tested in HepG2 cells transiently transfected with a vector encoding a 1.1mer of the HBV genome harboring known nucleoside/nucleotide resistance mutations.

ALG-0001075 retained antiviral activity against rtN236T and rtM204I, which reduced the activity of TDF (tenofovir disoproxil fumarate) ~ 3- and 6-fold, respectively. Similarly, the rtL180M+M204V+M250V+I169T and rtL180M+M204V+T184G+S202I mutations did not affect the antiviral activity of ALG-001075 but reduced the activity of entecavir and lamivudine (3TC) ≥ 45-fold.

Mutation	EC_{50} (nM)[1] Mean ± SD (Fold-shift)			
	ALG-001075	TDF	Entecavir	3TC
Wildtype	4.38±1.39	6.63±126	ND	ND
rtN236T	5.77±1.05 (1.3x)	19.0±4.26 (2.9x)	ND	ND
rtM204I	5.63±1.86 (1.3X)	38.31±14.2 (5.8x)	ND	ND
Wildtype	4.93±0.97	9.00±4.09	5.26±0.80	222±80.2
rtL180M+M204V+ M250V+I169T	11.5±4.07 (2.3x)	ND	1300±891 (247x)	>100,000 (>450x)
rtL180M+M204V+ T184G+S202I	10.3±1.69 (2.1x)	9.59±4.74 (1.1x)	1933±1432 (368X)	>10,000 (>45x)

[1]: n≥3.rt: reverse transcriptase; ND: not determined

Antiviral activity of ALG-001075 against core resistance mutations

The antiviral activity of ALG-001075 was tested in HepG2 cells transiently transfected with a vector encoding a 1.1mer of the HBV genome harboring known CAM resistance mutations in core. ALG-001075 retained antiviral activity against the majority of core resistance mutations, with a fold shift ≤ 3.0-fold. T33N is to date the only known core resistance mutation causing a major loss in activity of ALG-001075 of ~ 28-fold, which is considerably lower than the fold shifts reported for other CAMs. Minor 7.2- and 6.1-fold losses of activity were observed with T33P and V124G, respectively.

Mutation	Natural prevalence (%) #	fold shift (compared to WT, based on EC_{50})		
		ALG-001075	GLS4	JNJ-6379
F23Y	0.1	3.0	4.5	ND
P25A	-	1.3	ND	2.4
T33N	0.03	26.9/28.0 *	218	65
T33P	0.01	7.2	ND	11.2
L37Q	0.03	2.3	ND	8.2
Y38F	3.8	1.2	1.1	ND
I105F	1.7	2.1	4.6	ND
I105L	0.7	0.7	0.3	ND
I105T	0.7	2.1	1.7	ND
T109I	0.2	0.6	4.7	ND
T109M	0.8	0.6	1.8	ND
F110I	0.01	2.4	ND	11.5
V118F	0.3	1.2	2.6	ND
V124G	0.04	6.1	0.7	ND
T128I	0.03	0.5	0.6	ND

Verbinnen T et al., 2020; * T33N was tested in two different assay systems; ND: not determined

The I105T mutation was shown to be naturally present in the two genotype E isolates LP797739 and HE974384. To address the impact of I105T on the antiviral activity of ALG-001075, this mutation was introduced into the genotype D isolate U95551 and the I105T mutation in the genotype E isolate HE974384 was reverted back to I105. Introduction of the I105T mutation into the U95551 background increased the EC_{50} of ALG-001075 and GLS4 approximately 2-fold while reversion of the I105T mutation from the HE974384 background reduced the EC_{50} approximately 2-fold.

		U95551 WT	U95551 I105T	HE974384 WT	HE974384 T105I
ALG-001075	EC_{50} (nM)[1]	5.20±2.48	10.8±1.90	31.6±6.82	16.4±13.3
	fold shift	1.0x	2.1x	1.0x	0.5x
GLS4	EC_{50} (nM)[1]	14.4±1.89	23.8±1.33	95.0±49.6	47.8±30.2
	fold shift	1.0x	1.7x	1.0x	0.5x

[1] mean ± SD, n≥3

Conclusions

With sub-nanomolar EC_{50} activity in cell-based assays, ALG-001075 is among the most potent CAM-Es reported to date. The compound demonstrated pan-HBV activity against genotypes A-J. ALG-001075 has a favorable resistance profile with T33N identified to date as the only core resistance mutation causing a major loss of activity.

ALG-000184, the prodrug of ALG-001075, is currently advancing through clinical development, where it has demonstrated best-in-class reductions of HBV DNA, RNA and HBsAg. Please visit poster # 1483 for further details.

Financial disclosures

All authors except AAS are Aligos Therapeutics, Inc., employees and may own Aligos stock.