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CAPSID ASSEMBLY MODULATORS SUCH AS ALG-001075 INDUCE PROFOUND HBV DNA KNOCKDOWN AND DIRECTLY TARGET HBEAG IN VITRO

Cheng Liu[1], Jordi Verheyen[2], Dieudonné Buh Kum[1], Hannah Vanrusselt[2], Tse-I Lin[2], Julian Symons[1], Lawrence Blatt[1], Andreas Jekle[1] and Yannick Debing[2]
[1] Aligos Therapeutics, South San Francisco, CA; [2] Aligos Belgium, Leuven, Belgium.

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BACKGROUND

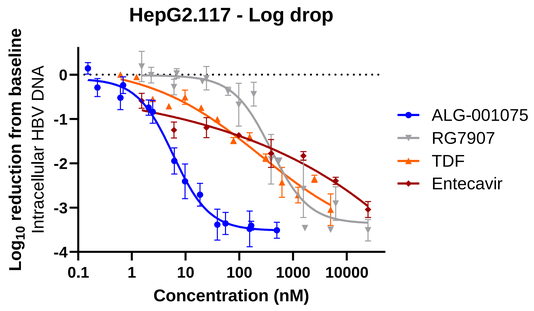
The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic hepatitis B (CHB).[1] ALG-000184 is a prodrug of ALG-001075, a novel capsid assembly modulator leading to the formation of empty capsids (CAM-E).[2] ALG-000184 has demonstrated best-in-class reductions of HBV DNA, RNA, HBsAg, HBcrAg and HBeAg in CHB patients.[3] Here, we reproduce the superior HBV DNA reductions over the nucleoside analog entecavir in vitro and show that CAMs directly target HBeAg.

METHODS

HBV DNA knockdown was assessed in HBV-expressing HepG2.117 cells with additional DNase digestion steps to remove non-encapsidated HBV DNA, allowing for the determination of EC_{90}, EC_{99} and $EC_{99.9}$ values (log drop assay). Cellular effects on HBeAg expression were studied using transient HBeAg expression in Huh7 cells, with a plasmid encoding either wild-type or T33N-mutated HBeAg. Interactions between CAMs and purified HBeAg were further investigated using microscale thermophoresis (MST), spectral shift, and size exclusion chromatography with multi-angle light scattering (SEC-MALS).

ALG-001075 REDUCES HBV DNA PRODUCTION BY MORE THAN 3 LOG_{10}

The HepG2.117 cell line contains a stably integrated genotype D HBV genome and is highly suitable to assess effects on HBV DNA production.[4] DNase digestion steps were included to remove background genomic/integrated HBV DNA and to exclusively detect encapsidated HBV DNA by qPCR, allowing to determine accurate EC_{90}, EC_{99} and $EC_{99.9}$ values. ALG-001075 proved highly effective in this regard with EC_{90}, EC_{99} and $EC_{99.9}$ values of 2.65, 6.99 and 22.3 nM, compared to 8.56, 1,480 and 26,700 nM for nucleoside analogue entecavir, respectively (Figure 1). ALG-001075 was also considerably more potent than tenofovir disoproxil fumarate (TDF) and reference CAM RG7907.[5]

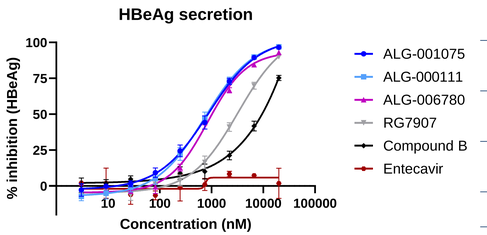


HepG2.117	EC_{90} (nM)	EC_{99} (nM)	$EC_{99.9}$ (nM)
ALG-001075	2.65	6.99	22.3
RG7907	192	528	2,150
Entecavir	8.56	1,480	26,700
TDF	30.9	378	6,110

Figure 1: Dose-response curves for ALG-001075-induced inhibition of HBV DNA in HepG2.117 cells in the log drop assay setup. Values represent mean ± SEM from ≥3 independent experiments.

ALG-001075 AND OTHER CAMS STRONGLY REDUCE HBEAG SECRETION

Given the fast initial HBeAg declines induced by ALG-000184 in CHB patients[3] and the closely related structures of HBc and HBeAg, we investigated if ALG-001075 and other CAMs[5-8] could also directly target HBeAg. Hereto, Huh7 cells were transiently transfected with an HBeAg-expressing plasmid and treated with different CAMs and nucleoside analogue entecavir. All tested CAMs but not entecavir reduced HBeAg levels in cell culture in a dose-dependent with ALG-001075, CAM-E ALG-000111[7] and non-HAP CAM-A ALG-006780[8] doing so most potently (Figure 2). Concentrations required for HBeAg inhibition were considerably higher than those required for HBV DNA inhibition.

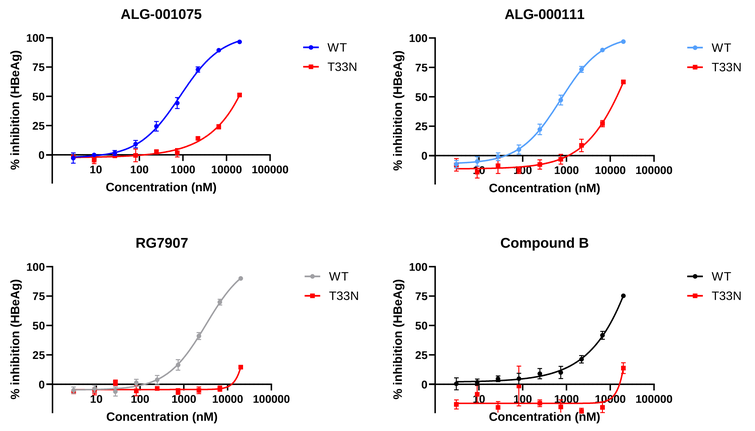


nM	HBeAg EC_{50}	HBV DNA EC_{50}*
ALG-001075	855	0.64
ALG-000111	814	0.99
ALG-006780	1,020	7.76
RG7907	3,120	65.6
Compound B[6]	9,470	8.81
Entecavir	>20,000	0.58

Figure 2: Dose-response curves for compound-induced inhibition of HBeAg levels in cell culture medium of HBeAg-overexpressing Huh7 cells. Values represent mean ± SEM from ≥3 independent experiments. * HBV DNA EC_{50} as determined in HepG2.117 cells.

HBEAG INHIBITION BY CAMS IS SENSITIVE TO KNOWN CAM HBC RESISTANCE MUTATION T33N

To investigate whether the CAM effect on HBeAg is mediated by a similar binding mode as its modulation of HBc assembly, a plasmid encoding HBeAg carrying the well-characterized T33N CAM resistance mutation[9] was transfected into Huh7 and treated with different CAMs. T33N resulted in a pronounced right shift of the dose-response curve (Figure 3). This suggests the observed effect is likely due to direct binding of CAMs to HBeAg.



HBeAg EC_{50} (nM)	ALG-001075	ALG-000111	RG7907	Compound B
WT	855	814	3,120	9,470
T33N	19,400	14,100	>20,000	>20,000

Figure 3: Dose-response curves for compound-induced inhibition of HBeAg levels in cell culture medium of plasmid-transfected Huh7 cells, either wild-type (WT) or containing the T33N mutation. Values represent mean ± SEM from ≥2 independent experiments.

CAMS DIRECTLY BIND TO HBEAG IN MST AND SPECTRAL SHIFT ASSAYS

Further evidence for direct binding of CAMs to HBeAg was obtained using biophysical MST and spectral shift assays on NT-650-maleimide-labeled wild-type HBeAg. CAM-E ALG-000111 yielded K_D values of 2.14 and 1.73 µM in MST and spectral shift, respectively; whereas CAM-A ALG-006780 showed K_D values of 1.12 and 0.81 µM (Figure 4). The nucleoside analogue entecavir showed no binding, as expected.

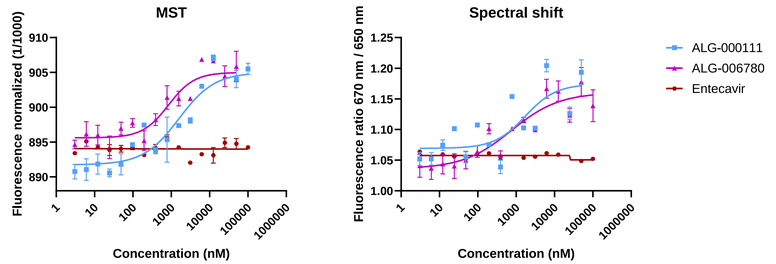


Figure 4: Dose-response curves for wild-type HBeAg binding in MST (left) and spectral shift (right). Values represent mean ± SEM from 2 independent runs.

CAM-A AND CAM-E INDUCE DIFFERENT EFFECTS ON HBEAG IN SEC-MALS

To understand the effect of CAM binding to HBeAg, wild-type HBeAg was incubated with 1% DMSO or 100 µM of ALG-000111 or ALG-006780 and analyzed by SEC-MALS. HBeAg in the DMSO control condition showed a retention volume of 14.7 mL and a calculated molecular weight of 40.8 kDa, indicating the expected dimeric state for HBeAg. Treatment with CAM-E ALG-000111 slightly decreased the retention volume to 14.4 mL but increased the calculated molecular weight to 56.3 kDa, suggesting a clear impact of CAM binding to HBeAg. CAM-A ALG-006780 showed intrinsic absorption at 280 nm, explaining the main peak at retention volume 28.1 mL (exceeding the column volume). However, the broad peak at lower volumes suggest that HBeAg in the presence of ALG-006780 elutes in non-specific protein species, potentially differently-sized soluble aggregates. This is in line with its CAM-A properties, inducing aggregation of not just HBc but also HBeAg.[5,10]

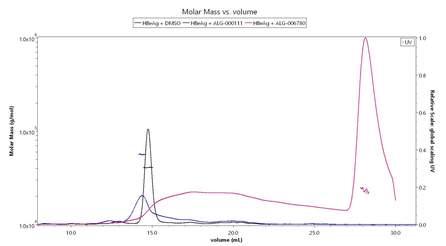


Figure 5: Elution profiles for HBeAg in the presence of DMSO (black), ALG-000111 (blue) or ALG-006780 (pink) in SEC-MALS analysis. Curly lines represent the calculated molar mass.

CONCLUSION

ALG-001075 demonstrated highly pronounced in vitro reductions in HBV DNA, in line with clinical observations for its prodrug ALG-000184.[3] It also showed specific inhibition of HBeAg secretion, which may be due to direct HBeAg binding. ALG-000184, the prodrug of ALG-001075, is currently advancing through clinical development, where it demonstrated best-in-class reductions of HBV DNA, RNA, HBsAg and HBeAg. Please visit poster # 1213 for further details.

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