Best-in-class preclinical characteristics of ALG-000184, a prodrug of the capsid assembly modulator ALG-01075 for the treatment of chronic hepatitis B

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Background
Capсид assembly modulators (CAMS) represent a clinically validated strategy for the treatment of chronic hepatitis B. We recently reported on ALG-01075, 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-010184, a prodrug of ALG-01075.

Methods
Antiviral activity on HBV DNA was determined using qPCR. The biochemical characteristics were studied using electron microscopy, size-exclusion chromatography and fluorescence microscopy. PC properties of ALG-01075 were evaluated in dogs following oral dosing of ALG-010184 administered as solutions in organic or aqueous vehicle or as suspensions.

ALG-01075 has nanomolar antiviral activity in cell-based assays

In cell-based assays using HepG2.2.15 and HepG2.117 cells, ALG-01075 and ALG-010184 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. For side comparisons, ALG-01075 was substantially more active than other class I and II CAM reference compounds such as GLS4, RO194389, JN1-62 and AS-423. The potent inhibition of HBV replication by the prodrug ALG-010184 indicates that it is efficiently metabolized to the parent ALG-01075 inside the cells. Subsequent experiments were therefore performed only with ALG-01075.

Table 1: Antiviral activity and cytotoxicity of ALG-01075 and its prodrug ALG-010184 compared with reference CAMs in HepG2.2.15 and HepG2.117 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>EC100 (nM)</th>
<th>CC50 (nM)</th>
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<tbody>
<tr>
<td>ALG-01075</td>
<td>53.0 ± 0.37</td>
<td>184.1 ± 13.9</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>ALG-000184</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GLS4</td>
<td>3.52 ± 0.61</td>
<td>11.6 ± 5.30</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>RO7043889</td>
<td>4.17 ± 0.08</td>
<td>16.5 ± 2.50</td>
<td>&gt; 50,000</td>
</tr>
<tr>
<td>JN1-62</td>
<td>ND</td>
<td>ND</td>
<td>87.0 ± 25.9</td>
</tr>
<tr>
<td>AB-423</td>
<td>ND</td>
<td>ND</td>
<td>54.8 ± 13.5</td>
</tr>
</tbody>
</table>

Figure 1: Broad-spectrum antiviral activity of ALG-010184 against 37 clinical isolates from HBV genotypes A to J. HBV sequences were cloned into the pDONR1 vector as a 1:1mer and transiently transfected into HepG2 cells. Intracellular HBV DNA was quantified to calculate antiviral activity.

ALG-01075 displays broad-spectrum antiviral activity against HBV genotypes A to J

ALG-01075 demonstrated broad antiviral activity when tested against 37 clinical isolates covering the HBV genotypes A to J. The mean EC50 against all 37 isolates was 6.137±9.4 nm (range: 0.80 nm to 37.12 nm). Exclusion of 2 genotype I isolates with the known CAM resistance mutation I105T resulted in a mean EC50 against the remaining 35 isolates of 4.44±2.95 nm (range: 0.80 nm to 13.76 nm) and a mean EC100 of ≤10 nm against each of the ten genotypes (Figure 2). These results indicate that ALG-01075 has broad antiviral activity against all HBV genotypes.

Figure 2: Resistance profile of ALG-01075

ALG-01075 retains activity against Nucleos(t)ide resistance mutations

ALG-01075 retains antiviral activity against the HBV polymerase conferring resistance to nucleos(t)ide inhibitors such as rtN362M and rtM204I or rtM204C analogs or mutations that are included in the G180N panel. However, ALG-01075 retains >50% of its activity against the rtM204I panel (Figure 3). This result indicates that ALG-01075 has broad antiviral activity against nucleos(t)ide resistance mutations.

Figure 3: Electron microscopy performed on recombinant core protein protein incubated alone (A) or in presence of Class II CAM JN1-62 (B), Class I CAM GLS4 (C) or ALG-01075 (D). In the absence of a CAM, the capsid is homogenous consisting of multiple capsomers with a diameter of ~30 nm (A). The Class I CAM JN1-632 induced a mixture of small and evenly shaped spherical capsids (~30 nm diameter) and other heterogeneous particles of partially assembled capsids as indicated with arrow (B). In the presence of Class II CAM GLS4, large aggregates with a diameter of >50-60 nm are formed (C). ALG-01075 in the presence of GLS150 induced the formation of empty capsids with a diameter of ~30 nm, indicative of the Class II CAM phenotype (D).

ALG-01075 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-01075 induces the formation of small ~30 nm and evenly shaped spherical particles consistent with a CAM I phenotype.

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

ALG-01075 had an aqueous solubility >120 mg/mL. This product was stable in simulated gastric and intestinal fluids (50% FBS and gave demonstrated overall high permeability and low efflux ratio across Caco-2 cells. Following oral administrations, ALG-000184 was converted to ALG-01075 efficiently (exposure typically >20% of ALG-01075) and resulted in high exposures to ALG-01075 in preclinical species.

In dogs, following oral dose of ALG-000184, ALG-01075 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 based on terms of ALG-01075 exposure in comparison to that of following iv dose of ALG-00175 ranged between 93.3% to 108%.

Food intake had minimal effect on systemic ALG-01075 exposure in dogs following ALG-000184 oral administration in an aqueous solution

The high aqueous solubility of ALG-01075-reduced oral effect. High fat diet delayed oral absorption of ALG-000184, as shown by ALG-01075 AUC(t)[>500] (from 0.83 to 2.67 h). However, it only reduced ALG-01075 Cmax by 23% and 16%, respectively.

References

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