EASL CONGRESS
7-10 May 2025
Amsterdam, the Netherlands

Abstract # 1924 (THU-242)

Viral kinetics and sequence analysis of a Phase I monotherapy study in subjects with chronic hepatitis B reveals a high barrier of resistance to the capsid assembly modulator ALG-000184

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ALIGOS THERAPEUTICS

BACKGROUND AND AIMS

ALG-000184 is a prodrug of ALG-001075, a novel hepatitis B virus (HBV) Capsid Assembly Modulator, which induces the formation of empty but otherwise intact capsids (CAM-E). ALG-000184 demonstrated substantial reductions in HBV DNA, RNA and viral antigens during the Phase I clinical trial ALG-000184-201 (NCT4536337). In Part 4 Cohort B of this trial, 10 HBeAg-positive and 11 HBeAg-negative treatment-naïve (TN) or currently not treated (CNT) chronic hepatitis B (CHB) subjects are currently being treated with 300 mg ALG-000184 monotherapy (open label) for up to 96 weeks.

In this study, baseline samples of all 21 subjects and the last available on-treatment sample with an HBV DNA viral load of ≥1000 IU/mL were sequenced to identify the presence of known CAM resistance mutations.

Results: Viral Kinetics

300 mg ALG-000184 monotherapy in TN/CNT subjects resulted in a rapid, multi-$\log_{10}$ HBV DNA decline in HBeAg-positive and HBeAg-negative subjects. **No viral breakthrough or viral non-response** as defined by HBV DNA levels were observed. In the HBeAg-positive group, 6 out of 10 subjects reached the lower limit of quantification (LLOQ, 10 IU/mL) at week 48, and 9 out of 9 at week 96. All HBeAg-negative subjects reached LLOQ by week 20 (**Figure 1**). Once subjects reached LLOQ, viral suppression was maintained at this level during monotherapy, indicating sustained viral response.

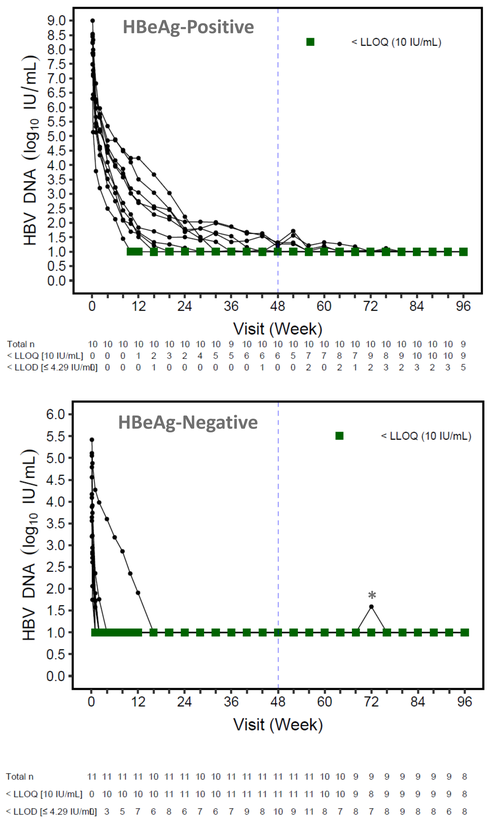

Figure 1: HBV DNA viral load in TN/CNT HBeAg-positive (top) and HBeAg-negative (bottom) subjects receiving 300 mg ALG-000184 monotherapy in Part 4 Cohort B of ALG-000184-201. * Viral blip observed for one subject at a single timepoint. This subject tested LLOQ at the next time point.

MAIN FINDINGS

- ALG-000184 is a prodrug of ALG-001075, a novel and highly potent hepatitis B virus (HBV) Capsid Assembly Modulator (CAM-E).
- In a Phase I trial, **300 mg ALG-000184 monotherapy in treatment-naïve or currently not treated subjects resulted in rapid, multi-$\log_{10}$ HBV DNA decline** in HBeAg-positive and HBeAg-negative subjects.
- **No viral breakthrough or viral non-response** based on HBV DNA levels was observed during monotherapy for up to 96 weeks.
- Consistently, **the four major ALG-0001075 in vitro resistance mutations** (T33N, T33P, T33Q and V124G) **were not detected** in any baseline or on-treatment sample (frequency ≤ 1%).
- The lack of viral breakthrough and emerging resistance, combined with the significant reduction in HBV DNA, positions ALG-000184 as a promising candidate for further clinical development as a best-in-class CAM-E.
- **For more information on ALG-000184, visit posters THU-261 and THU-256**

Methods

Next-generation sequencing was performed on the baseline samples of all 10 HBeAg-positive and 11 HBeAg-negative participants; in addition, at least one on-treatment sample of the HBeAg-positive participants and one HBeAg-negative was analyzed. The minimum plasma HBV DNA concentration for sequencing was 1000 IU/mL. Sequences were compared to their respective genotype reference. Reported here are amino acid substitutions in the HBV core protein with a frequency of ≥1%, with a focus on the 4 known major ALG-001075 resistance mutations, which cause a >4-fold reduction in antiviral activity in vitro (T33N, T33P, T33Q and V124G), minor ALG-001075 resistance mutations (3- to 4-fold loss of antiviral activity in vitro, e.g. F23Y and R127H), and other core protein polymorphisms and CAM resistance mutations (**Table 1**). In general, the in vitro loss in antiviral activity for ALG-001075 was smaller compared to other CAMs.

ALG-001075 In Vitro Resistance Mutations

Category	Core Protein Amino Acid Substitution	Decrease of in vitro antiviral activity (Fold EC_{50} change compared to Wildtype)			
		ALG-001075	Bersacapavir (JNJ-6379)[1]	Vebicorvir (ABI-H0731)[2]	Morphothiadin (GLS-4)[1,2]
Major ALG-001075 in vitro resistance mutations	T33N	28.0	85	21	350
	T33P	7.2	14	n.d.	n.d.
	T33Q	16.4	n.d.	n.d.	n.d.
	V124Q	6.1	>35	n.d.	1.1
Minor ALG-001075 in vitro resistance mutation	F23Y	3.0	5.2	n.d.	n.d.
	R127H	3.2	3.7	n.d.	n.d.
Other core protein polymorphism and CAM resistance mutations	D29G	0.7	4.4	20	0.9
	Y38F	1.2	1.4	3.3	3.7
	I105L	0.7	0.5	0.7	0.6
	I105T	2.1	2.7	2.2	0.9
	I105V	1.2	1.4	n.d.	n.d.
	T109M	0.6	1.2	>68	0.9
	Y118F	1.2	6.6	14	4.7

Table 1: Impact of core mutations on the cell-based antiviral activity of ALG-001075 and other CAMs. The fold change was calculated by dividing the EC_{50} for a specific mutation by the EC_{50} for wildtype.

(1): Verbinnen T et al., J Antimicrob Chemother 2020; (2): Huang Q et al., Antimicrob Agts Chemother 2020

Sequence Analysis of ALG-000184-201 P4CB Samples

All baseline samples and all on treatment samples except one were successfully amplified and sequenced with an average read coverage of > 55,000 reads/base.

The four known major ALG-001075 resistance mutations, namely T33N, T33P, T33Q and V124G were not detected (frequency ≤ 1%) in any of the baseline or on-treatment samples.

Similarly, R127H, which causes a minor, 3.2-fold loss in antiviral activity in vitro, was also not detected in any of the samples.

F23Y, which reduces the in vitro ALG-001075 activity 3-fold, was detected in the baseline sample of one subject at a frequency of 5.1%. The viral load in this subject declined rapidly and reached LLOQ by Day 14. No on-treatment plasma sample with a sufficiently high viral load (1000 IU/mL) was available for sequencing.

Y118F, which causes a major loss in antiviral activity of bersacapavir, vebicorvir and morphothiadin but not ALG-001075, was detected in the baseline sample of one subject at a frequency of 8.60%. T109M, a major vebicorvir resistance mutation was detected in the baseline sample of one subject at a frequency of 99.82%.

I105L, I105T and I105V were detected in the baseline samples of 4 subjects at frequencies up to 99.97%; I105L/T/V do not substantially (<3-fold) affect the antiviral activity of ALG-001075 or other CAMs. The viral load of the subjects harboring Y118F, T109M and I105L/T/V reached LLOQ by Day 14 and no on-treatment sample for sequencing was available.

D29G was detected in the Day 42 sample of one subject at a frequency of 1.06% in one subject; it was not present at a frequency ≥1% in the baseline sample of this subject. No follow-up sample with sufficiently high viral load for sequencing was available. D29G reduces the in vitro antiviral activity of bersacapavir and vebicorvir 4.4- and 20-fold, respectively, but does not affect the activity of ALG-001075.

Subject	HBeAg Status	Time Point	HBV DNA (IU/mL)	Core Mutation associated with CAM Resistance	Frequency [%]
A	Negative	Day 1	1.23E+04	F23Y	5.10
				I105V	18.33
				Y118F	8.60
B	Negative	Day 1	6.17E+04	I105L	1.98
				I105V	65.88
				T109M	99.82
C	Negative	Day 1	1.48E+04	I105L	11.16
				I105T	6.52
				I105V	30.01
D	Negative	Day 1	1.59E+03	Y38F	97.67
				I105T	99.97
E	Positive	Day 1	2.78E+8	none	n.a.
		Day 42	1.68E+3	D29G	1.06

Table 2: Core amino acid substitutions associated with resistance to CAMs, which were detected at a frequency of ≥ 1% in any subjects of ALG-000184-201 Part 4 Cohort B.

No viral breakthrough or viral non-response based on HBV DNA levels was observed during **monotherapy with 300 mg ALG-000184 for up to 96 weeks** in 10 HBeAg-positive and 11 HBeAg-negative TN/CNT subjects in Part 4 Cohort B of the Phase I clinical trial ALG-000184-201 (NCT4536337). Consistently, **the four major ALG-001075 resistance mutations T33N, T33P, T33Q and V124G were not detected** in any of the samples. The lack of viral breakthroughs and genotypic resistance, combined with the substantial, multi-$\log_{10}$ reductions in HBV DNA, HBV RNA and viral antigens, positions ALG-000184 as a promising candidate for further clinical development as a **best-in-class CAM-E.**

Financial disclosure: AJ, TV, JR, MM, JAS, LB, RV, KL, HEA, TL and MW are current or former employees of Aligos Therapeutics Inc.