

# Capsid Assembly Modulator ALG-001075 Binds and Directly Targets HBeAg

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## BACKGROUND

The hepatitis B virus (HBV) capsid assembly process has emerged as a key target for the treatment of chronic HBV infection (CHB).<sup>1</sup> Pevifoscorvir sodium, also known as ALG-000184, is a prodrug of ALG-001075, a capsid assembly modulator that leads to the formation of empty capsids (CAM-E).<sup>2</sup> It has demonstrated meaningful reductions of HBV DNA, RNA, HBsAg, HBcrAg and HBeAg in CHB patients. Pevifoscorvir sodium monotherapy induced an immediate 0.4 log<sub>10</sub> PEIU/mL reduction in the first two weeks of treatment of HBeAg-positive patients,<sup>3</sup> suggesting a potential direct effect of the CAM-E ALG-001075 on HBeAg. The effects of CAM-As (aberrant) on HBeAg were reported earlier.<sup>4,5</sup>

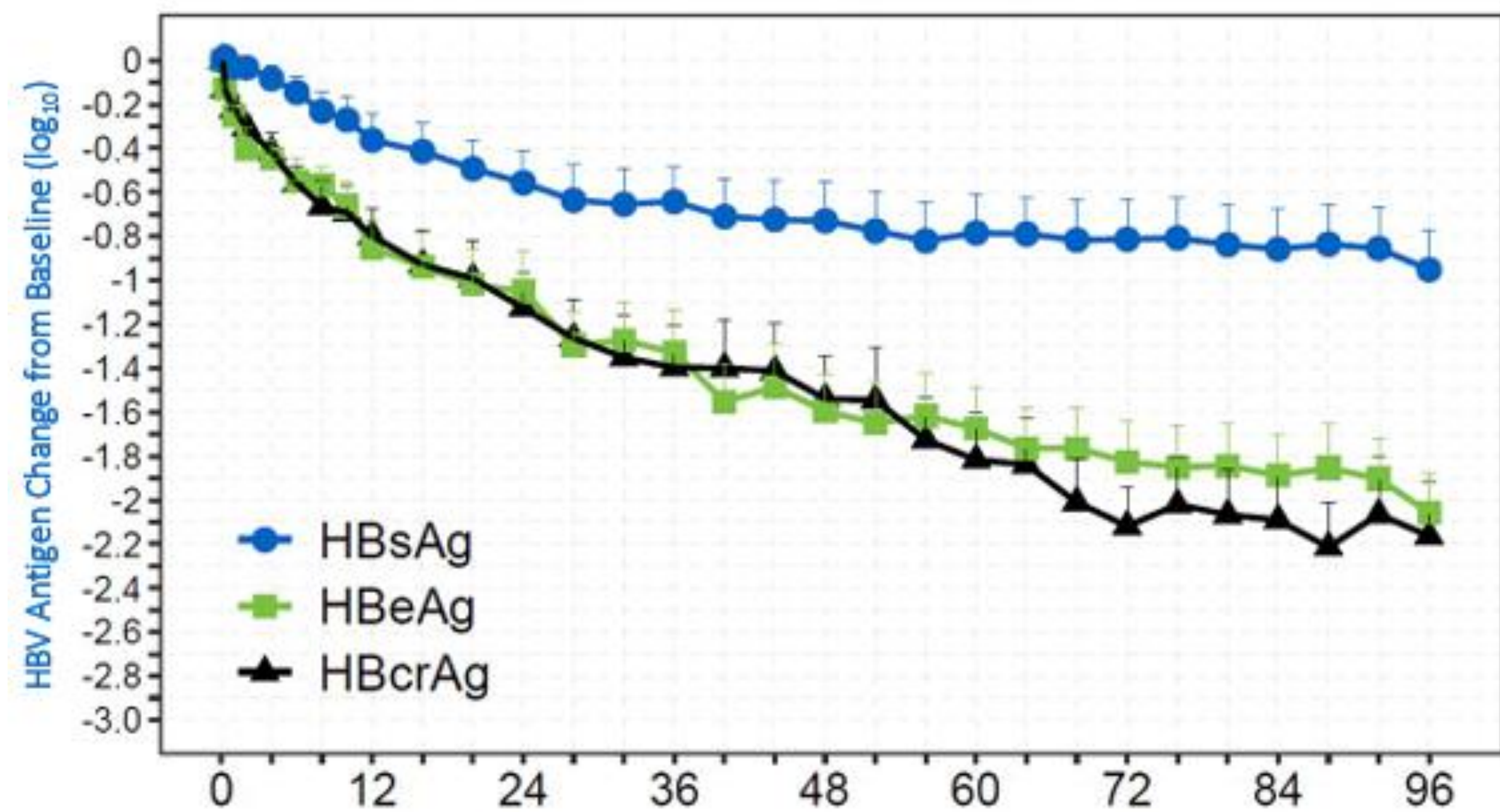


Figure 1: Graph shows multiple-log reductions in HBsAg, HBeAg and HBcrAg in 10 Treatment Naïve/current-not-treated HBeAg-positive subjects who received 300 mg ALG-000184 for ≤ 96 weeks. Values represent mean ± SEM of HBV antigen change in HBeAg-positive subjects.

## METHODS

The impact of CAMs on HBeAg biogenesis was studied using transient HBeAg expression in Huh7 cells and stably transfected HepG2 cells, with plasmids encoding either wild-type or T33N-mutated HBeAg. The effect on HBeAg secretion was assessed utilizing a Chemiluminescent Immunoassay (CLIA) to determine EC<sub>50</sub> values, while a Western blot analysis was conducted to characterize the impact of CAMs on HBeAg and its precursors intracellularly. Interactions between CAMs and purified HBeAg were further investigated using biophysical assays such as spectral shift and isothermal titration calorimetry (ITC).

## ALG-001075 INHIBITS HBeAg SECRETION IN VITRO

Given the rapid initial HBeAg declines induced by pevifoscorvir sodium in HBeAg-positive CHB patients<sup>3</sup> and the closely related structures of HBc and HBeAg, we investigated whether ALG-001075 could directly target HBeAg. To this end, an HepG2-derived cell line stably expressing HBeAg, was generated to study the effect of CAMs on HBeAg. After 13 days of treatment, a CLIA was performed to quantify the amount of secreted HBeAg (Figure 2). A Western blot was also performed as a secondary method, loading equal volumes of culture medium. The obtained image was quantified using ImageJ and an EC<sub>50</sub> value was calculated. ALG-001075 inhibited HBeAg secretion with an EC<sub>50</sub> of 1,542 nM and 2,310 nM according to the CLIA and ImageJ analysis, respectively.

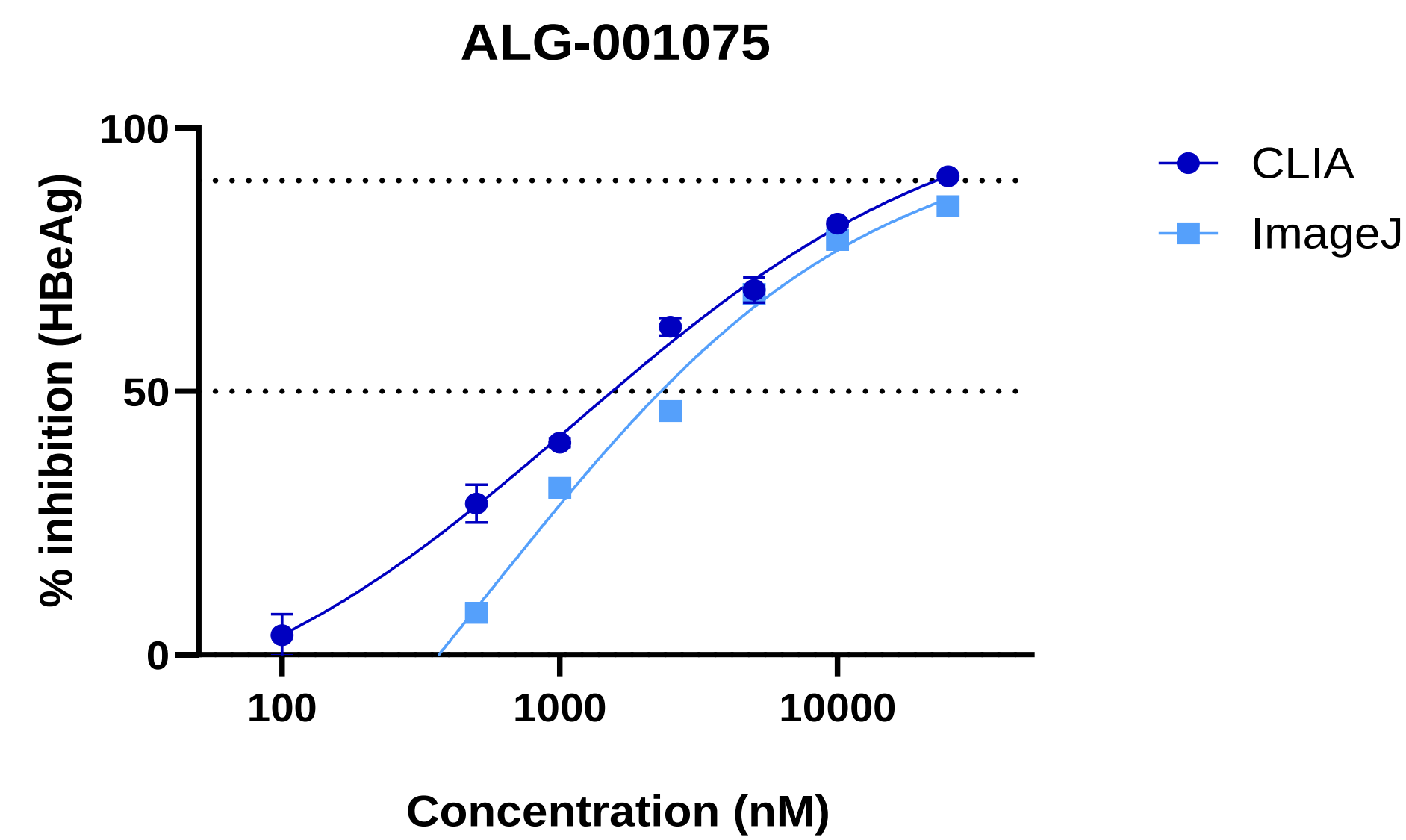


Figure 2: Top graph shows HBeAg % inhibition values obtained from CLIA and ImageJ analysis. CLIA values represent mean ± SEM of 3 technical replicates. Western blot below was performed on the culture medium of stable HBeAg-overexpressing HepG2 cells treated with ALG-001075 at different concentrations.

## ALG-001075 INHIBITS HBeAg FORMATION INTRACELLULARLY

To further characterize the effect of ALG-001075 on HBeAg, a Western blot was performed using intracellular lysates. Two bands became visible at approximately 24 and 17 kDa in size with a different dose-response effect. The signal of the upper band (likely p22) seemed to keep accumulating with increasing compound concentration while the signal of the lowest band (p17) increased up to the 5,000 nM condition, then decreased. These suggest that the inhibition of HBeAg secretion might be due to both blocking of secretion and of processing through binding of ALG-001075 to HBeAg precursors.

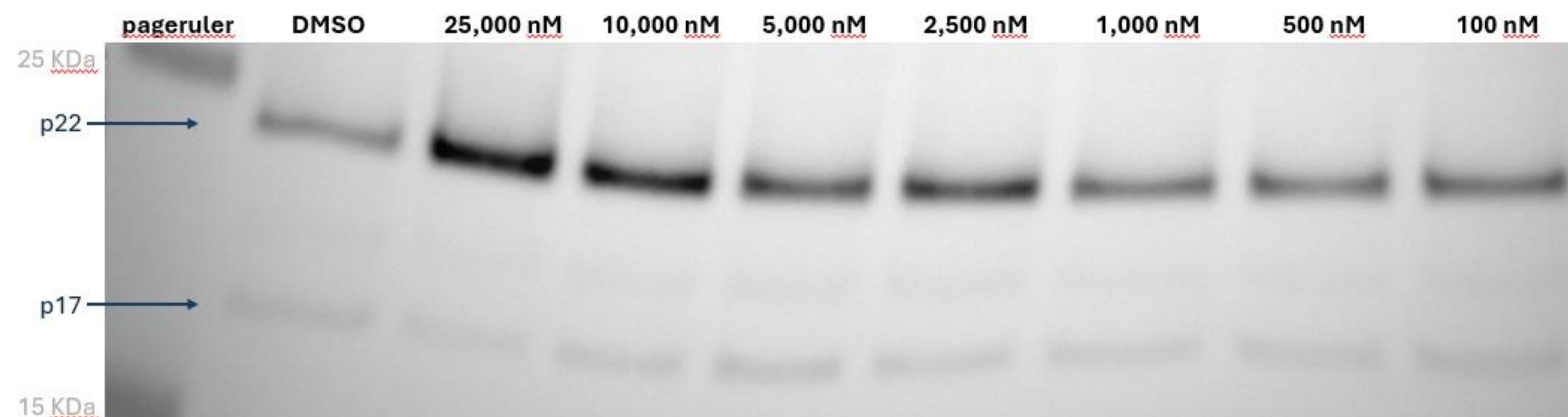
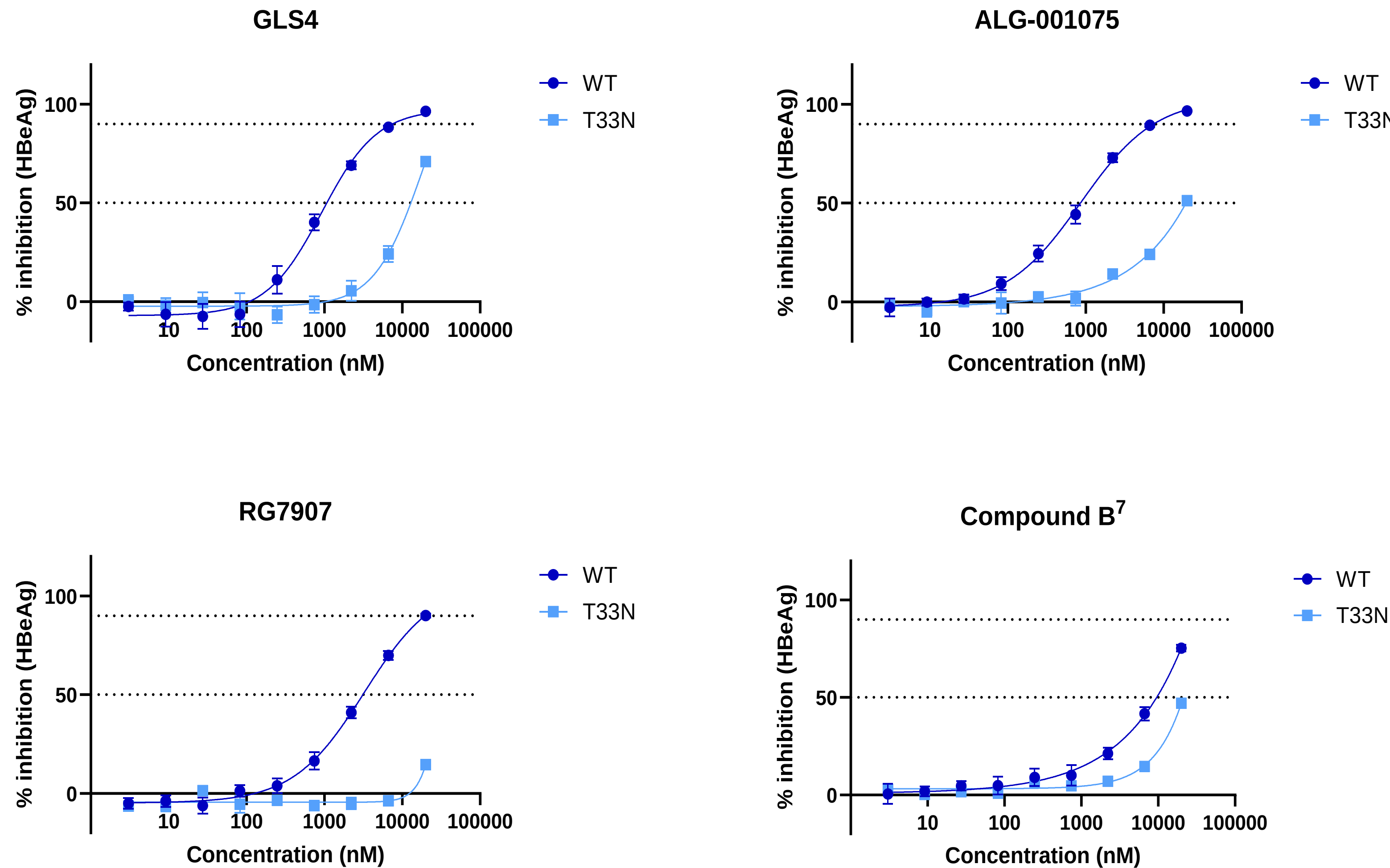


Figure 3: Western blot performed on stable HBeAg-overexpressing HepG2 cells treated with ALG-001075 at different concentrations. In each lane, 40 µg of total protein sample, determined by BCA, was loaded.

## CAM HBc RESISTANCE MUTATION T33N ALSO INDUCES RESISTANCE IN HBeAg

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 was transfected into Huh7 and treated with different CAMs.<sup>6-10</sup> T33N resulted in a pronounced right shift of the dose-response curve (Figure 4). This suggests the CAM binding site in HBeAg overlaps with the binding site in HBc.



|      | (nM)             | GLS4    | ALG-001075 | RG7907  | Compound B <sup>7</sup> |
|------|------------------|---------|------------|---------|-------------------------|
| WT   | EC <sub>50</sub> | 1,075   | 855        | 3,124   | 9,570                   |
|      | EC <sub>90</sub> | 7,501   | 7,456      | 19,696  | >20,000                 |
| T33N | EC <sub>50</sub> | 12,901  | 19,355     | >20,000 | >20,000                 |
|      | EC <sub>90</sub> | >20,000 | >20,000    | >20,000 | >20,000                 |

Figure 4: Dose-response curves for compound-induced inhibition of HBeAg in culture medium of plasmid-transfected HBeAg-overexpressing Huh7 cells, either wild-type (WT) or containing the T33N mutation. Values represent mean ± SEM of 3 individual experiments.

## BIOPHYSICAL BINDING OF ALG-001075 TO HBeAg

With the goal of confirming that CAMs directly bind to HBeAg, the biophysical methods spectral shift and ITC were used with purified HBeAg, produced in *E. coli*. Both methods confirmed binding of ALG-001075 to WT HBeAg with ITC yielding a molar ratio of 0.36, meaning 1 CAM binds to ≥2 HBeAg monomers or 1 HBeAg dimer. Additionally, no binding was observed in spectral shift when the known CAM resistance mutation T33N was introduced (Figure 5). This again indicates an overlap in binding site for CAMs on HBeAg and on HBc.

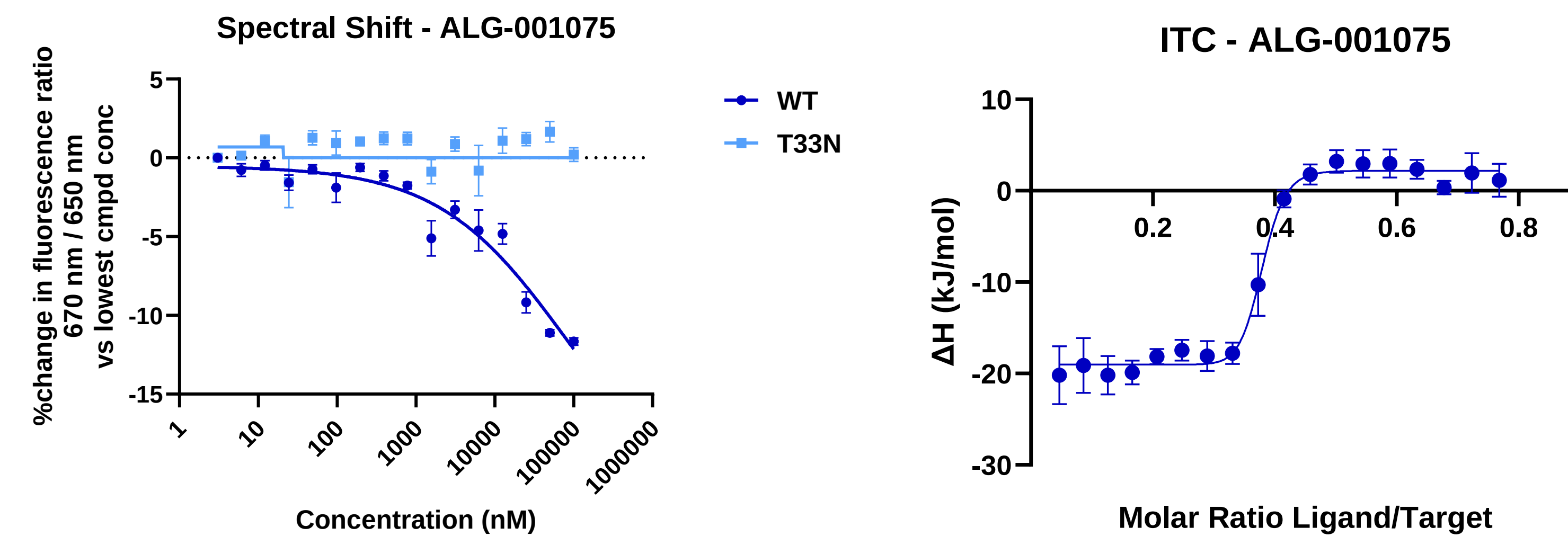


Figure 5: Dose-response curves for ALG-001075 binding to either wild-type (WT) or T33N-containing HBeAg in spectral shift (left) and ITC (right). Values represent mean ± SEM of ≥2 individual runs.

## CAM-HBeAg KINETICS

In order to investigate the kinetics of HBeAg secretion and how CAMs impact this, two different approaches were tried. In the left graph, DMSO or CAM ALG-000111 (a close structural analog of ALG-001075) was added at the same time to HBeAg-overexpressing HepG2 cells receiving fresh medium and samples were collected at different timepoints. This approach measures the secretion of HBeAg under influence of ALG-000111 or DMSO. In the 2nd approach (right graph), HBeAg-producing cells were treated for different time periods with DMSO or compound added at different time points and samples collected at the same time which measures the effect of ALG-000111 on preformed HBeAg. In both approaches a difference can be seen between CAM treatment and DMSO starting at around 24 hours (Figure 6).

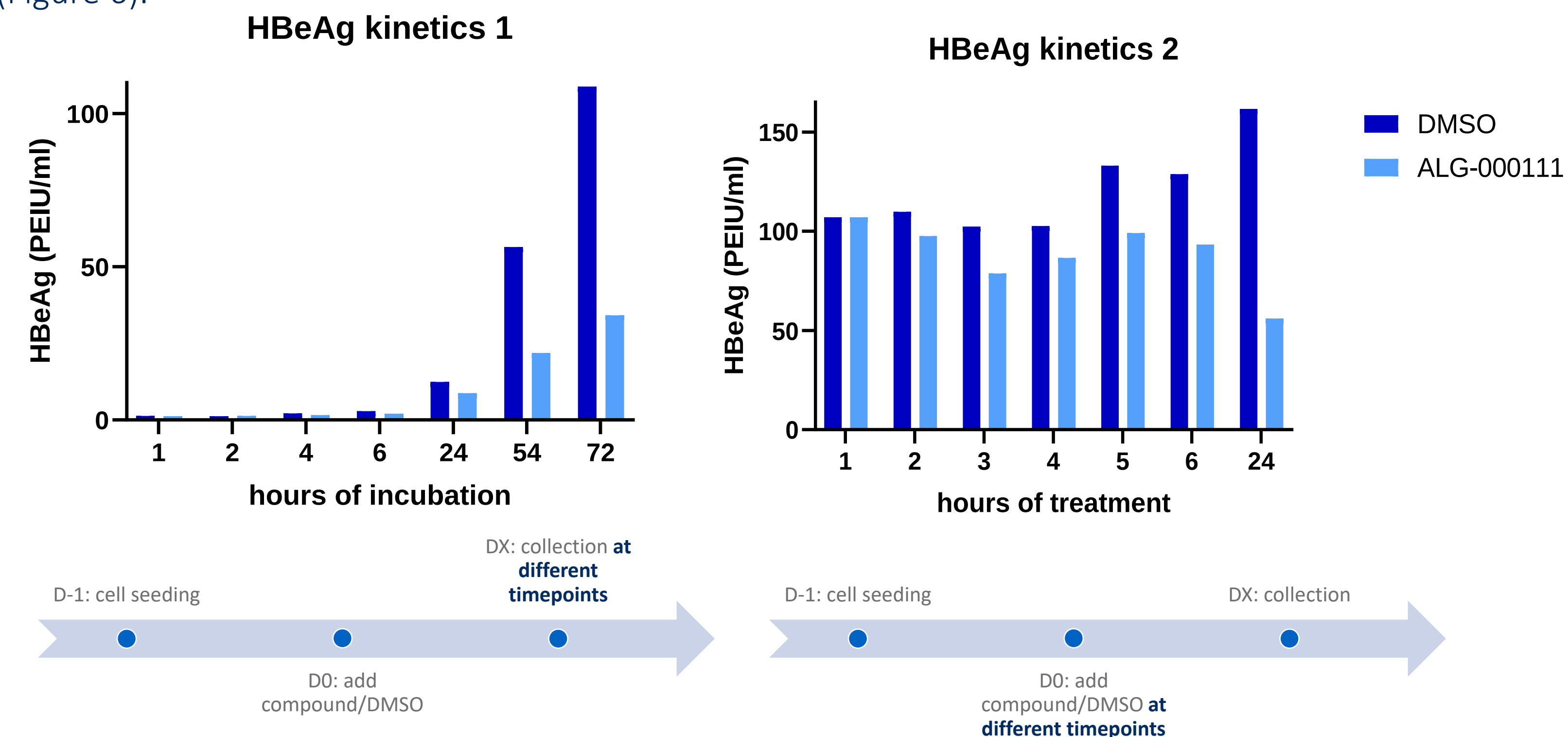


Figure 6: Graphs show HBeAg values (PEIU/mL) in culture medium from stable HBeAg-overexpressing HepG2 cells treated with either DMSO or ALG-000111 (10,000 nM) at different timepoints. Values are obtained from a single experiment.

## CONCLUSION

ALG-001075 strongly reduce the levels of secreted HBeAg, suggesting a direct effect on HBeAg. EC<sub>50</sub> values for HBeAg inhibition were considerably higher than for HBV DNA inhibition but strongly increased upon introduction of the T33N mutation into HBeAg, indicating a similar binding site to HBc. Western blot analysis showed CAMs also impacted the HBeAg precursor, contributing to HBeAg secretion inhibition. Finally, biophysical analysis through spectral shift and ITC confirmed direct binding of CAMs to wild-type HBeAg but not to T33N HBeAg.

## REFERENCES

1. Taverniti et al 2022 J Clin Med; 11:1349.
2. Vendeville et al 2024 J Med Chem; 67:21126-42.
3. Yuen et al 2025 EASL Congress; THU-261.
4. Lahlali et al 2018 AAC; 62:e00835-18.
5. Yan et al 2019 Hepatology; 70:11-24.
6. Kum et al 2023 Hepatology; 78:1252-65.
7. Lenz et al 2019; WO2019175657A1.
8. Debing et al 2021 EASL Congress; poster 1386.
9. Vanrusselt et al 2024 EASL Congress; TOP-358-YI.
10. Verbrinnen et al 2023 Antiviral Res; 216:105660.