# Systematic *In Vitro* Evaluation of Current and Experimental Hepatitis B Therapeutics: Potential Utility for Combinations Exploiting Multiple and Diverse Mechanisms of Action

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## INTRODUCTION

- Current maintenance therapy for chronic hepatitis B virus (HBV) infection is limited to nucleoside/nucleotide analogues (NA), and interferon α-2a (IFN); cures are rare, and there are no available combination treatment regimens for improving the clinical outcome.
- Arbutus is developing several distinct classes of anti-HBV agents with diverse mechanisms of action, with the goal of introducing novel combination regimens. Three agents, the capsid inhibitor AB-423, and the siRNA agents ARB-1467 and ARB-1740, are currently in Phase I and II clinical trials:
  - AB-423 is a Class II small molecule HBV encapsidation inhibitor, distinct from the Class I HBV capsid polymerization modulators.
  - ARB-1467 and ARB-1740 are siRNA agents formulated in a proprietary lipid nanoparticle (LNP) delivery vehicle; both siRNA agents cleave all HBV RNAs, inhibiting antigen production as well as replication.
- Effects of combination treatments of ARB-1740, AB-423 and other capsid inhibitors with standard of care (SOC) agents for hepatitis B were evaluated in different HBV cell culture systems.

**Table 1: ABUS agents in study**

| Agent | Class | Development Status |
|---|---|---|
| ARB-1740 | LNP-formulated siRNA | Phase II |
| AB-423 | Encapsidation Inhibitor | Phase I |
| ARB-880 | Encapsidation Inhibitor | Preclinical |
| ARB-1820 | Encapsidation Inhibitor | Preclinical |
| ARB-168786 | Encapsidation Inhibitor | Preclinical |

## OBJECTIVE

- To evaluate the potential for mechanistic interaction of several ABUS agents with standard of care or other experimental agents, as defined by demonstration of synergistic, additive, or antagonistic effects in HBV cell culture models.

## METHODS AND STUDY DESIGN

- Compounds: Tenofovir alafenamide (TAF), tenofovir disoproxil fumarate (TDF), entecavir (ETV), and GLS-4 were purchased or synthesized, and solubilized in DMSO. PEGylated human interferon α2a was purchased as an aqueous solution.
- Combination treatment in inducible HBV cell lines (AML12-HBV10 and HepDE19) were run on 6X10 titration matrices, with dose ranges priorly determined by single compound titrations. After 48 or 144 hrs, intracellular HBV DNA levels were measured by branched DNA assay, and % inhibition was calculated against DMSO-only controls. Cytotoxicity was similarly determined by CellTiter-Glo assay on cell monolayers of replica plates.
- in infected primary human hepatocytes used FRG chimeric mouse-derived PHH, which were culture plated and infected. Combination treatments were run on 5X7 titration matrices, with dose ranges priorly determined by single compound titrations. After 168 hrs, extracellular HBV DNA, HBsAg and HBeAg levels were measured by qPCR and ELISA, and % inhibition was calculated against DMSO-only controls. Cytotoxicity was similarly determined by CellTiter-Glo assay on the cell monolayers
- Combination effects on inhibition levels were analyzed by the MacSynergy II (Pritchard-, Aseltine, and Shipman model) computational tool, and conclusions were derived according to the guidelines: At 95% confidence interval, volumes under 25 units$^2$% (log volume <2)=probably insignificant synergy/antagonism (if negative) ; 25-50 (log volume >2 and < 5)=minor but significant; 50-100 (log volume >5 and <9)=moderate, may be important in vivo; >100 (log volume >9) = strong, probably important in vivo; approaching 1000 (log volume >90) = unusually high, check data

## RESULTS

**Table 2: Combination results in HBV- infected primary human hepatocytes**

| HBV Assay Endpoint | Inhibitor A | Inhibitor B | Inhibitor A $EC_{50}$ | Inhibitor B $EC_{50}$ (nM) | Synergy Volume (units$^2$%)* | Synergy Log Volume* | Antagonism Volume (units$^2$%)* | Antagonism Log Volume* | Conclusion* |
|---|---|---|---|---|---|---|---|---|---|
| HBV DNA | IFNα2a | AB-423 | 2.154 IU/mL | 876.5 | 34.73 | 7.91 | -3.87 | -0.88 | Synergistic |
| HBsAg | IFNα2a | AB-423 | 13.8 IU/mL | 7793 | 24.11 | 5.49 | 0 | 0 | Synergistic |
| HBeAg | IFNα2a | AB-423 | 10.24 IU/mL | 8580 | 103.04 | 23.46 | 0 | 0 | Synergistic |
| HBV DNA | TAF | AB-423 | 0.405 nM | 876.5 | 30.5 | 6.94 | -1.73 | -0.39 | Synergistic |
| HBsAg | TAF | AB-423 | >100 nM | 7793 | 64.13 | 14.6 | 0 | 0 | Synergistic |
| HBeAg | TAF | AB-423 | >100 nM | 8850 | 5.0 | 1.14 | 0 | 0 | Additive |
| HBV DNA | TAF | ARB-880 | 0.405 nM | 1020 | 8.07 | 1.84 | 0 | 0 | Additive |
| HBsAg | TAF | ARB-880 | >100 nM | 12,800 | 9.09 | 2.07 | -2.14 | -0.49 | Additive |
| HBeAg | TAF | ARB-880 | >100 nM | 10,740 | 0 | 0 | -3.25 | -0.74 | Additive |
| HBV DNA | IFNα2a | ARB-880 | 2.154 IU/mL | 1020 | 311.72 | 70.96 | 0 | 0 | Synergistic |
| HBsAg | IFNα2a | ARB-880 | 13.8 IU/mL | 12,800 | 8.59 | 1.96 | 0 | 0 | Additive |
| HBeAg | IFNα2a | ARB-880 | 10.24 IU/mL | 10,740 | 0 | 0 | 0 | 0 | Additive |
| HBV DNA | TDF | ARB-1820 | 5.62 nM | 229.6 | 60.83 | 13.85 | 0 | 0 | Synergistic |
| HBsAg | TDF | ARB-1820 | >100 nM | 4.36 | 45.85 | 10.44 | 0 | 0 | Synergistic |
| HBeAg | TDF | ARB-1820 | >100 nM | 4.53 | 3.73 | 0.85 | 0 | 0 | Additive |
| HBV DNA | TDF | ARB-168786 | 5.16 nM | 181.6 | 586.54 | 133.52 | 0 | 0 | Synergistic |
| HBsAg | TDF | ARB-168786 | >100 nM | ~1104 | 166.48 | 37.9 | 0 | 0 | Synergistic |
| HBeAg | TDF | ARB-168786 | >100 nM | 1087 | 0 | 0 | 0 | 0 | Additive |
| HBV DNA | TAF | ARB-001820 | 0.405 nM | 229.6 | 1.89 | 0.43 | -8.64 | -1.97 | Additive |
| HBsAg | TAF | ARB-001820 | >100 nM | 4.36 | 0 | 0 | 0 | 0 | Additive |

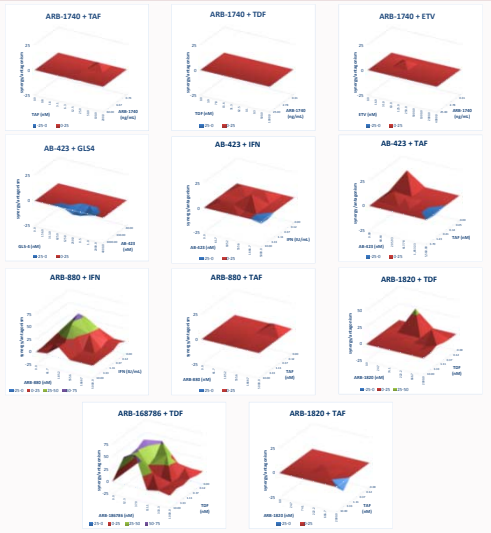
*at 99.9% confidence interval

**Table 3: Combination results of HBV DNA inhibition using inducible cell lines**

| HBV System | Inhibitor A | Inhibitor B | Inhibitor A $EC_{50}$ | Inhibitor B $EC_{50}$ (nM) | Synergy Volume (units $^2$%)* | Synergy Log Volume | Antagonism Volume (units$^2$%)* | Antagonism Log Volume | Conclusion |
|---|---|---|---|---|---|---|---|---|---|
| AML12-HBV10 | ARB-1740 | TAF | 0.624 ng/mL | 44.52 | 6.26 | 0.9 | 0 | 0 | Additive* |
| AML12-HBV10 | ARB-1740 | TDF | 0.947 ng/mL | 89 | 0 | 0 | 0 | 0 | Additive* |
| AML12-HBV10 | ARB-1740 | ETV | 0.906 ng/mL | 1.780 | 5.37 | 0.77 | 0 | 0 | Additive* |
| HepDE19 | AB-423 | GLS-4 | 272 nM | 77 | 0 | 0 | -15.72*, -29.85# | -2.17*, -4.13# | Additive*, Minor Antagonism# |

*at 99.9% confidence interval
#at 95% confidence interval

**Figure 1: Prichard-Shipman plots of inhibition of HBV DNA synthesis from combination treatment in HBV cell culture systems.** Combinations were tested in HBV-infected PHH, induced HepDE19, or induced AML12-HBV10, as indicated in Tables 2 and 3.




## CONCLUSIONS

- ABUS agents tested in combination treatment matrices with NAs and IFN resulted in either additive or significantly synergistic effects. No effect was seen in testing combinations for cytotoxicity under equivalent conditions.
- In PHH, significant synergy was observed for multiple endpoints in combinations with IFN, indicating that innate immune modulation may effectively potentiate direct acting antivirals.
- Minor antagonism was observed only from a combination of the Class II encapsidation inhibitor AB-423 and the Class I capsid polymerization modulator GLS-4, at a confidence interval of 95%; seen as additive at 99.9% confidence interval.
- The results confirm the complementarity of ABUS agents with SOC, and their potential utility in novel clinical combination regimens.

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