Combination Treatment with HBV-Targeting GalNAc-siRNA and Small-Molecule PD-L1 Inhibitor Increases HBV Specific Immune Responses in a Chronic Hepatitis B Infection Mouse Model

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Arbutus BIOPHARMA Curing Chronic Hepatitis B

INTRODUCTION

Curative strategies for chronic HBV (CHB) will likely require combination of drugs with different modes of action. AB-729, an HBV-targeting GalNAc-siRNA, has been shown to enhance HBV-specific T cell responses in CHB patients, potentially through its reduction of HBsAg[1,2]. PD-1/PD-L1 checkpoint inhibition by antibodies has been associated with HBsAg loss and seroconversion in CHB patients with low baseline HBsAg[3,4].

Here we examine a preclinical proof-of-concept combination strategy of HBV GalNAc-siRNA and small-molecule-mediated PD-L1 inhibition, with the goal of potentiating HBV immune responses that would benefit functional cure.

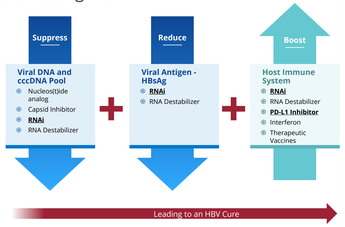


Figure 1. Combination strategies potentially leading to HBV cure

OBJECTIVES

- Assess ability of combination treatment with PD-L1 small-molecule compound AB-101 and HBV-targeting siRNA to reinvigorate HBV-specific T cell and humoral activity in an AAV-HBV mouse model

BACKGROUND

PD-1:PD-L1 checkpoint axis plays a key role in antiviral immune tolerization in CHB

- PD-L1 expression is upregulated during HBV infection[5,6]
- PD-1 expression is upregulated on HBV-specific T- and B-cells[5,6]
- Inhibition associated with HBsAg loss in some CHB patients[3,4]
- Preclinical data in an AAV-HBV mouse model suggests enhanced HBV-specific T cell activity after combination treatment with an HBV-targeting RNA interference agent and antibody-mediated PD-L1 inhibition[7]

AB-101 is an oral small-molecule inhibitor of PD-L1

Advantages of small-molecule PD-L1 inhibitor approach:

- Enables oral dosing
- Minimizes systemic safety issues seen with antibodies
- Tunable control of checkpoint inhibition
- Better tissue penetrance and potential for increased efficacy

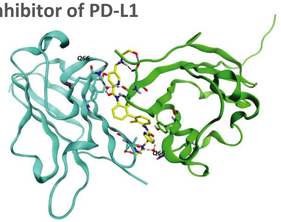
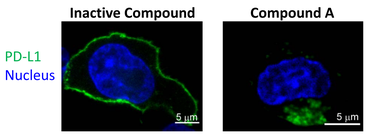


Figure 2. Co-crystal structure of representative small-molecule PD-L1 inhibitor (Compound A) and PD-L1 protein. Compound interaction with PD-L1 results in dimerization of two PD-L1 monomers (cyan and green, chains A and B)[8].

RESULTS

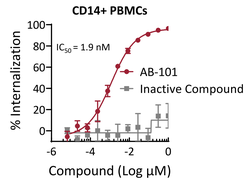
1. PD-L1 inhibitor compounds reduce PD-L1 expression on cell surface through a novel internalization mechanism that is reversible upon compound removal

A Small-molecule-mediated dimerization of PD-L1 protein results in internalization from cell membrane to endosomal structures.

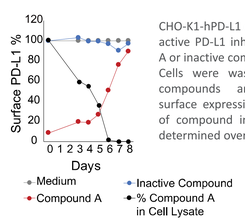


Confocal microscopy of CHO-K1 cells expressing human PD-L1 incubated with PD-L1 inhibitor (Compound A) or inactive compound at 1 µM[8].

B AB-101 mediates PD-L1 internalization in human myeloid cells



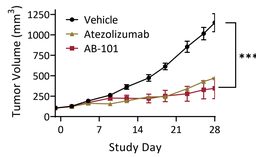
C Effect of PD-L1 inhibitors is reversible, with rapid recovery of PD-L1 surface expression upon compound removal



CHO-K1-hPD-L1 cells treated with active PD-L1 inhibitor Compound A or inactive compound at 1 µM. Cells were washed to remove compounds and PD-L1 cell surface expression and presence of compound in cell lysate was determined over time[8].

2. AB-101 treatment results in *in vivo* anti-tumor activity

AB-101 treatment results in anti-tumor effects comparable to anti-PD-L1 monoclonal antibody.

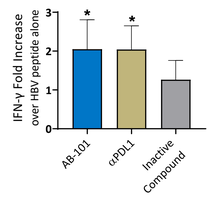


AB-101 dosed at 10 mpk *q.d. p.o.* for 28 days
Atezolizumab dosed at 0.3 mpk *t.i.w. i.p.*
***p<0.001 by Welch's t-test

3. AB-101 treatment reinvigorates HBV-specific T cell activation

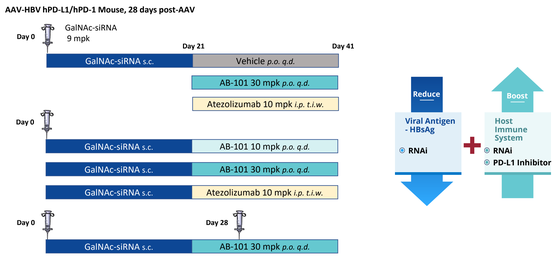
AB-101 reinvigorates HBV-specific T cell responses *ex vivo*; effect is comparable to anti-PD-L1 antibody.

N = 9 CHB patients
*p<0.05 by One-way ANOVA



4. Combination treatment of PD-L1 inhibitor + HBV-siRNA

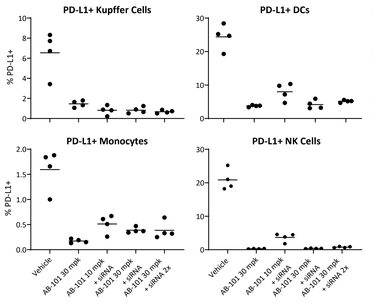
Effect of mono- or combination sequential treatment was assessed in an AAV-HBV mouse model.



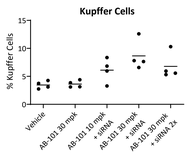
+ Vehicle, Naïve and positive immune response control (pHBV1.3 HDI C57BL/6) groups

mpk = mg/kg

5. AB-101 treatment reduces PD-L1 in liver



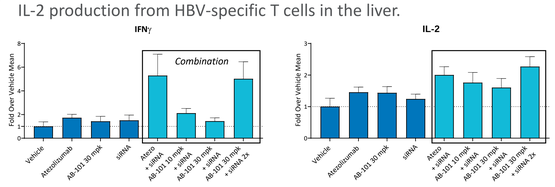
- Liver target engagement confirmed
- PD-L1 reduced in liver immune cells
- Increased Kupffer cell frequency in combination groups



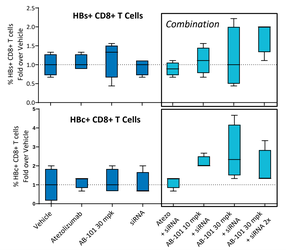
Day 42; PD-L1 reduction statistically significant in all AB-101 groups
% Kupffer cell increase statistically significant in AB-101 30 mpk + siRNA groups

6. AB-101 + siRNA combination increases HBV-specific T cell & humoral activity

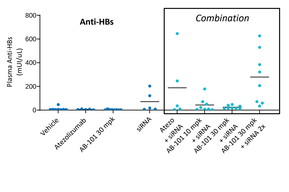
A PD-L1 inhibitor + siRNA combination was associated with greater IFNγ and IL-2 production from HBV-specific T cells in the liver.



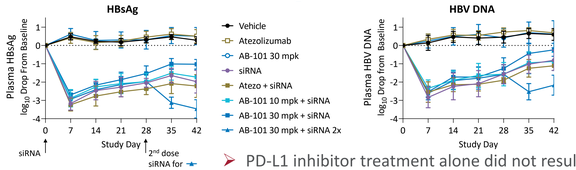
B Increased frequencies of HBV-specific CD8+ T cells were observed in combination treatment groups.



C Greater anti-HBs antibody production was observed with repeat siRNA dosing.



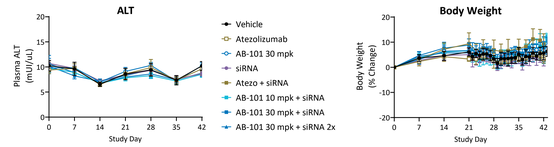
7. siRNA mediated reductions in HBsAg and HBV DNA



- PD-L1 inhibitor treatment alone did not result in viral marker reductions

8. PD-L1 inhibitor treatment alone or in combination with siRNA was not associated with ALT elevation

Treatments were well-tolerated; no changes in liver function markers or decreases in body weight observed



CONCLUSIONS

- Oral small-molecule PD-L1 inhibitors have been identified which function through a novel internalization mechanism distinct from antibody approaches
- Combination treatment with AB-101, a small-molecule PD-L1 inhibitor, and HBV-targeting siRNA resulted in activation of HBV-specific T cell and humoral responses in an AAV-HBV mouse model
- This favorable preclinical profile suggests this combination treatment strategy may provide additional benefit in increasing HBV immune responses, a key driver of CHB functional cure

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METHODS

- Live cell confocal imaging was assessed as described previously[9]
- PD-L1 internalization assessments: CHO-K1-hPD-L1 or PBMCs from healthy donors were incubated with or without compound and PD-L1 cellular surface expression was determined by flow cytometric analysis using PE-conjugated αPD-L1
- HBV-specific T cell activation assay: PBMCs from CHB patients were incubated with HBV overlapping peptides spanning surface antigen and core protein in the presence or absence of compounds. IFN-γ production was determined by Luminex assay
- MC38 tumor efficacy assessments were conducted as described previously[9]
- HBV-specific T cell activity in AAV-HBV mouse was assessed by fluorospot of liver mononuclear cells and flow cytometric analysis of blood with tetramer staining
- Anti-HBs antibody was measured with CLIA

CONTACT AND DISCLOSURES

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