Preclinical activity of small-molecule oral PD-L1 checkpoint inhibitors capable of reinvigorating T cell responses from chronic hepatitis B patients

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INTRODUCTION

HBV-specific T cell tolerance is a critical driver in maintaining chronic hepatitis B (CHB) infection. The PD-1/PD-L1 checkpoint axis plays a key role in tolerization and inhibition of this axis by antibody approaches has been associated with loss of hepatitis B surface antigen and seroconversion in CHB patients.

AB-101 is an oral small-molecule inhibitor of PD-L1 with potential for tunable on-target engagement and better tissue penetration and improved efficacy.

Here we report the preclinical *in vitro* activity of AB-101 and other novel PD-L1 inhibitor compounds with demonstrated *in vivo* activity and ability to reinvigorate HBV-specific T cells from CHB patients.

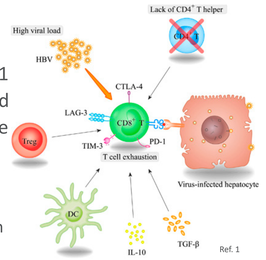


Figure 1.
HBV-induced T cell tolerance in CHB infection

OBJECTIVES

- Assess preclinical activity of PD-L1 inhibitor compounds in cell culture models and determine *in vivo* efficacy in a transgenic MC38 tumor mouse model
- Assess PD-L1 inhibitor compound ability to reinvigorate HBV-specific T cell activity in PBMCs from CHB patients

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[2,3]
- PD-1 expression is upregulated on HBV-specific T- and B-cells[2,3]
- Inhibition associated with HBsAg loss in some CHB patients[4,5]
- 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 PD-L1 inhibition[6]

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

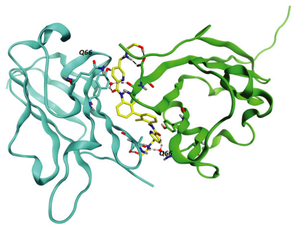
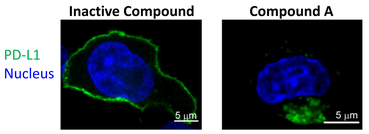


Figure 2. Co-crystal structure of representative small-molecule PD-L1 inhibitor (Compound A) and PD-L1 protein. Compound interaction with PD-L1 results in dimerization of two PD-L1 monomers (cyan and green, chains A and B)[7].

RESULTS

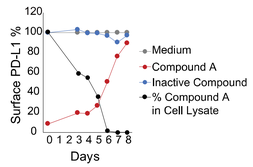
1. PD-L1 inhibitor compounds reduce PD-L1 expression on cell surface through a novel internalization mechanism

A Dimerization of PD-L1 protein results in internalization from cell membrane to endosomal structures post-treatment with PD-L1 small-molecule inhibitor



Confocal microscopy of CHO-K1 cells expressing human PD-L1 incubated with PD-L1 inhibitor or inactive compound at 1 µM[7].

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



PD-L1 expression was assessed in CHO-K1-hPD-L1 cells after treatment with active PD-L1 inhibitor Compound A or inactive structurally related 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[7].

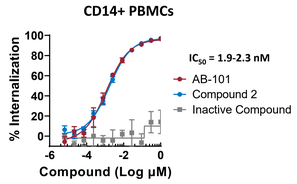
2. PD-L1 inhibitor treatment mediates T cell activation

A Compounds are highly potent in inducing PD-L1 internalization in CHO-K1-hPD-L1 cells and T cell activation in a Jurkat T cell reporter activity assay

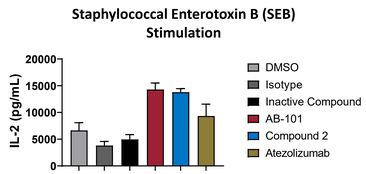
Compound	Internalization Assay CHO-K1-hPD-L1	T Cell NFAT Reporter Assay
Potency	IC_{50} ± SD (µM)	EC_{50} ± SD (µM)
AB-101	0.019 ± 0.005	0.018 ± 0.009
Compound 2	0.024 ± 0.013	0.013 ± 0.005
Anti-PD-L1 mAb	No activity	0.002 ± 0.0009

Jurkat T cell line expressing PD-1 and a luciferase reporter driven by NFAT-response element that produces light when T cells are activated were co-cultured with CHO-K1-hPD-L1 cells. Inhibition of PD-1:PD-L1 interaction results in NFAT reporter activity.

B PD-L1 inhibitors mediate PD-L1 internalization in primary human myeloid cells

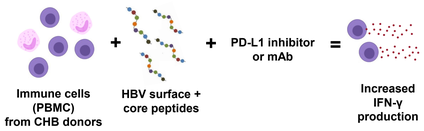


C PD-L1 reduction in myeloid cells is associated with increased human T cell activation upon antigen stimulation



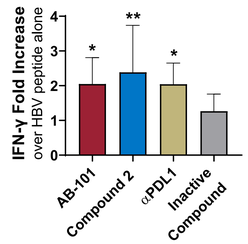
N = 6 donors
Representative data from one donor shown
Compounds tested at 1.25 µM; Atezolizumab at 0.25 µg/mL

3. PD-L1 inhibitor treatment reinvigorates HBV-specific T cell responses



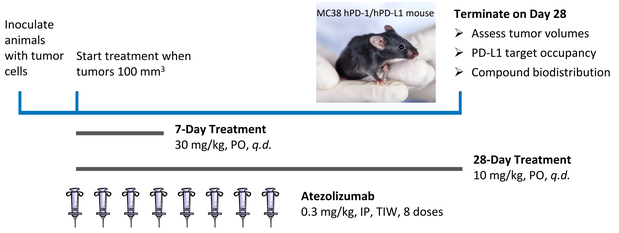
Treatment of PBMCs from CHB donors with anti-PD-1/PD-L1 antibodies has been shown to enhance HBV-specific T cell responses *ex vivo*.[8]

A AB-101 and another small-molecule PD-L1 inhibitor (Compound 2) reinvigorates HBV-specific T cell responses *ex vivo;* effect is comparable to anti-PD-L1 antibody

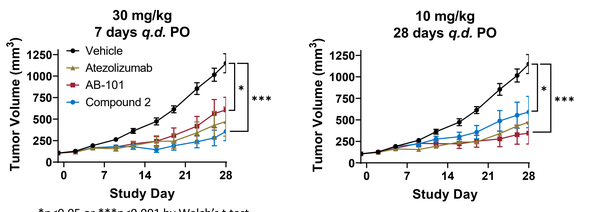


N = 9 CHB patients
*p<0.05 or **p<0.01 by One-way ANOVA

4. PD-L1 inhibitors mediate anti-tumor efficacy in MC38 tumor mouse model

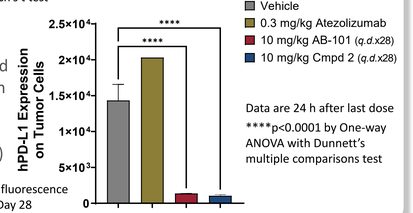


A PD-L1 inhibitor treatment results in anti-tumor effects comparable to anti-PD-L1 monoclonal antibody



*p<0.05 or ***p<0.001 by Welch's t test

B Anti-tumor activity is associated with reduced PD-L1 tumor expression and increased CD8+ T cell infiltration into tumors (data not shown)



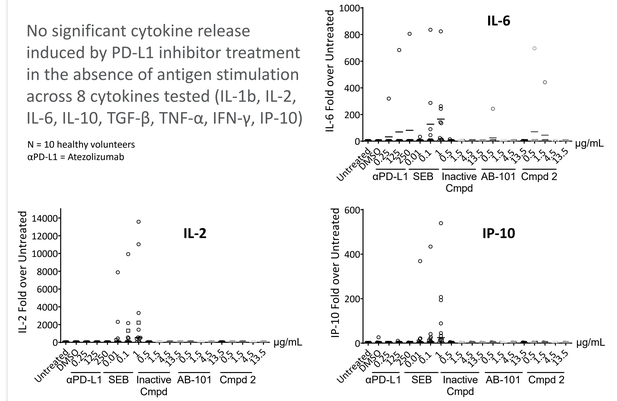
hPD-L1 expression measured as median fluorescence intensity in flow cytometric analysis on Day 28

Data are 24 h after last dose
****p<0.0001 by One-way ANOVA with Dunnett's multiple comparisons test

5. PD-L1 inhibitors do not stimulate cytokine release in human whole blood

No significant cytokine release induced by PD-L1 inhibitor treatment in the absence of antigen stimulation across 8 cytokines tested (IL-1b, IL-2, IL-6, IL-10, TGF-β, TNF-α, IFN-γ, IP-10)

N = 10 healthy volunteers
αPD-L1 = Atezolizumab



CONCLUSIONS

- Oral small-molecule PD-L1 inhibitors have been identified which function through a novel internalization mechanism distinct from antibody approaches
- Once daily oral administration of AB-101 resulted in profound tumor reduction that was associated with T cell activation in a MC38 tumor mouse model
- AB-101 treatment of PBMCs from CHB patients resulted in activation and reinvigoration of HBV-specific T cells
- This favorable preclinical profile supports further development of AB-101 as a therapeutic modality for CHB treatment

REFERENCES

1. Ye et al., 2015, Cell Death and Disease; 6: e1694
2. Fisicaro et al., 2012, Gastroenterology; 143(6):1576-1585
3. Fisicaro et al., 2010, Gastroenterology; 138(2):682-93
4. Gane et al., 2019, J Hepatology; 71(5):900-907
5. Wang, et al., Abstract L012, AASLD The Liver Meeting, November 12-15, 2021
6. Thi et al., Poster 929, AASLD The Liver Meeting, October 20-24, 2017
7. Park et al., 2021, Nat Communications; 12:1222
8. Boni et al., 2007, J Virology; 81(8):4215-25

METHODS

- Live cell confocal imaging was assessed as described previously[7]
- 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 APC-conjugated αPD-L1
- NFAT T cell activation assay: PD-1 Jurkat effector cells were co-cultured with hPD-L1-aAPC/CHO-K1 cells with or without compound treatment and luciferase activity was assessed per manufacturer's protocol (Promega)
- 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[7]

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