Discovery of oral PDL1 small molecule inhibitors specifically designed for the treatment of chronic hepatitis B

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KEY TAKE HOME MESSAGES

Rationale

PD1/PDL1 is a major pathway of T-cell exhaustion in CHB

Problem

Systemic toxicities limit optimum dosing of PD1/L1 antibodies

Our Solution

Liver-targeted PDL1 SMi to localize T-cell activation to the liver

Current Status

High liver/plasma ratio PDL1 SMi (ALG-093453) shows better efficacy than durvalumab in a liver in vivo model

INTRODUCTION AND OBJECTIVES

In chronic hepatitis B (CHB) patients, upregulation of both PD-1 on HBV-specific T cells and PDL1 on liver cells causes T-cell exhaustion and persistent viral infection. Therefore, inhibiting the PD1/PDL1 pathway has recently emerged as an attractive therapeutic strategy to reverse immune tolerance in CHB. Seven PD1/PDL1 antibodies are currently approved as cancer therapies and 2 antibodies (nivolumab and ASC22) have demonstrated some clinical efficacy in CHB patients. However, the systemic immune-related adverse effects associated with antibodies limit their therapeutic window and therefore, there is a need to develop better tolerated PD1/PDL1 inhibitors for CHB patients. Here, we rationally designed liver-targeted oral PDL1 small molecule inhibitors to localize T cell activation to the liver and thereby potentially mitigate systemic toxicity in CHB patients.

METHODS

Biochemical PD1/PDL1 interaction and PDL1 dimerization were assessed by AlphaLISA. Cellular activity was measured using a co-culture assay of PD1 expressing Jurkat NFAT-luciferase T cells with PDL1-expressing CHO cells. HBV-specific T cell activation assays were performed in PBMCs from an HBV-infected patient. Pharmacokinetics were performed in C57BL/6 mice and in vivo efficacy was assessed using humanized-PDL1 MC38 cells implanted either subcutaneously or in the liver.

RESULTS

Discovery of Highly Potent PDL1 Small Molecule Inhibitors

		Nivolumab	Durvalumab	ALG-093453	ALG-093578
Biochemical Activity	PD1/PDL1 Interaction IC_{50} (nM)	0.159 ± 0.007 (n=2)	0.025 ± 0.009 (n=2)	0.014 ± 0.004 (n=3)	0.040 ± 0.027 (n=3)
	PDL1 Dimerization IC_{50} (nM)	*n.a*	*n.a*	84.1 ± 35.7 (n=3)	338.4 ± 141.7 (n=2)
Cellular Activity	Jurkat PD1/PDL1 Blockade EC_{50} (nM)	3.3 ± 0.3 (n=2)	0.3 ± 0.1 (n=4)	1.4 ± 0.5 (n=13)	15 ± 8 (n=4)
	Jurkat PD1/PDL1 Blockade Emax (% to Atezolizumab)	119 ± 8 (n=2)	102 ± 18 (n=4)	143 ± 18 (n=13)	129 ± 17 (n=4)

Table 1: Biochemical and cellular activities of Aligos PDL1 inhibitors vs. FDA-approved antibodies
PD1/PDL1 interaction and PDL1 dimerization were assessed by Alpha-LISA. Cellular activity was measured using a co-culture reporter assay in which TCR-mediated NFAT activity of Jurkat T cells is constitutively inhibited by the engagement of PD1 by PDL1 expressing CHO cells. *n.a:* no activity

ALG-093453 and ALG-093578 Induce PDL1 Internalization in Cells

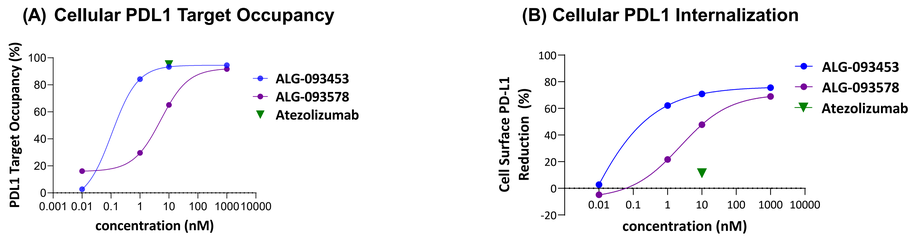

Figure 1: Effect of Aligos PDL1 inhibitors on PDL1 cell surface expression
PDL1-expressing CHO cells were incubated for 24 hours in presence of PDL1 inhibitors. PDL1 target engagement and PDL1 surface expression were assessed by FACs using MIHI and Abcam 28.8 anti-PDL1 antibodies, respectively.

ALG-093453 and ALG-093578 Show High Liver/Plasma Ratios in Mice

(A) Mouse PK Parameters

	ALG-093453	ALG-093578
PO Dose (mg/kg)	50	52 (Prodrug)
T_{max} (h)	5.33	1.50
C_{max} (ng/mL)	2422	1628
$AUC_{0\text{-}12\ h}$ (ng.h/mL)	23,831	8974
Liver/Plasma	14.9 at 24hr	48.6 at 12hr
Liver/Lung	0.80 at 24hr	31.4 at 12hr

(B) ALG-093578 Mouse Tissue Distribution

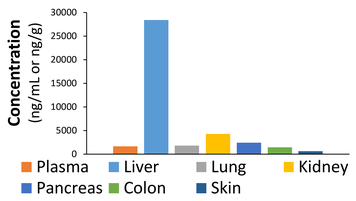

Figure 2: Mean plasma and tissue concentrations of Aligos PDL1 inhibitors in C57BL/6 mice
(A) Mouse PK parameters following a single oral dose of ALG-093453 and ALG-093578 prodrug.
(B) Mouse tissue distribution of ALG-093578 2 hours of post dosing of prodrug at 100 mg/kg.

ALG-093453 Orally-dosed Shows Similar Efficacy to Durvalumab in Subcutaneous Tumor, but Better Efficacy When the Same Tumor are Implanted in the Liver

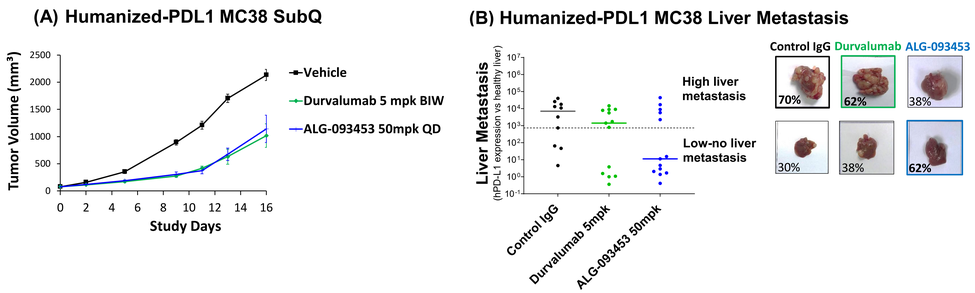

Figure 3: In vivo anti-tumor activity of ALG-093453 in humanized-PDL1 MC38 subcutaneous tumor and hu-PDL1 MC38 liver metastasis
(A) hu-PDL1 MC38 cells were implanted subcutaneously, and mice dosed with vehicle or indicated compounds. (B) hu-PDL1 MC38 cells were injected intra-splenic to generate liver metastasis and mice dosed for 20 days. Liver metastasis were quantitated ex-vivo by measuring hu-PDL1 mRNA expression using Q-PCR. Durvalumab was dosed intra-peritoneally (IP) at 5mpk BIW and ALG-093453 was dosed orally (PO) at 50 mpk QD.

ALG-093453 and ALG-093578 Activate HBV-specific T-cells From Patients

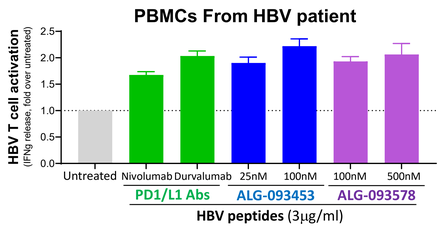

Figure 4: Ex-vivo HBV-specific T cell activity of Aligos PDL1 inhibitors
HBV-specific T cell activation assays were performed in PBMCs from an HBV-infected patient and assessed by measuring IFNγ release with ELISA. Nivolumab and Durvalumab were used at 10 μg/ml.

CONCLUSIONS

- Identified oral PDL1 small molecule inhibitors with similar potency to FDA-approved antibodies
  - ALG-093453 demonstrates a high liver/plasma ratio and ALG-093578 shows liver tropism (high liver/tissues)
- ALG-093453 shows similar in vivo efficacy to durvalumab in a systemic model but enhanced efficacy in a liver in vivo model; confirming our liver-targeted approach
- Our lead molecules activate HBV-specific T cells ex-vivo to a similar extent as Nivolumab and Durvalumab
- The ALG-093578 compound series with high liver tropism is currently under in vivo evaluation

REFERENCES

Li et al *Frontiers in Immunology* 2020; Gane et al *Journal Hepatology* 2019; Wang et al AASLD 2022

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