Poster PB-010

Discovery of a Liver-Targeted PD-L1 Small Molecule Inhibitor for the Treatment of Chronic Hepatitis B and Liver Cancer

Heleen Roose[1], Sarah Stevens[2], Cheng Liu[2], Kristina Rekstyte-Matiene[1], Ruchika Jaisinghani[2], Qingling Zhang[2], Andreas Jekle[2], Antitsa Stoycheva[2], Francois Gonzalvez[1], Tongfei Wu[1]

[1]*Aligos Belgium BV, Leuven, Belgium,* [2]*Aligos Therapeutics, Inc., South San Francisco, CA*

** contact emails: hroose@aligos.com; twu@aligos.com*

Background

The PD-1/PD-L1 immune checkpoint pathway has emerged as an attractive target to reverse immune tolerance in chronic hepatitis B (CHB). However, due to systemic immune related adverse events associated with antibodies, lower doses of PD-1/PD-L1 antibodies have been used in clinical trials for CHB patients compared to cancer patients. Here, we report the discovery of a liver-targeted PD-L1 small molecule inhibitor that preferentially and significantly partitions into the liver and thereby may potentially mitigate extra-hepatic on target related toxicity.

Methods

Biochemical interaction of PD-1/PD-L1 and PD-L1 dimerization were assessed by AlphaLISA. Cellular activity was measured using a co-culture assay of PD-1 expressing Jurkat NFAT-luciferase T cells with PD-L1 expressing CHO cells. HBV-specific T cell activation assays were performed with PBMCs from an HBV-infected patient. Pharmacokinetic studies were performed in C57BL/6 mice. In vivo PD-L1 target occupancy and tumor growth inhibition was assessed using either a MC38 humanized-PD-L1 subcutaneous mouse model or a liver metastasis mouse model.

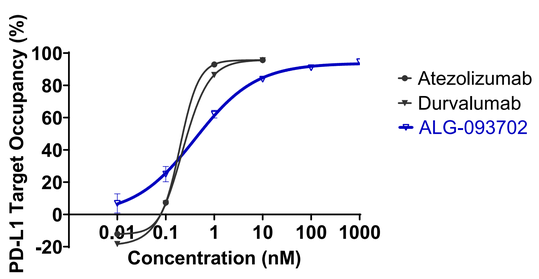
Results 1 - In Vitro Biochemical and Cellular Potency

		Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	ALG-093702 PD-L1 SMi
Biochemical Activity	Human PD-1/PD-L1 Interaction IC_{50} (nM)	0.159 (n=2)	0.025 (n=2)	0.048 (n=2)
	Human PD-L1 Dimerization EC_{50} (nM)	No dimerization	No dimerization	5.5 (n=2)
Cellular Activity	Jurkat PD-1/PD-L1 Blockade EC_{50} (nM)	3.3 (n=2)	0.3 (n=4)	5.9 (n=8)

Table 1*:* Biochemical and cellular activities of Aligos PD-L1 inhibitor vs. FDA-approved antibodies
PD-1/PD-L1 interaction and PD-L1 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 PD-1 by PD-L1 expressing CHO cells.

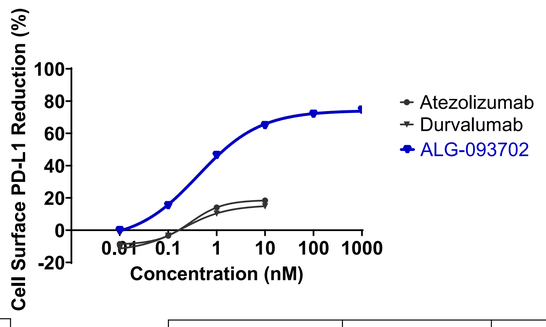
Results 2 - Mechanism of Action of PD-1/PD-L1 Blockade

A. Cellular PD-L1 Target Occupancy
FACS using MIH1 PD-L1 antibody which competes with PD-L1 inhibitors



	Atezolizumab	Durvalumab	ALG-093702
Target Occupancy EC_{50} (nM)	0.22	0.28	0.49

B. Cellular Surface PD-L1 Reduction
FACS using Abcam 28.8 PD-L1 antibody which cannot compete with PD-L1 inhibitors



	Atezolizumab	Durvalumab	ALG-093702
PD-L1 reduction EC_{50} (nM)	No effect	No effect	1.5

Figure 1: Effect of Aligos PD-L1 inhibitor on PD-L1 cell surface expression
PD-L1-expressing CHO cells were incubated for 24 hours in presence of PD-L1 inhibitors. PD-L1 target engagement and PD-L1 surface expression were assessed by FACs using MIH1 and Abcam 28.8 anti-PDL1 antibodies, respectively.

Results 3 - ALG-093702 Exhibits Liver Targeted Tissue Distribution

A. Mouse PK Parameters

	ALG-093702 Mouse PK
PO Dose (mg/kg)	52 (Prodrug ALG-093701)
T_{max} (hr)	2.0
C_{max} (ng/mL)	1807
$AUC_{0\text{-}inf}$ (ng.h/mL)	8913
Oral bioavailability	41%

B. Mouse Tissue Distribution at 6 hours of Post Dosing

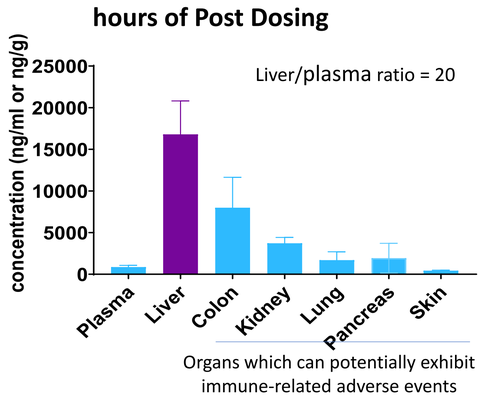
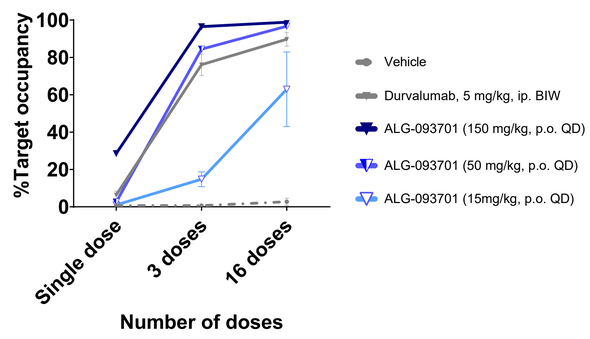


Figure 2: Mean plasma and tissue concentrations of Aligos PDL1 inhibitor in C57BL/6 mice
A. Mouse PK parameters following a single oral dose of ALG-093702 prodrug.
B. Mouse tissue distribution of ALG-093702 at 2 hours of post dosing of prodrug

Results 4 - ALG-093702 Demonstrated Target Occupancy and Efficacy in a Mouse Sub-Q Tumor Model

A.In vivo PD-L1 Target Occupancy in a MC38-human-PD-L1 Sub-Q Model



B. In vivo Tumor Growth Inhibition (TGI) in a MC38-human-PD-L1 Sub-Q Model

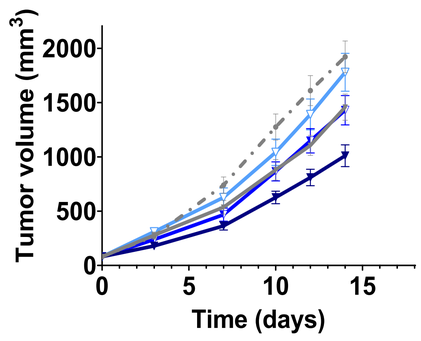
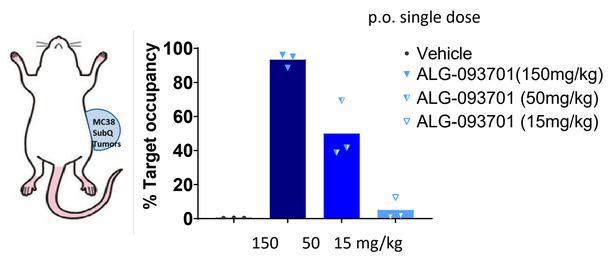


Figure 3: In vivo PD-L1 Target occupancy and anti-tumor activity of ALG-093702 in humanized-PDL1 MC38 subcutaneous tumor
hu-PD-L1 MC38 cells were implanted subcutaneously, and mice dosed with vehicle or indicated compounds. A. Target occupancy was measured at 24 hours of post dosing by FACs. B. Anti tumor activity of ALG-093702 was assessed by measuring tumor volume.

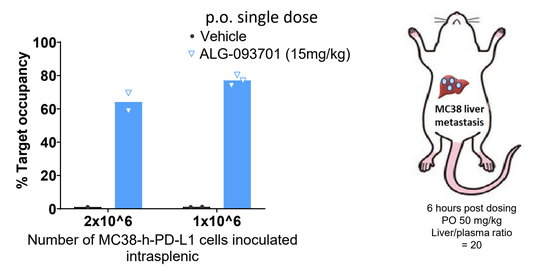
Results 5 - ALG-093702 Requires a Lower Minimum Efficacious Dose in a Liver Metastasis Model vs. a Sub-Q Model

A. In vivo PD-L1 Target Occupancy in a MC38-human-PD-L1 Sub-Q Model



15 mg/kg PO of an ALG-093702 prodrug **cannot** achieve significant PD-L1 target occupancy in Sub-Q tumors

B. In vivo PD-L1 Target Occupancy in a MC38-human-PD-L1 Liver Metastasis Model



15 mg/kg PO of an ALG-093702 prodrug **can** achieve significant PD-L1 target occupancy in liver metastasis tumors

Figure 4: In vivo PD-L1 target occupancy of ALG-093702 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, target occupancy was measured at 6 hours post dosing by FACs. B. hu-PDL1 MC38 cells were injected intra-splenic to generate liver metastasis, target occupancy was measured at 6 hours post dosing by FACs.

Results 6 - ALG-093702 Reactivates HBV-specific T-cells from an HBV-infected Patient

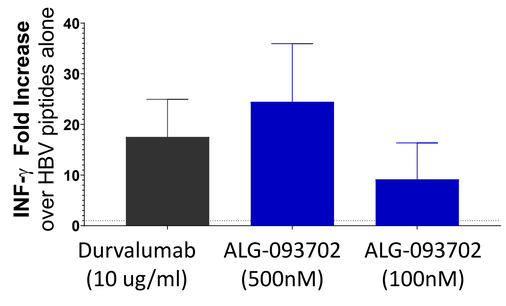


Figure 5: Ex-vivo HBV-specific T cell activity of ALG-093702
HBV-specific T cell activation assays were performed in PBMCs from an HBV-infected patient and assessed by measuring IFNγ release with ELISA.

Conclusions:

We discovered a liver-targeted PD-L1 small molecule inhibitor, ALG-093702, with a different mechanism of action of PD-1/PD-L1 blockade compared to PD-L1 antibodies. The compound blocked PD-1/PD-L1 interaction while also reducing cell surface PD-L1. ALG-093702 had similar in vivo and ex vivo potency to a PD-L1 antibody drug, durvalumab. Overall, these data suggest that ALG-093702 has the potential to mitigate immune related systemic toxicity and could potentially be used for the treatment of chronic hepatitis B, hepatocellular carcinoma and liver metastatic patients.

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