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Discovery of ALG-093989, a Highly Potent and Orally Bioavailable Small Molecule PD-L1 Inhibitor for the Treatment of Cancers

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Background

PD-1/PD-L1 antibody-based therapies have demonstrated tremendous success in the treatment of a variety of cancers. However, these antibody drugs are associated with several disadvantages, such as weak tumor penetration, immune-related adverse events (irAEs) due to their long half-life and development of anti-drug antibodies. Recently, PD-L1 small molecule inhibitors have been developed, e.g., INCB086550 which demonstrated clinical response in a phase I study.[1] Here, we report the discovery of ALG-093989, a highly potent and orally bioavailable PD-L1 small molecule inhibitor, that may overcome the limitation of PD-1/PD-L1 antibodies.

Methods

The biochemical interaction of PD-1/PD-L1 and PD-L1 dimerization was assessed by AlphaLISA®. 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. T cell viability was assessed in Jurkat T cells using Cell Titer Blue. Pharmacokinetic (PK) studies were performed in mouse, rat and Cynomolgus monkey. Percentage target engagement and cell surface PD-L1 reduction was calculated using median fluorescent intensity compared with an untreated control. In vivo PD-L1 target occupancy was assessed 6 hours following a single oral dose in a humanized-PD-L1 MC38 subcutaneous mouse model.

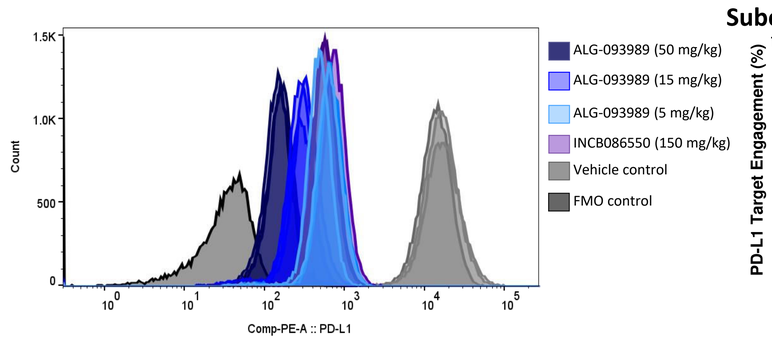
ALG-093989 is a Highly Potent and Selective PD-L1 Inhibitor

		Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-093989 PD-L1 SMi
Biochemical Activity	Human PD-1/PD-L1 Interaction IC_{50} (nM)	0.159 (n=2)	0.025 (n=2)	0.011 (n=2)	0.014 (n=1)
	Human PD-L1 Dimerization EC_{50} (nM)	No dimerization	No dimerization	66 (n=1)	13 (n=1)
Cellular Activity	Jurkat PD-1/PD-L1 Blockade EC_{50} (nM)	2.4 (n=9)	0.4 (n=10)	11.5 (n=239)	0.95 (n=21)
	Jurkat T cell viability CC_{50} (nM)	>500	>500	7166 (n=64)	7147 (n=10)
	Selectivity Index T cell CC_{50}/Blockade EC_{50}			623	7523

Table 1: Biochemical and cellular activities of ALG-093989 vs. INCB086650 and FDA-approved PD-L1 antibodies

ALG-093989 Demonstrates In Vivo Target Engagement in a Humanized PD-L1 MC38 Subcutaneous Tumor Model

A. Histogram of flow cytometry analysis



B. PD-L1 Target engagement in hPD-L1-MC38 Subcutaneous Tumors

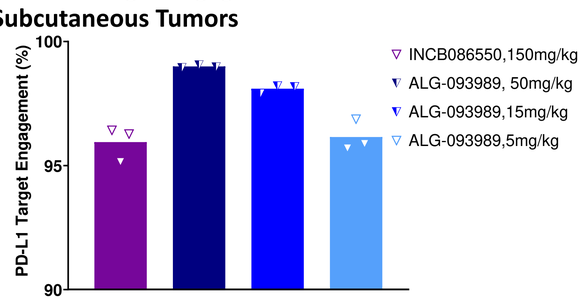
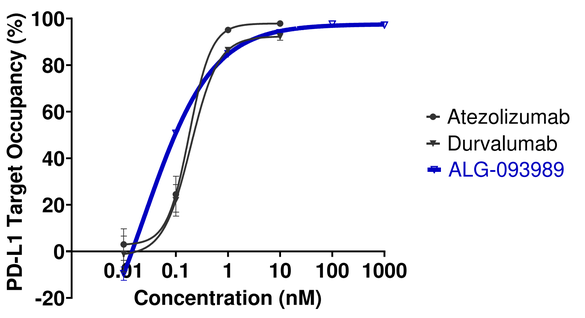


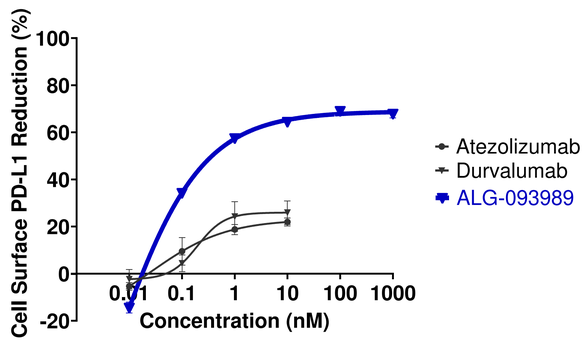
Figure 3: In vivo PD-L1 target engagement of ALG-093989 in humanized-PD-L1 MC38 subcutaneous tumor
hu-PD-L1 MC38 cells were implanted subcutaneously, and mice were dosed with vehicle or indicated compounds. Target engagement was measured 6 hours post dosing by flow cytometry.
A. Histogram of flow cytometry analysis of unoccupied h-PD-L1 on the cell surface
B. Dose-dependent increase target engagement was observed; PO 5 mg/kg ALG-093989 was as efficacious as PO 150 mg/kg INCB086550

ALG-093989 Binds Cellular PD-L1 and Reduces Cell Surface PD-L1

A. Cellular PD-L1 Target Engagement
Flow cytometry using competitive MIH1 PD-L1 antibody



B. Cellular Surface PD-L1 Reduction
Flow cytometry using non-competitive 28.8 PD-L1 antibody



	Atezolizumab	Durvalumab	ALG-093989
Target Engagement EC_{50} (nM)	0.18	0.22	0.1
PD-L1 Cell Surface Reduction EC_{50} (nM)	No effect	No effect	0.37

Figure 1: Effect of ALG-093989 vs. FDA-approved PD-L1 antibodies 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 flow cytometry using competitive MIH1 and non-competitive 28.8 anti-PDL1 antibodies, respectively.

ALG-093989 In Vitro ADME Tox Profile

A. ALG-093989 in vitro ADME profile

Caco-2 Papp (10^{-6} cm/s) A→B (Efflux Ratio)	0.32 (4.2)
Hepatocyte Stability $T_{1/2}$ (min) mouse/rat/dog/monkey/human	43/46/103/48/75
CYP Inhibition at 10 µM CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4	All < 40%
CYP3A4 PXR Activation up to 10 µM	No activation
GSH Conjugation	No adduct
PPB (% bound) mouse/rat/dog/monkey/human	>99

B. ALG-093989 in vitro toxicology profile

hERG/NaV/CaV IC_{50} (µM)	9.6 / >10 />10
AMES Screening TA98, TA100, TA1535, TA97a, WP2 uvrA, pKM101	Negative
CEREP Safety Functional Panel 78 targets	GABAA antagonist EC_{50} 18 µM Others E/IC_{50} > 10 µM
CEREP 58 Kinases at 10 µM	No significant inhibition

Table 2: In vitro ADME (A) and Tox (B) profile of ALG-093989

- Low potential for CYP450-mediated DDIs
- Low potential for generation of reactive metabolite
- No inhibition of NaV and CaV channel up to 10 µM
- Inhibition of hERG at 9.6 µM, approximately 50,000-fold over IC_{50} for PD-1/PDL-1 interaction

ALG-093989 Demonstrates Broad Tissue Distribution

A. Mouse Plasma PK Parameters

	ALG-093989
PO Dose (mg/kg)	50
C_{max} (µM)	7.30
T_{max} (hr)	1.00
$AUC_{0\text{-}12hr}$ (µM·hour)	57.5

B. Mouse Tissue Distribution at 12 hours of Post Dosing

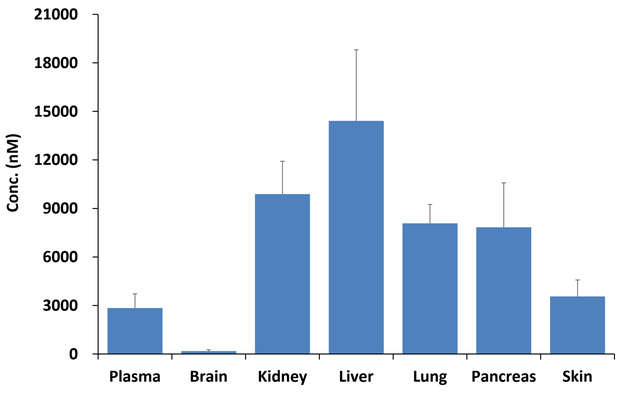


Figure 2: Mean plasma and tissue concentrations of ALG-093989 in C57BL/6 mice
A. Mouse PK parameters following a single oral dose of ALG-093989
B. Mouse tissue distribution of ALG-093989 at 12 hours of post dosing of ALG-093989

ALG-093989 Pharmacokinetic Properties

	Mouse	Rat		Monkey
	PO	IV	PO	IV
Dose (mg/kg)	5	2	15	1
C_0 or C_{max} (µM)	0.50	5.67	1.78	3.75
T_{max} (hour)	2.00	-	4.67	
Cl_obs (mL/min/kg)	-	9.48	-	8.86
Vss_obs (L/kg)	-	2.19	-	2.23
$t_{1/2}$ (hour)	2.76	2.96	3.06	4.31
$AUC_{0\text{-}inf}$ (µM·hour)	2.83	5.59	17.0	2.82
Oral Bioavailability (F%)			40%	

Table 3: ALG-093989-pharmacokinetic parameters in tested preclinical species
ALG-093989 was formulated in 50% or 60% PEG400 in water as clear solution.
PK studies were conducted in C57BL/6 mice, male Wistar Han rat (fasted for IV) and male cynomolgus monkey.

Conclusion

We have discovered ALG-093989, a highly potent PD-L1 small molecule inhibitor, which shows similar T-cell activation potency as Durvalumab, and ~10-fold improved T-cell activation potency vs. INCB086550. ALG-093989 has the similar target occupancy in mice following oral dosing at a 30-fold lower dose than INCB086550. ALG-093989 will be further evaluated as a potential candidate for drug development.

Financial Disclosure: Authors are employees of Aligos Therapeutics, Inc.

1. Koblish HK, Wu L, Wang LS, et al. Characterization of INCB086550: A Potent and Novel Small-Molecule PD-L1 Inhibitor. *Cancer Discov*. 2022;12(6):1482-1499. doi:10.1158/2159-8290.CD-21-1156