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DISCOVERY AND PRECLINICAL CHARACTERIZATION OF ALG-093940, A POTENT AND ORALLY BIOAVAILABLE SMALL MOLECULE PD-L1 INHIBITOR FOR THE TREATMENT OF CANCER

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ALIGOS THERAPEUTICS

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 that demonstrated clinical responses in a phase I study.[1] Here, we report the discovery and preclinical characterization of ALG-093940, a potent and orally bioavailable small molecule PD-L1 inhibitor, that may overcome the limitations of PD-1/PD-L1 antibodies.

METHODS

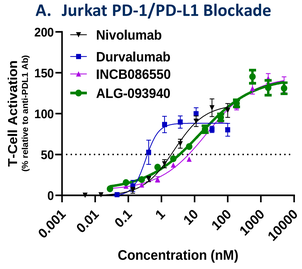
The biochemical interaction of PD-1/PD-L1 and PD-L1 dimerization was assessed by AlphaLISA®. Cellular activity was measured using a co-culture assay of PD-1 expressing Jurkat NFAT luciferase T cells with PD-L1 expressing CHO cells. In vitro ADME and the compound safety profile was established using standard assays. Pharmacokinetic (PK) studies were performed in mice, rat and cynomolgus monkey. In vivo inhibition of tumor growth, PD-L1 target occupancy and tumor infiltration of T-cells were assessed in a humanized-PD-L1 MC38 subcutaneous tumor mouse model.

ALG-093940 IS A POTENT AND SELECTIVE PD-L1 SMALL MOLECULE INHIBITOR

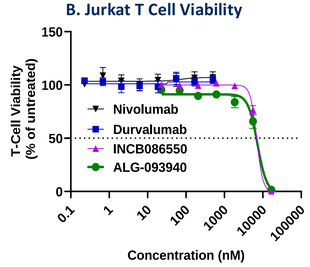
Biochemical activity	Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-093940 PD-L1 SMi
Human PD-1/PD-L1 Interaction IC_{50} (nM)	0.159 (n=2)	0.025 (n=2)	0.043 (n=3)	0.048 (n=3)
Human PD-L1 Dimerization EC_{50} (nM)	**No dimerization**	**No dimerization**	63 (n=3)	79 (n=3)

Table 1: Biochemical activities of ALG-093940 vs. FDA-approved PD-L1 antibodies and INCB086550

A. Jurkat PD-1/PD-L1 Blockade



B. Jurkat T Cell Viability

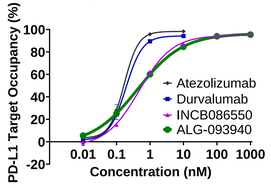


Cellular activity	Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-093940 PD-L1 SMi
Jurkat PD-1/PD-L1 Blockade EC_{50} (nM)	2.4 (n=9)	0.4 (n=10)	11 (n=239)	10.7 (n=32)
Jurkat T cell viability CC_{50} (nM)	**>500**	**>500**	**7166** (n=64)	**6969** (n=11)

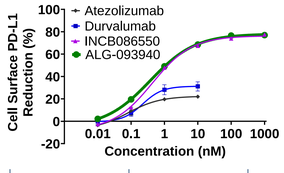
Figure 1. Cellular activities of ALG-093940 vs. FDA-approved PD-L1 antibodies and INCB086550

ALG-093940 BINDS CELLULAR PD-L1 AND REDUCES CELL SURFACE PD-L1

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



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

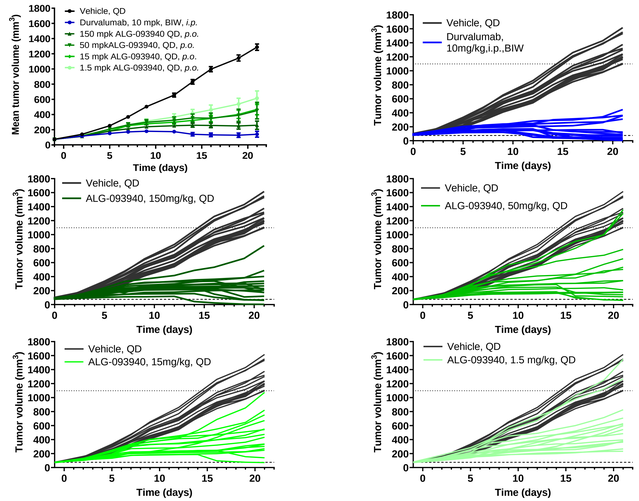


	Atezolizumab	Durvalumab	INCB086550	ALG-093940
Target Occupancy EC_{50} (nM)	0.18	0.22	0.59	0.52
PD-L1 Cell Surface Reduction EC_{50} (nM)	**<50%**	<50%	1.3	1.1

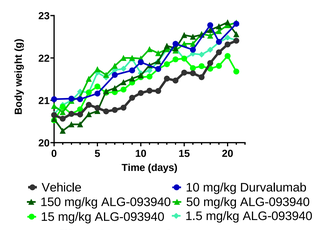
Figure 2: Effect of ALG-093940 vs. FDA-approved PD-L1 antibodies and INCB086550 on PD-L1 cell surface expression
PD-L1-expressing CHO cells were incubated for 24 hours in presence of PD-L1 inhibitors. PD-L1 target engagement (A) and PD-L1 cell surface expression (B) were assessed by flow cytometry using competitive MIH1 and non-competitive 28.8 anti-PDL1 antibodies, respectively.

ALG-093940 DEMONSTRATES DOSE DEPENDENT TUMOR GROWTH INHIBITION, PD-L1 RECEPTOR OCCUPANCY AND TUMOR INFILTRATING LYMPHOCYTES IN A HUMANIZED PD-L1 MC38 SUBCUTANEOUS MOUSE TUMOR MODEL

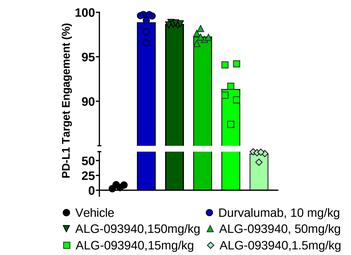
A. Tumor Growth Inhibition



B. Body weight changes



C. Receptor Occupancy 24h Post-Dosing, Day 21



D. Tumor Infiltrating Lymphocytes

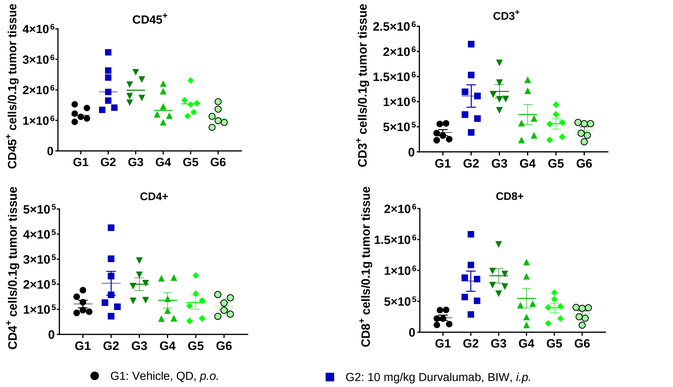


Figure 3: In vivo efficacy of ALG-093940 in a hPDL1 MC38 subQ tumor model in C57BL/6-hPDL1 mice. hPD-L1 MC38 cells were implanted subcutaneously, and mice were dosed with vehicle or indicated compounds. Dosing started at an average TV of 80 mm^3. PDL-1 receptor occupancy on $CD45^-$ cells and $CD45^+$, $CD3^+$, $CD4^+$ and $CD8^+$ tumor infiltrating lymphocytes isolated from the tumors was measured 24h after the last dose on day 21 with flow cytometry.

ALG-093940 HAS A FAVORABLE IN VITRO ADME AND TOX PROFILE

A. ALG-093940 in vitro ADME profile

Caco-2 Papp (10^{-6} cm/s) A→B (Efflux Ratio)	0,6 (2.7)
Hepatocyte Stability $T_{1/2}$ (min) mouse/rat/dog/monkey/human	All > 60
CYP Inhibition at 10 µM CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4	All < 40%
CYP3A4 PXR Activation 0.1 µM, 1.0 µM, 10 µM	No activation
GSH Conjugation	No adduct
PPB (% bound) mouse/rat/dog/monkey/human	All > 99%

B. ALG-093940 in vitro Tox profile

hERG/NaV/CaV IC_{50} (µM)	All > 10
In Vitro Micronucleus Screening in TK6 cells	Negative
AMES Screening TA98, TA100, TA1535, TA97a, WP2 uvrA, pKM101	Negative
CEREP Safety Functional Panel 78 targets E/IC_{50} (µM)	All > 10
CEREP 58 Kinases at 10 µM	No significant inhibition

Table 2: ALG-093940 in vitro ADME and Tox profile

ALG-093940 EXHIBITS FAVORABLE PHARMACOKINETIC PROPERTIES

	Mouse		Rat		Monkey	
	IV	**PO**	**IV**	**PO**	**IV**	**PO**
Dose (mg/kg)	**2.0**	**15**	**2.0**	**15**	**1.0**	**10**
C_0 or C_{max} (µM)	1.91	4.62	2.51	2.69	4.18	2.81
T_{max} (hour)		2.00	-	8.00		6.0
Cl_obs (mL/min/kg)	12.0	-	9.27	-	5.34	-
Vss_obs (L/kg)	2.86	-	2.78	-	1.94	-
$t_{1/2}$ (hour)	3.45	2.38	3.56	-	4.59	5.4
$AUC_{0\text{-}inf}$ (µM·hour)	4.14	49.6	5.36	35.5	4.82	28.4
Oral Bioavailability (F%)		160%		89%		59%

Table 3: ALG-093940 pharmacokinetic parameters in mouse, rat, and monkey. ALG-093940 was formulated in 40% -80% PEG400 in water as a clear solution. PK was performed in female C57BL/6J mouse, male Wistar Han rat (fed) and male cynomolgus monkey (fasted).

CONCLUSIONS

- **ALG-093940 is a potent PD-L1 small molecule inhibitor that blocked the interaction between PD-1 and PD-L1 with sub-nanomolar IC_{50} values in a biochemical assay. Unlike antibodies, the compound induced dimerization of PD-L1.**
- **ALG-093940 caused dose-dependent PD-L1 receptor occupancy in PD-L1 expressing CHO cells with similar efficiency to INCB086550.**
- **ALG-093940 demonstrated excellent dose-dependent tumor growth inhibition and dependent infiltration of lymphocytes, particularly CD8+ T-cells, into tumors in a humanized PD-L1 MC38 subcutaneous mouse model. At oral QD doses as low as 15 mg/kg, significant reductions in tumor volume (TV) were observed.**
- **The favorable in vitro ADME and safety profile of ALG-093940 included a low potential for CYP-450-mediated drug-drug interactions, a low potential to generate reactive metabolites and a low risk for cardiovascular liabilities and genotoxicity.**
- **Optimal PK properties were observed across all tested preclinical species, with low clearance and a moderate volume of distribution and high oral bioavailability.**
- **These favorable properties of ALG-093940 warrant further development as a potential clinical candidate for the treatment of cancer.**
- **ALG-093940 is currently advancing through pre-clinical toxicology studies**

REFERENCES

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

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