PRECLINICAL CHARACTERIZATION OF ALG-093940, A POTENT AND ORALLY BIOAVAILABLE SMALL MOLECULE PD-1/PD-L1 INHIBITOR TARGETING PD-L1 DIMERIZATION, INTERNALIZATION AND DEGRADATION

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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 and emergence of anti-drug antibodies. Here, we report the characterization of ALG-093940, a potent and orally bioavailable small molecule PD-1/PD-L1 inhibitor that binds to PD-L1 and promotes PD-L1 dimerization, internalization and degradation, offering an alternate mechanism of action with potential advantages over PD-1/PD-L1 antibody therapeutics.

METHODS

The interaction of PD-1/PD-L1 and PD-L1 dimerization were assessed by AlphaLISA®. Inhibition of PD-1/PD-L1 in cell culture was measured using PD-1 expressing Jurkat NFAT luciferase T cells and CHO-hPD-L1 cells. In vivo PK/PD/Efficacy were assessed in C57BL/6-hPDL1 mice engrafted with humanized-PD-L1 MC38 subcutaneous tumor. In vitro ADME tox profile studies used standard assays. Pharmacokinetic (PK) studies were conducted in mice, rat, dog, and cynomolgus monkeys. Safety and tolerability were evaluated following oral administration in rats and cynomolgus monkeys.

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

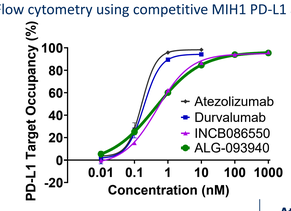
		Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-093940 PD-L1 SMi
Biochemical activity	Human PD-1/PD-L1 Interaction IC_{50} (nM)	0.159 (n=2)	0.025 (n=2)	0.043 (n=3)	0.048 (n=3)
	Human PD-L1 Dimerization EC_{50} (nM)	No dimerization	No dimerization	63 (n=3)	79 (n=3)
Cellular activity	Jurkat PD-1/PD-L1 Blockade EC_{50} (nM)	2.4 (n=9)	0.4 (n=13)	11 (n=367)	9.1 (n=34)
	Jurkat T cell viability CC_{50} (nM)	>500	>500	7800 (n=142)	6824 (n=11)

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

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

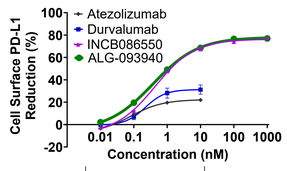
A. Cellular PD-L1 Target Occupancy

Flow cytometry using competitive MIH1 PD-L1 antibody



B. Cell Surface PD-L1 Reduction

Flow cytometry using non-competitive 28.8 PD-L1 antibody

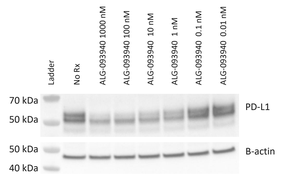


	Atezolizumab	Durvalumab	INCB086550	ALG-093940
Target Occupancy EC_{50} (nM)	0.18	0.22	0.59	0.52
PD-L1 Cell Surface Reduction EC_{50} (nM)	<50%	<50%	1.3	1.1

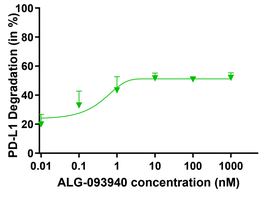
Figure 1: Effect of ALG-093940 vs. INCB086550 and FDA-approved PD-L1 antibodies on PD-L1 cell surface expression
PD-L1-expressing CHO cells were incubated for 24 hours in presence of PD-L1 inhibitors. PD-L1 target engagement (A) and PD-L1 cell surface expression (B) were assessed by flow cytometry using competitive MIH1 and non-competitive 28.8 anti-PD-L1 antibodies, respectively.

ALG-093940 INDUCES PD-L1 DEGRADATION WHICH IS REVERSIBLE AFTER TREATMENT WITHDRAWAL

A. PD-L1 Degradation Induced by ALG-093940



B. Quantification PD-L1 Degradation



C. Restoration of PD-L1 Protein Levels After Withdrawal of Treatment of ALG-093940

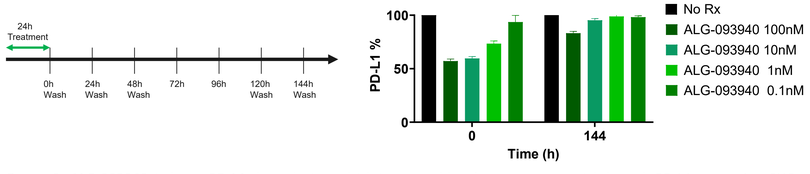
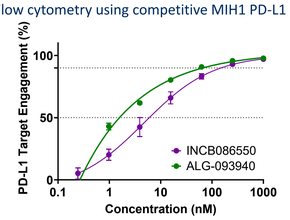


Figure 2: ALG-093940 induces PD-L1 degradation which is reversible after treatment withdrawal. PD-L1-expressing CHO cells were incubated for 24 hours in presence of ALG-093940. Western blot analysis on total protein level PD-L1 (A) and quantification (B) of PD-L1 degradation in response to increasing concentrations of ALG-093940. (C) Restoration of PD-L1 protein levels after withdrawal of treatment of ALG-093940. PD-L1-expressing CHO cells were incubated for 24 hours in presence of ALG-093940. After 24 hours treatment, the cells were washed daily to remove the compound out of the medium. Total level of PD-L1 was analyzed immediately after 24 hours treatment and 144 hours after treatment withdrawal. The total level of PD-L1 was almost completely restored 144 h after treatment withdrawal compared to no treatment condition. Total levels of PD-L1 were quantified via flow cytometry (n=3).

ALG-093940 DEMONSTRATED IN EX VIVO HUMAN PBMC ASSAYS T -CELL ACTIVATION AND IMMUNE MEDIATED CELL KILLING

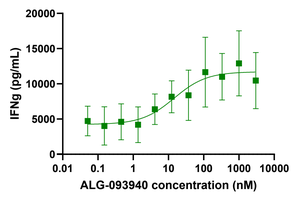
A. PD-L1 Target Occupancy in hPBMC

Flow cytometry using competitive MIH1 PD-L1 antibody



	INCB086550	ALG-093940
Target Engagement EC_{50} (nM)	6.0	1.58
Target Engagement EC_{90} (nM)	150	66

B. T-cell Activation



C. PBMC Induced Tumor Cell Killing

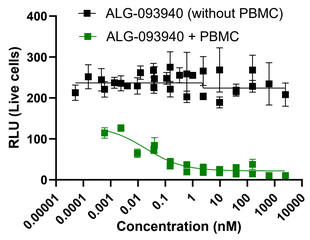
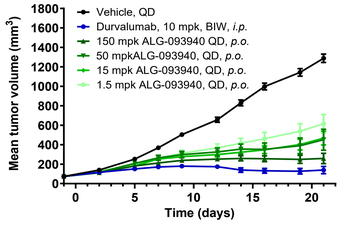


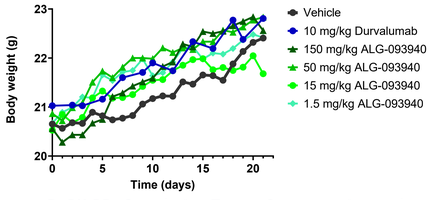
Figure 3: In ex vivo human PBMC assays, ALG-093940 demonstrated PD-L1 target engagement, T cell activation and immune cell mediated tumor cell killing. (A) hPBMC were activated with 1ng/ml IFNγ and incubated for 16 hours in presence of PD-L1 inhibitors. PD-L1 target engagement was assessed by flow cytometry using competitive MIH1 anti-PD-L1 antibodies. (B) ALG-093940 increased human T cell IFNγ secretion in a mixed lymphocyte reaction. CD4+ T cells and monocyte-derived dendritic cells from separate healthy donors were combined and IFNγ was measured in the culture supernatant after 5 days (n=4). (C) A375-eGFP cells and SEB-stimulated human PBMCs were incubated together with ALG-093940, which dose-dependently reduced the number of live cells as measured by eGFP (RLU). In the absence of PBMCs, the same concentrations of ALG-093940 had no effect on cell survival.

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

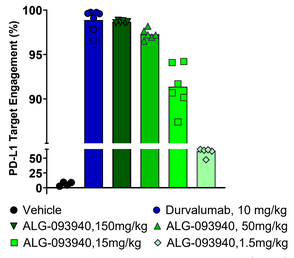
A. Tumor Growth Inhibition



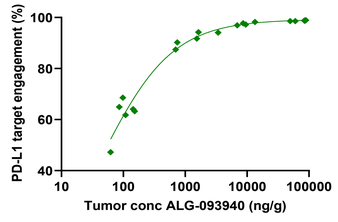
B. Body Weight Changes



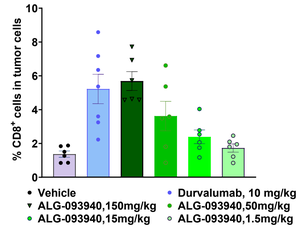
C. Receptor Occupancy 24h Post-Dosing, Day 21



D. PK-PD: Correlation Tumor Concentration and Receptor Occupancy 24h Post-Dosing, Day 21



E. Tumor Infiltrating Lymphocytes (TILs) 24h Post-Dosing, Day 21



F. PD-Efficacy: Correlation TILs and Tumor Volume 24h Post-Dosing, Day 21

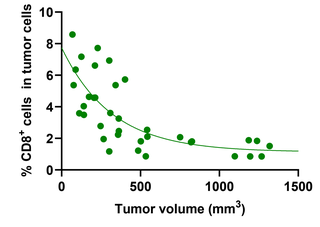


Figure 4: In vivo efficacy of ALG-093940 in hPD-L1 MC38 subQ tumor model in C57BL/6-hPD-L1 mice. hPD-L1 MC38 cells were implanted subcutaneously, and mice were dosed with vehicle or indicated compounds. Dosing started at an average TV of 80 mm3. PD-L1 receptor occupancy on CD45- cells and CD8+ tumor 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 (2.6)
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 (fed), male Wistar Han rat (fed) and male cynomolgus monkey (fasted).

ALG-093940 DEMONSTRATED A FAVORABLE TOX PROFILE

	Wistar Han Rat	Cynomolgus Monkey
Study Design	Doses of 0 (vehicle), 15, 50, 150 mg/kg/day for 14 days (N=6/sex/group), 150mg/kg/day was the limit of exposure	Single ascending phase of study with doses of 25, 75, 150, 300, 500 mg/kg followed by multiple dose phase of 300 mg/kg/day for 3 days (N=1/sex)
End Points	clinical observations, hematology, clinical chemistry, cytokine analysis, toxicokinetics and histopathology	clinical observations, hematology, coagulation, clinical chemistry, cytokine analysis and toxicokinetics
Results	Well tolerated in rats up to the highest dose tested (NOAEL) with no significant toxicity observed	Well tolerated, slight elevation of bilirubin (average ≤ 1 mg/dL) of at doses of ≥ 300 mg/kg

Table 4: ALG-093940 dose-range finding tox study results. ALG-093940 was formulated in 50 mM citrate buffer in deionized water, pH 3.3 (± 0.05)

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, internalization and degradation of PD-L1.**
- **In ex vivo human PBMC assays, ALG-093940 demonstrated PD-L1 target engagement, T cell activation and immune cell mediated tumor cell killing.**
- **ALG-093940 demonstrated excellent dose-dependent tumor growth inhibition and infiltration of $CD8^+$ 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.**
- **ALG-093940 demonstrated no in vitro liabilities for CYP-mediated drug-drug interactions, off target toxicity, cardiovascular safety, or genotoxicity.**
- **Optimal PK properties were observed across all tested preclinical species, with low clearance and a moderate volume of distribution and high oral bioavailability.**
- **ALG-093940 was well tolerated when orally administered in rats for 14 days up to the highest dose tested at 150 mg/kg/day (up to the saturation of exposure), and in cynomolgus monkeys as a single dose up to 500 mg/kg and multiple doses for 3 days at 300 mg/kg/day.**
- **These favorable properties of ALG-093940 warrant further development as a potential clinical candidate for the treatment of cancer.**

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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