PRECLINICAL CHARACTERIZATION OF ALG-094295, A HIGHLY POTENT AND ORALLY BIOAVAILABLE SMALL MOLECULE PD-1/PD-L1 INHIBITOR TARGETING DIMERIZATION, INTERNALIZATION AND DEGRADATION OF PD-L1

Heleen Roose[1], Kristina Rekstyte-Matiene[1], Sarah Stevens[2], Kusum Gupta[2], Sandra Chang[2], Nour Fayyad[1], Cheng Liu[2], Vladimir Serebryany[2], Lillian Adame[2], Kha Le[2], Antitsa Stoycheva[2], Dinah Misner[2], Lawrence M. Blatt[2], Sushmita Chanda[2], Dave Smith[2], Julian A. Symons[2], Andreas Jekle[2], Tongfei Wu[1]
[1]Aligos Belgium BV, Leuven, Belgium; [2]Aligos Therapeutics, Inc., South San Francisco, CA, USA; Contact emails: hroose@aligos.com; twu@aligos.com

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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 discovery and characterization of ALG-094295 as a highly 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 a different 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®. Cellular activity was measured using PD-1 expressing Jurkat NFAT luciferase T cells and CHO-hPD-L1 cells. In vivo PD-L1 target engagement, tumor growth inhibition and tumor infiltration of T-cells were assessed in C57BL/6-hPDL1 mice engrafted with humanized-PD-L1 MC38 subcutaneous tumor. In vitro ADME tox profile was established using standard assays. Pharmacokinetic (PK) studies were conducted in rat, dog and cynomolgus monkey.

ALG-094295 IS A HIGHLY POTENT AND SELECTIVE PD-L1 SMALL MOLECULE INHIBITOR

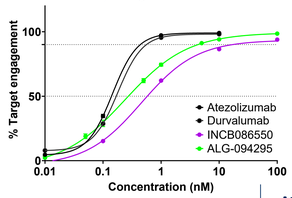
		Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-094295 PD-L1 SMi
Biochemical activity	**Human PD-1/PD-L1 Interaction** IC_{50} (nM)	0.159 (n=2)	0.025 (n=2)	0.043 (n=3)	0.089 (n=3)
	Human PD-L1 Dimerization EC_{50} (nM)	No dimerization	No dimerization	63 (n=3)	5.3 (n=3)
Cellular activity	**Jurkat PD-1/PD-L1 Blockade** EC_{50} (nM)	2.4 (n=9)	0.4 (n=13)	11 (n=367)	0.9 (n=14)
	Jurkat T cell viability CC_{50} (nM)	>500	>500	7800 (n=142)	12,335 (n=5)

Table 1: Biochemical and cellular activities of ALG-094295 vs. INCB086550 and FDA-approved PD-L1 antibodies.

ALG-094295 BINDS CELLULAR PD-L1 AND REDUCES CELL SURFACE PD-L1

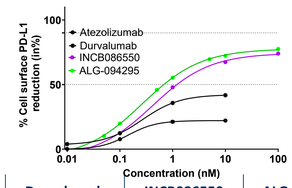
A. Cellular PD-L1 Target Occupancy

Flow cytometry using competitive MIH1 PD-L1 antibody



B. Cell Surface PD-L1 Reduction

Flow cytometry using non-competitive 28.8 PD-L1 antibody

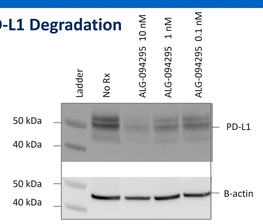


	Atezolizumab	Durvalumab	INCB086550	ALG-094295
Target Occupancy EC_{50} (nM)	0.22	0.26	0.86	0.28
PD-L1 Cell Surface Reduction EC_{50} (nM)	<50%	<50%	1.7	0.62

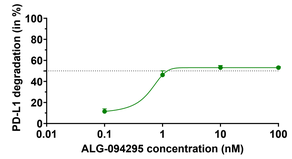
Figure 1: Effect of ALG-094295 vs. INCB086550 and FDA-approved PD-L1 antibodies on PD-L1 cell surface expression
PD-L1-expressing CHO cells were incubated for 24 hours in presence of PD-L1 inhibitors. PD-L1 target engagement (A) and PD-L1 cell surface expression (B) were assessed by flow cytometry using competitive MIH1 and non-competitive 28.8 anti-PD-L1 antibodies, respectively.

ALG-094295 INDUCES PD-L1 DEGRADATION WHICH IS REVERSIBLE AFTER TREATMENT WITHDRAWAL

A. PD-L1 Degradation



B. Quantification PD-L1 Degradation



C. Restoration of PD-L1 Protein Levels After Withdrawal of Treatment of ALG-094295

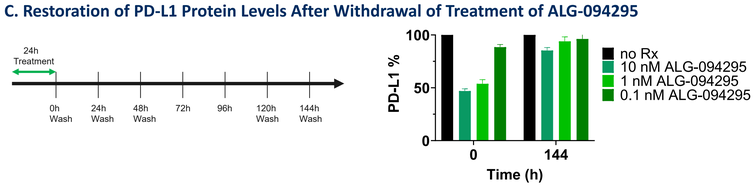
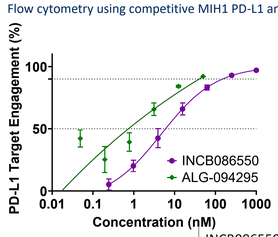


Figure 2: ALG-094295 induces PD-L1 degradation which is reversible after treatment withdrawal. PD-L1-expressing CHO cells were incubated for 24 hours in presence of ALG-094295. Western blot analysis on total protein level PD-L1 (A) and quantification (B) of PD-L1 degradation in response to increasing concentrations of ALG-094295 was measured via flow cytometry (n=4). (C) Restoration of PD-L1 protein levels after withdrawal of treatment of ALG-094295. PD-L1-expressing CHO cells were incubated for 24 hours in presence of ALG-094295. 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).

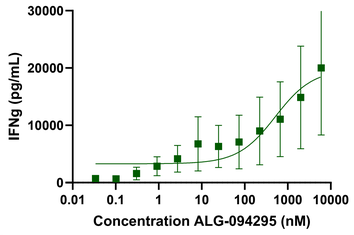
ALG-09394295 DEMONSTRATED IN EX VIVO HUMAN PBMC ASSAYS TARGET OCCUPANCY AND T -CELL ACTIVATION

A. PD-L1 Target Occupancy in hPBMC

Flow cytometry using competitive MIH1 PD-L1 antibody



B. T-cell Activation

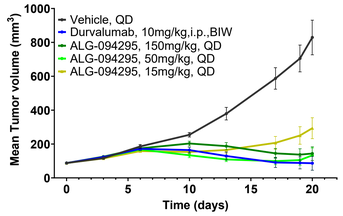


	INCB086550	ALG-094295
Target Engagement EC_{50} (nM)	6.0	0.70
Target Engagement EC_{90} (nM)	150	40

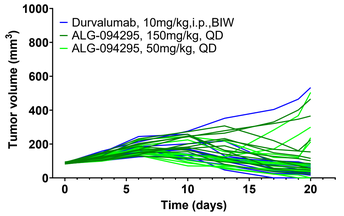
Figure 3: In ex vivo human PBMC assays, ALG-094295 demonstrated PD-L1 target engagement and T cell activation.
(A) hPBMC were activated with 1ng/ml IFNγ and incubated for 16 hours in presence of PD-L1 inhibitors. PD-L1 target engagement was assessed by flow cytometry using competitive MIH1 anti-PD-L1 antibodies. (B) ALG-094295 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=3).

ALG-094295 DEMONSTRATES DOSE DEPENDENT TUMOR GROWTH INHIBITION, PD-L1 RECEPTOR OCCUPANCY AND TUMOR INFILTRATING LYMPHOCYTES IN A HUMANIZED PD-L1 MC38 SUBCUTANEOUS MOUSE TUMOR MODEL

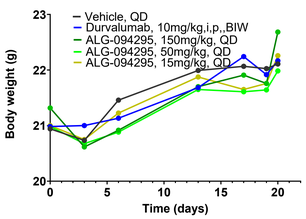
A. Tumor Growth Inhibition



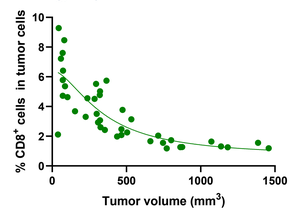
B. Individual Tumor Growth Curves



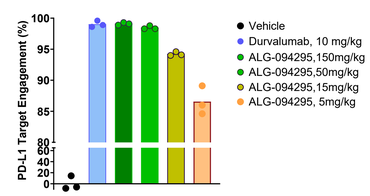
C. Body weight changes



D. PD-Efficacy: Correlation Tumor Infiltrating Lymphocytes and Tumor Volume 24h Post-Dosing, Day 21



E. Receptor Occupancy 24h Post-Single Dosing



F. PK-PD: Correlation Tumor Concentration and Receptor Occupancy 24h Post-Single Dosing

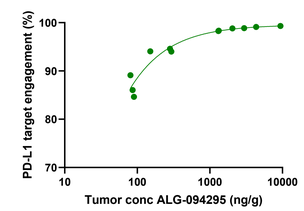


Figure 4: In vivo efficacy of ALG-094295 in hPDL1 MC38 subQ tumor model in C57BL/6-hPDL1 mice. hPD-L1 MC38 cells were implanted subcutaneously, and mice were dosed with vehicle or indicated compounds. Dosing started at an average TV of 80 mm3. PD-L1 receptor occupancy on CD45- cells (after single dose) and $CD8^+$ tumor infiltrating lymphocytes isolated from the tumors (after the last dose) was measured 24h post dosing with flow cytometry.

ALG-094295 HAS A FAVORABLE IN VITRO ADME AND TOX PROFILE

A. ALG-094295 *in vitro* ADME profile

Liver microsomal Stability $T_{1/2}$ (min) mouse/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%

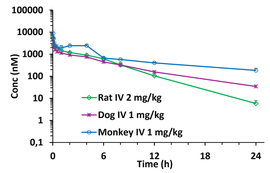
B. ALG-094295 in *vitro* Tox profile

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

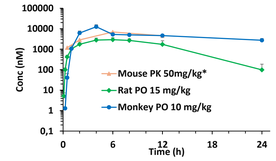
Table 2: ALG-094295 in vitro ADME and Tox profile

ALG-094295 EXHIBITS FAVORABLE PHARMACOKINETIC PROPERTIES

A. IV PK in Preclinical Species



B. PO PK in Preclinical Species



*Abbreviated PK with last collection at 12 h

	Mouse	Rat		Dog	Monkey	
	PO	IV	PO	IV	IV	PO
Dose (mg/kg)	50	2.0	15	1.0	1.0	10
C_0 or C_{max} (µM)	6.93	9.29	2.93	7.57	12.6	12.5
T_{max} (hour)	6.0	-	5.33	-	-	4.00
Cl_obs (mL/min/kg)	-	4.90	-	2.63	1.03	-
Vss_obs (L/kg)	-	1.17	-	0.95	0.62	-
$t_{1/2}$ (hour)	-	2.77	3.09	4.83	10.0	20.3
$AUC_{0\text{-}inf}$ (µM·hour)	56.3*	9.19	33.6	8.44	19.1**	112**
Oral Bioavailability (F%)	NA		48.7			58.5

Table 3: ALG-094295 pharmacokinetic parameters in mouse, rat, and monkey. ALG-094295 was formulated in 80% PEG400 in water as a clear solution. PK was performed in female C57BL/6J mouse (fed), male Wistar Han rat (fed), beagle dog (fasted), and male cynomolgus monkey (fasted).*$AUC_{0\text{-}12}$ as collections were through 12 h. **AUC_{last}

CONCLUSIONS

- **ALG-094295 is a highly 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-094295 demonstrated PD-L1 target engagement and T-cell activation.**
- **ALG-094295 demonstrated excellent dose-dependent tumor growth inhibition in a humanized PD-L1 MC38 subcutaneous mouse model. Oral dosing of ALG-094295 achieved similar efficacy as durvalumab.**
- **ALG-094295 demonstrated PK/PD/efficacy correlation; ALG-094295 concentration in tumor correlates with target engagement (PK-PD), and cytotoxic $CD8^+$ T-cell infiltration correlates with tumor volume (PD-efficacy).**
- **ALG-094295 has no in vitro liabilities for CYP-mediated drug-drug interactions, off target toxicity, cardiovascular safety, or genotoxicity.**
- **ALG-094295 PK across species is characterized by low clearance, low to moderate volume of distribution, good oral bioavailability and plasma half-life suggestive of QD potential in human.**
- **These favorable properties of ALG-094295 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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