Abstract Number: 1382

# Discovery of ALG-094103, a Liver-Targeted and Orally Bioavailable Small Molecule PD-L1 Inhibitor for the Treatment of Liver Cancer

Heleen Roose[1], Kristina Rekstyte-Matiene[1], Sarah Stevens[2], Kusum Gupta[2], Sandra Chang[2], Cheng Liu[2], Vladimir Serebryany[2], Lillian Adame[2], Kha Le[2], Antitsa Stoycheva[2], Lawrence M. Blatt[2], Leonid Beigelman[2], Sushmita Chanda[2], David B. 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*

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

## Background

PD-1/PD-L1 antibody-based therapies have demonstrated success in the treatment of liver cancers. Most systemic immune-related adverse events (irAEs) associated with PD-1/PD-L1 antibodies are mild to moderate, but severe irAEs can be life threatening, due to the long half-lives of antibodies. Recently, PD-L1 small molecule inhibitors (SMi) have been developed, e.g., INCB086550 which demonstrated clinical response in a phase I study.[1] Here, we report the discovery of an orally bioavailable PD-L1 small molecule inhibitor, ALG-094103, which preferentially partitions into the liver and may thereby mitigate extra-hepatic irAEs.

## 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) and tissue distribution studies were performed in C57BL/6 mice and PK in Wistar Han rats 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.

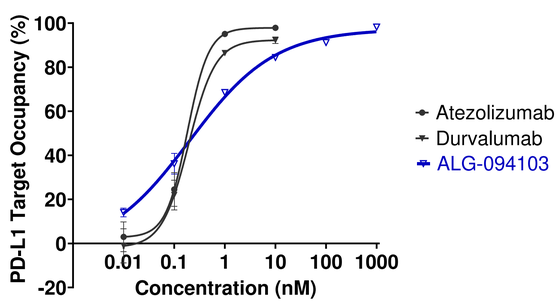
## ALG-094103 is a Potent and Selective PD-L1 Small Molecule Inhibitor

| | | Nivolumab PD-1 antibody | Durvalumab PD-L1 antibody | INCB086550 PD-L1 SMi | ALG-094103 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) | 0.012 (n=3) |
| | Human PD-L1 Dimerization $EC_{50}$ (nM) | No dimerization | No dimerization | 63 (n=3) | 143 (n=3) |
| Cellular Activity | Jurkat PD-1/PD-L1 Blockade $EC_{50}$ (nM) | 2.4 (n=9) | 0.4 (n=10) | 11 (n=239) | 11 (n=15) |
| | Jurkat T cell viability $CC_{50}$ (nM) | >500 | >500 | 7166 (n=64) | 31438 (n=6) |
| | Selectivity Index T cell $CC_{50}$/Blockade $EC_{50}$ | | | 623 | 2715 |

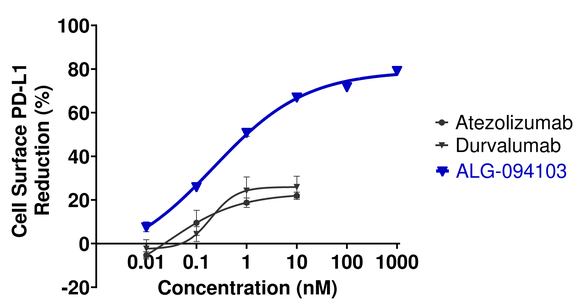
**Table 1: Biochemical and cellular activities of ALG-094103 vs. FDA-approved PD-L1 antibodies and INCB086550**

## ALG-094103 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. Cellular Surface PD-L1 Reduction**
Flow cytometry using non-competitive 28.8 PD-L1 antibody

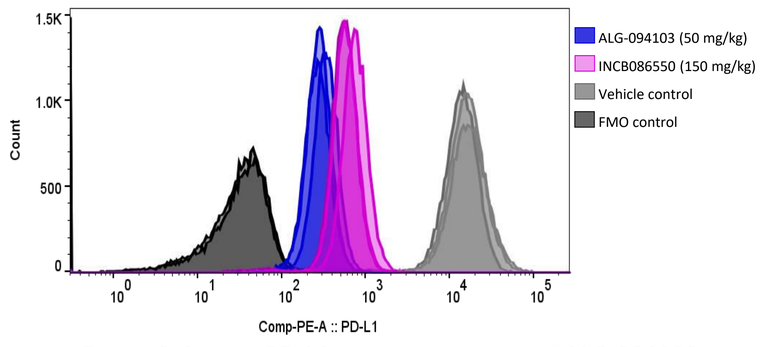



| | Atezolizumab | Durvalumab | ALG-094103 |
|---|---|---|---|
| Target Occupancy $EC_{50}$ (nM) | 0.18 | 0.22 | 0.26 |
| PD-L1 Cell Surface Reduction $EC_{50}$ (nM) | No effect | No effect | 1.0 |

**Figure 1: Effect of ALG-094103 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 (A) and PD-L1 surface expression (B) were assessed by flow cytometry using competitive MIH1 and non-competitive 28.8 anti-PDL1 antibodies, respectively.

## ALG-094103 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**

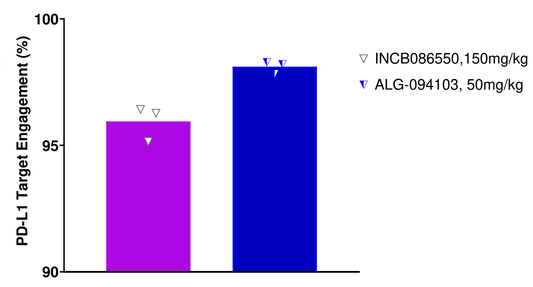



**Figure 3: In vivo PD-L1 target occupancy of ALG-094103 in humanized-PD-L1 MC38 subcutaneous tumor**
hu-PD-L1 MC38 cells were implanted subcutaneously, and mice were dosed with vehicle or indicated compounds.
A. Histogram of flow cytometry analysis of unoccupied h-PD-L1 on the cell surface
B. PD-L1 Target engagement of 50 mg/kg ALG-094103 was more efficacious than 150 mg/kg INCB086550

## ALG-094103 Has a Favorable In Vitro ADME Tox Profile

**A. ALG-094103 in vitro ADME profile**

| | |
|---|---|
| Caco-2 Papp ($10^{-6}$ cm/s) A→B (ER) | 1.1 (18.5) |
| Liver Microsomal Stability $T_{1/2}$ (min) mouse/rat/dog/Monkey/human | All > 60 |
| CYP Inhibition @ 10 µM CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 | All < 20% |
| CYP3A4 PXR Activation 0.1 µM, 1.0 µM, 10 µM | No activation |
| GSH Conjugation | No adduct |
| PPB (% bound) mouse/rat/dog/Monkey/human | 96.06 -98.30 |

**B. ALG-094103 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-094103 in vitro ADME Tox profile**

- Low potential for CYP450-Mediated DDIs
- Low potential for generating reactive metabolite
- Low potential for cardiovascular safety liability, gene tox liability and other safety related off target effects

## ALG-094103 Exhibits Liver Targeted Tissue Distribution

**A. Mouse Plasma PK Parameters**

| | ALG-094103 |
|---|---|
| PO Dose (mg/kg) | 50 |
| $C_{max}$ (µM) | 21.1 |
| $T_{max}$ (hour) | 0.50 |
| $AUC_{0\text{-}12hr}$ (µM.hour) | 82.5 |

**B. Mouse Tissue Distribution at 12 hours of Post Dosing**

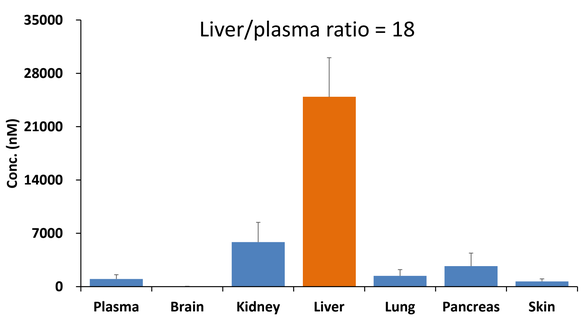



**Figure 2: Mean plasma and tissue concentrations of ALG-094103 in C57BL/6 mice**
A. Mouse PK parameters following a single oral dose of ALG-094103
B. Mouse tissue distribution of ALG-094103 at 12 hours of post dosing of ALG-094103

## ALG-094103 Exhibits Favorable Pharmacokinetic Properties

| | Rat | | Monkey | |
|---|---|---|---|---|
| | IV | PO | IV | PO |
| Dose (mg/kg) | 2.0 | 15 | 1.0 | 10 |
| $C_0$ or $C_{max}$ (µM) | 6.34 | 1.16 | 22.2 | 22.1 |
| $T_{max}$ (hour) | - | 3.00 | | 2.67 |
| Cl_obs (mL/min/kg) | 23.1 | - | 3.27 | - |
| Vss_obs (L/kg) | 1.82 | - | 0.63 | - |
| $t_{1/2}$ (hour) | 1.06 | 2.08 | 3.78 | 4.63 |
| $AUC_{0\text{-}inf}$ (µM·hour) | 2.33 | 6.97 | 9.26 | 106 |
| Oral Bioavailability (F%) | | 40% | | 115% |

**Table 3: ALG-094103 pharmacokinetic parameters in rats and monkeys**
ALG-094103 was formulated in 40% -60% PEG400 in water as a clear solution.
PK was performed in male Wistar Han rat and cynomolgus monkey, fasted for IV.

## Conclusion

We have discovered ALG-094103 as a novel liver-targeted and orally bioavailable PD-L1 small molecule inhibitor. The properties of ALG-094103 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