AASLD The Liver Meeting® Nov. 10-14, 2023

Discovery of a Liver Targeted Oral PD-L1 Small Molecule Inhibitor for the Treatment of Chronic Hepatitis B and 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, USA; Contact emails: hroose@aligos.com; twu@aligos.com

ALIGOS THERAPEUTICS

Poster #: 1466-C

Background

PD-1/PD-L1 antibody-based therapies have demonstrated success in the treatment of liver cancer and have exhibited potential for achieving functional cure of chronic hepatitis B (CHB)[1]. However, the systemic immune-related adverse events (irAEs) associated with PD-1/PD-L1 antibodies can be life threatening, due to their long half-lives. Compared to antibodies, the shorter half life of PD-L1 small molecule inhibitors (SMi) might bring advantages for managing irAEs. Recently, PD-L1 small molecule inhibitors have been developed, e.g., INCB086550 which demonstrated clinical response in cancer in a phase I study.[2]

Our first-generation liver targeted PD-L1 small molecule inhibitor, ALG-093702, activated PBMC from an HBV-infected patient ex vivo with similar activity to durvalumab and demonstrated in vivo antitumor efficacy in a human PD-L1 MC38 subcutaneous mouse model. Further optimization led to our next generation liver targeted PD-L1 SMi, ALG-094103, which has similar in vitro potency to ALG-093702 with excellent oral bioavailability.

Methods

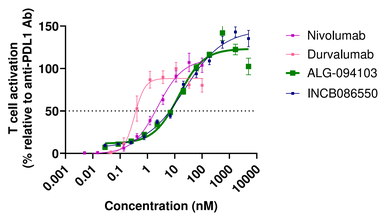
The biochemical interaction of PD-1/PD-L1 and PD-L1 dimerization was assessed by AlphaLISA®. Cellular activity was measured using a co-culture reporter assay in which TCR-mediated NFAT activity of Jurkat T cells is constitutively inhibited by the engagement of PD-1 by PD-L1 expressing CHO cells. T cell 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

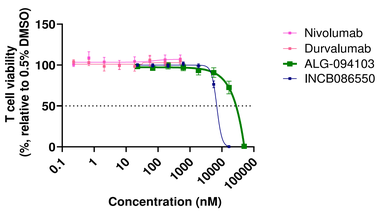
Biochemical activity	Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-094103 PD-L1 SMi
Human PD-1/PD-L1 Interaction IC_{50} (nM)	0.159 (n=2)	0.025 (n=2)	0.043 (n=3)	0.012 (n=3)
Human PD-L1 Dimerization EC_{50} (nM)	No dimerization	No dimerization	63 (n=3)	143 (n=3)

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

A. Jurkat PD-1/PD-L1 Blockade



B. Jurkat T Cell Viability



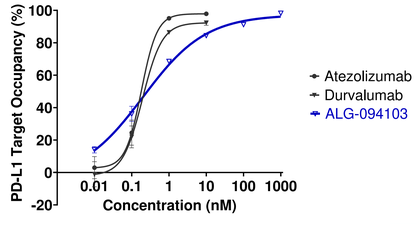
Cellular activity	Nivolumab PD-1 antibody	Durvalumab PD-L1 antibody	INCB086550 PD-L1 SMi	ALG-094103 PD-L1 SMi
Jurkat PD-1/PD-L1 Blockade EC_{50} (nM)	2.4 (n=9)	0.4 (n=10)	11 (n=239)	11 (n=15)
Jurkat T cell viability CC_{50} (nM)	>500	>500	7166 (n=64)	31438 (n=6)

Figure 1. 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

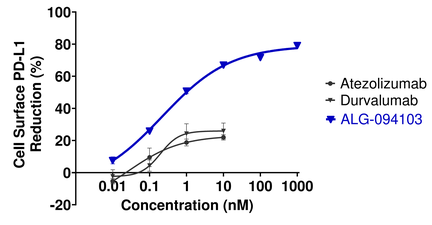
A. Cellular PD-L1 Target Occupancy

Flow cytometry using competitive MIH1 PD-L1 antibody



B. Cell 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 2: 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 cell surface expression (B) were assessed by flow cytometry using competitive MIH1 and non-competitive 28.8 anti-PDL1 antibodies, respectively.

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

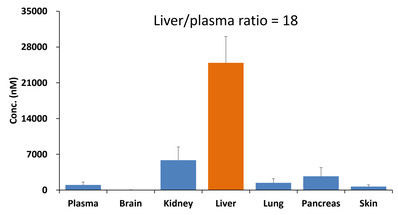
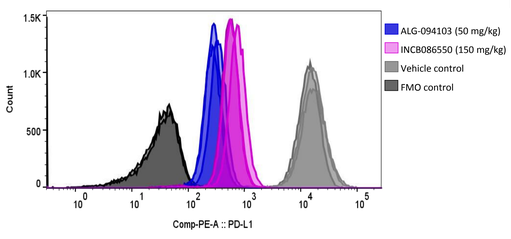


Figure 3: Mean plasma and tissue concentrations of ALG-094103 in C57BL/6 mice
A. Mouse PK parameters following a single oral dose of ALG-094103
B. Mouse tissue distribution of ALG-094103 at 12 hours of post dosing of ALG-094103

ALG-094103 Demonstrates In Vivo Target Occupancy in Humanized PD-L1 MC38 Subcutaneous Tumor Model

A. Histogram of flow cytometry analysis



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

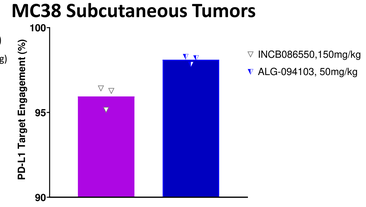


Figure 4: In vivo PD-L1 target occupancy of ALG-094103 in humanized-PD-L1 MC38 subcutaneous tumor
hu-PD-L1 MC38 cells were implanted subcutaneously, and mice were dosed with vehicle or indicated compounds.
A. Histogram of flow cytometry analysis of unoccupied h-PD-L1 on the cell surface
B. PD-L1 target 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 (Efflux Ratio)	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	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's in vitro ADME Tox profile

- Low potential CYP450-mediated DDIs
- Low potential for generating reactive metabolites
- Low potential for cardiovascular safety liability, genotoxicity and other safety related off target effects

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.

Disclosure: Authors are employees of Aligos Therapeutics, Inc.

1. Guiqiang Wang et. al, Abstract #OS091, The International Liver Congress™ EASL – European Association for the Study of the Liver, June 22-26, 2022
2. 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