Novel, heterocyclic small molecule inhibitors of PD-1/PD-L1 pathway

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SUMMARY

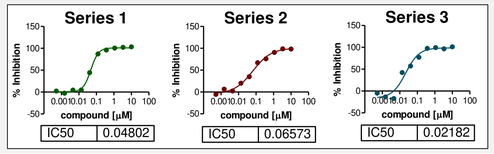
The PD-1/PD-L1 molecular pathway is one of the primary mechanisms of immune evasion deployed by cancer cells. Induction of PD-L1 expression on cancer cells is associated with inhibition of immune responses against cancer, thus permitting cancer progression and metastasis. Activation of PD-1/PD-L1 pathway induces apoptosis of activated T-cells, inhibits their proliferation, facilitates T-cell anergy and exhaustion and enhances the function of regulator T-cells. Therefore, blocking this pathway restores the proliferation and cytotoxicity of CTLs, inhibits the function of Tregs and results in decreased T-cell apoptosis. Although a number of therapeutic antibodies targeting PD-1/PD-L1 have been developed and approved for a number of malignancies, there is a still a need for potent, selective small molecule inhibitors of the PD-1/PD-L1 pathway.

Rational and structure guided *de novo* design approaches were used to design novel small molecule PD-1/PD-L1 pathway inhibitors; potency of these inhibitors was assessed in an *in-vitro* TR-FRET assay. Checkpoints signaling reporter assays as well as *ex-vivo* co-culture assays were used to assess the ability of the compounds to restore T-cell proliferation and function.

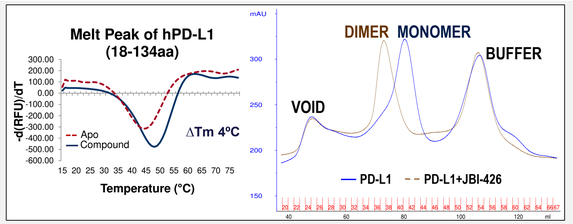
Three novel chemical series as potent PD-1/PD-L1 pathway inhibitors are being developed for the treatment of cancer. A representative inhibitor JBI-426 showcased here exhibited low nM potency *in vitro* and no cytotoxicity against cancer cell proliferation *per se*. JBI-426 showed good *in vitro* ADME properties in terms of aqueous solubility, metabolic stability, permeability and excellent oral bioavailability in mouse pharmacokinetics. In a RENCA syngeneic model, oral administration of JBI-426 at 50 mg/kg resulted in a strong tumor growth inhibition, comparable (or better) than the PD-L1 mAb, and was well tolerated. The effect of JBI-426 on tumor infiltrating lymphocytes was also assessed; a significant increase in CD8+ cytotoxic lymphocytes was observed. The anti-tumor effect of JBI-426 was also exhibited in CT-26 syngeneic model.

Biochemical characterization

	Two representative compounds		
	Series 1	Series 2	Series 3
IC_{50} (μM)	0.048	0.066	0.022
	0.043	0.083	0.039

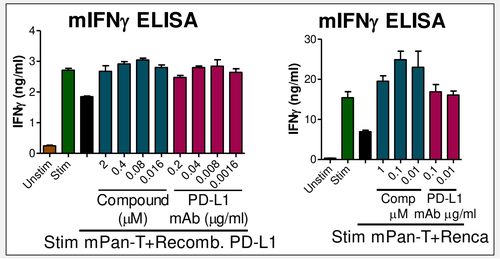


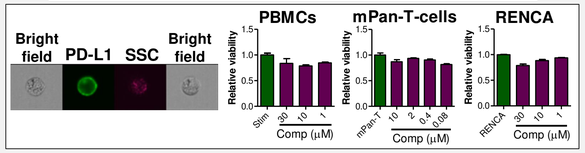
PD-1/PD-L1 inhibitor induces dimerization of PD-L1



PD-1/PD-L1 inhibitor induces dimerization of PD-L1 as indicated by SEC

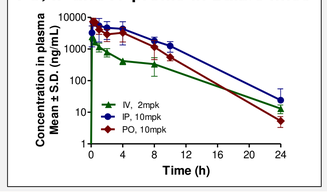
PD-1/PD-L1 inhibition restores T-cell function





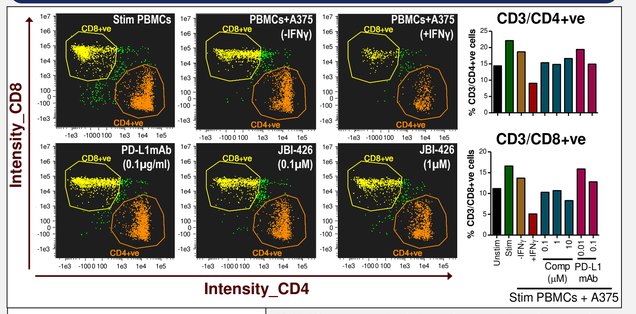
Top: Purified T-cells were isolated from 6-8wk C57BL/6 female mice and co-cultured with either Recombinant PD-L1 protein or RENCA cells. Secreted IFNγ levels was assessed by ELISA. *Bottom:* PD-L1 is endogenously expressed in RENCA cells; JBI-426 does not affect the viability of PBMCs, murine T-cells or RENCA

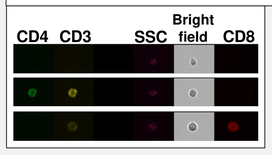
Pharmacokinetic profile of JBI-426



Parameter, PO	Mice
$t_{1/2}$ (hr)	2.07
Clearance (ml/min/kg)	3.98
C_{max} (ng/ml)	7395
$AUC_{(0-t)}$ (ng*hr/ml)	29715
$AUC_{(0-\infty)}$ (ng*hr/ml)	29731
t_{max} (hr)	0.25
t_{last} (hr)	24

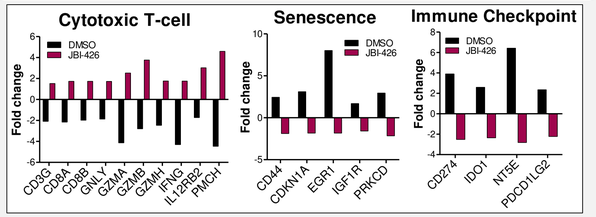
PD-1/PD-L1 inhibition leads to restoration of CD4+ and CD8+ T-cells





PBMCs were isolated from a healthy volunteer and co-cultured with A375 cells, treated with IFNγ. Post compound treatment for 48H, PBMCs were stained with CD3-PE, CD4-FITC and CD8-APC to assess the T-cell population. Image-based FACS acquisition on Amnis® Flowsight

PD-1/PD-L1 inhibition restores gene expression across immune subsets



Gene	PBMCs vs. CC	CC vs. JBI-426
PMCH	-4.47	4.59
IFNG	-4.3	1.75
GZMA	-4.14	2.53
IL17F	-3.47	8.09
LRRN3	-3.25	2.08
BIRC5	-3.23	2.19
CDK1	-2.87	2.06
TTK	-2.84	2.09
DPP4	-2.84	2.65
GZMB	-2.8	3.76
IL32	-2.75	1.96
ICOS	-2.57	1.75
GZMH	-2.46	1.81
TUBB	-2.42	1.5
PBK	-2.34	1.81
HAVCR2	-2.18	2.38
CD8A	-2.15	1.73
CD3G	-2.09	1.53
LTB	-1.99	1.92
CD8B	-1.97	1.73

Gene	PBMCs vs. CC	CC vs. JBI-426
IL1RL1	9.49	-7.87
IL13RA2	89.66	-7.6
CT45A1	117.66	-5.55
AXL	69.78	-5.44
APOE	4.71	-4.87
MAGEA3	28.3	-4.77
THBS1	13.43	-4.52
C1S	24.6	-4.23
TLR4	4.46	-4.13
TLR8	5.64	-3.82
IL24	174.05	-3.79
C2	7.03	-3.7
FCGR2A	3.94	-3.7
LRP1	5.39	-3.68
CLEC5A	4.46	-3.68
SPANXB1	18.17	-3.67
CYBB	6.7	-3.63
CD276	8.73	-3.48
APP	8.77	-3.34
LILRB3	5.48	-3.33

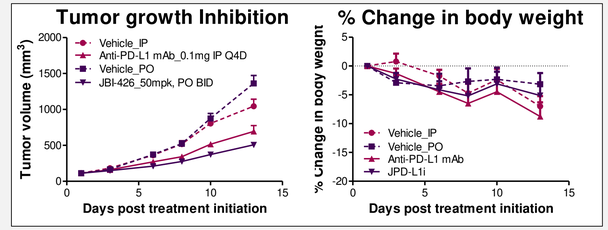
Upregulated Pathways	FDR
Natural killer cell mediated cytotoxicity	5.16e-02
T cell activation	1.8e-01
Jak-STAT signaling pathway	8.65e-01

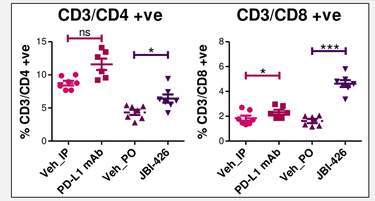
Downregulated Pathways	FDR
Chemokine signaling pathway	1.57e-01
PI3K-Akt signaling pathway	5.87e-01

CC: Co-culture

KEGG pathway analysis of differentially expressed genes in PBMCs co-cultured with A375, in the presence or absence of JBI-426

In vivo efficacy of JBI-426 in RENCA syngeneic model; increase in TILs

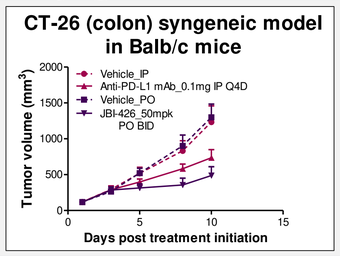




Antitumor activity of JBI-426 (administered 50mpk, BID) and Anti-PD-L1 mAb (0.1mg/mouse, Q4D) in RENCA syngeneic model.

TILs were assessed in the two treatment groups: JBI-426 administration resulted in a more significant increase in CD8+ TILs as compared to anti-PD-L1 mAb

JBI-426 is efficacious in CT-26 syngeneic model



Conclusions

Small molecule PD-1/PD-L1 inhibitors, in contrast to antibody therapies, can provide increased oral bioavailability, increased bio-efficiency and shorted half life activity for a more controllable treatment, particular in the case of auto-immune or other adverse effects.

Further studies to assess additional compounds from the three chemical series are underway. The oral administration route of these PD-1/PD-L1 inhibitors would provide an attractive alternate to the currently available antibodies in treating cancer either as a stand-alone therapy or in combination with other immuno-modulatory agents, as well as other standard of care agents.

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