Novel, small molecule inhibitors of PD-L1/PD-1 interaction

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Abstract Control # 1917

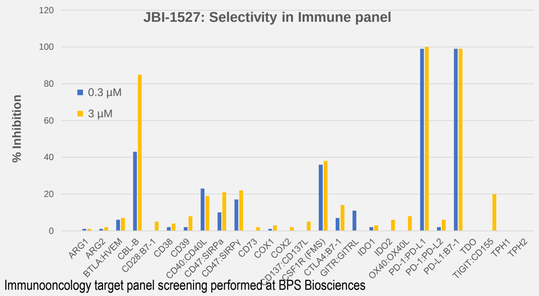
SUMMARY

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-1527 showcased here exhibited low nM potency *in vitro* and also highly selective against 27 immuno oncology targets. JBI-1527 also induces the dimerization of the protein and subsequent internalization of PD-L1 protein, thereby alleviating PD-L1-induced suppression of T cell activation. In Hs746T cells, JBI-1527 treatment showed the inhibitor competes with anti-PD-L1 blocking antibody. Similarly, in PD-L1 overexpressed tumor cells JBI-1527 reduced the recombinant PD-1 to PD-L1. In the Bio Map cancer panel screen, JBI-1527 showed the enhanced levels of IFNγ, IL-6, TNF-α, IP-10, Col-1, tPA and Keratin 20 and these modulation were similar to the Pembrolizumab. In a CT-26 syngeneic model, oral administration of JBI-1527 at 50 mg/kg resulted in a strong tumor growth inhibition, comparable to Anti-PD-L1 mAb, and was well tolerated.

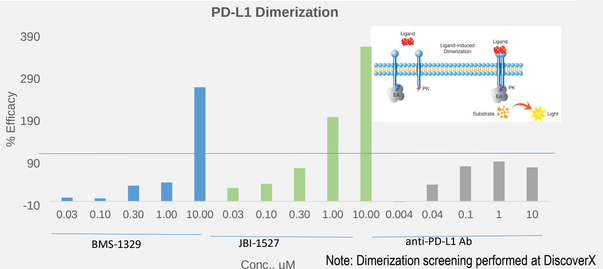
JBI-1527 is selective for PD-L1



Note: Immunooncology target panel screening performed at BPS Biosciences

JBI-1527 screened against 27 IO targets. JBI-1527 binds selectively to PD-L1 and only inhibits interactions between PD1/PD-L1 and B7-1/PD-L1; moderate binding with CBL-B observed; follow-up study clearly indicated that JBI-1527 did not have any inhibitory activity on CBL-B

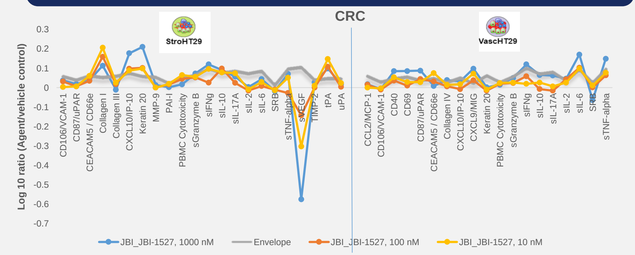
PD-1/PD-L1 inhibitor induces dimerization of PD-L1 (U2OS cells)



Note: Dimerization screening performed at DiscoverX

U20S cells engineered to co-express one receptor subunit fused to Enzyme Donor (ED), and a second dimer partner fused to Enzyme Acceptor (EA). Binding of an agonist to one receptor subunit induces it to interact with its dimer partner, which results the functional enzyme hydrolyzes a substrate to generate a chemiluminescent signal. JBI-1527 induces dimerization of PDL-1 in a concentration dependent manner

BioMAP Colorectal Cancer Panel



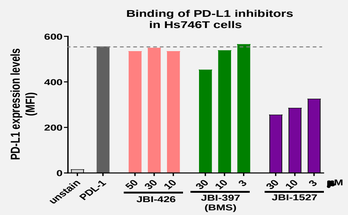
System	Cell Types
StroHT29	HT-29 colorectal adenocarcinoma cell line + Peripheral blood mononuclear cells + Primary human fibroblasts
VascHT29	HT-29 colorectal adenocarcinoma cell line + Peripheral blood mononuclear cells + Primary human endothelial cells

Biological and Disease relevance	JBI-1527	Pembrolizumab
Inflammation related	sTNF-a, IP10 ↑	sTNF-a
Matrix remodeling	Col-1↑	-
Angiogenesis	sVEGF↓, tPA↑	sVEGF↓
Tumor related	Keratin 20↑	-
Immune related	sIFNg, sIL6 ↑	sIFNg, sIL6

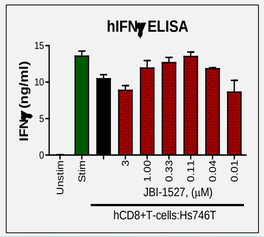
Modulation of JBI-1527 were comparable with the Pembrolizumab modulation which are shown in the above table

Note: Bio Map Panel screening performed at Eurofins

JBI-1527 competes with anti-PD-L1 blocking antibody and restores T-cell function

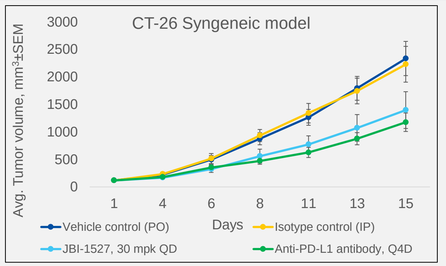


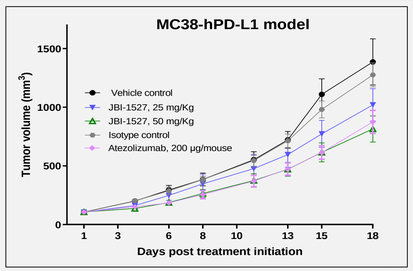
Hs746T treated with JBI-1527 for 1.5 h and incubated with Anti-PDL-1 PE (blocking) antibody for 30 min. Mean Fluorescence intensity was analyzed by FACS



Purified T-cells were isolated from human blood was and co-cultured with Hs746T cells over-expressing PD-L1 protein in the presence of JBI-1527; Secreted IFNγ levels was assessed by ELISA.

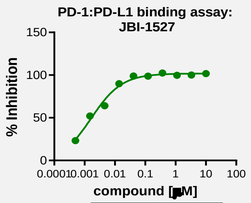
JBI-1527 is efficacious in CT-26 and MC38-hPD-L1 syngeneic model





JBI-1527 showed strong tumor growth inhibition, comparable to Anti- PD-L1 mAb/Atezolizumab, and was well tolerated

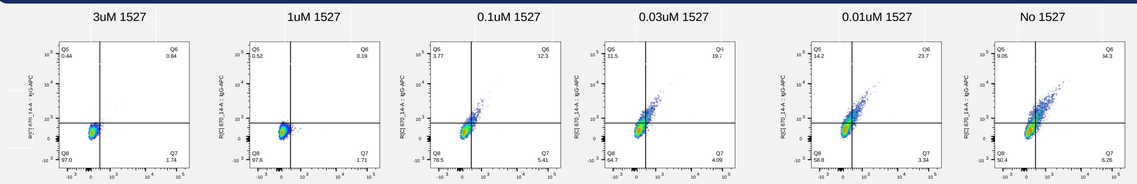
Biochemical characterization



	JBI-1527
IC50	0.001638

In vitro potency of JBI-1527 against hPD-1/hPD-L1 assessed by HTRF

JBI-1527 reduced rPD-1 binding to PD-L1 overexpressed in tumor cells



JBI-1527 was incubated with A375 cells over-expressing PD-L1 for 2h. Cells were then co-stained with recombinant PD-1 (and subsequently a fluorochrome conjugated secondary antibody that binds the Fc tail of the rhPD-1) and a fluorochrome conjugated PDL1 antibody; JBI-1527 reduced rPD-1 binding to PD-L1

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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