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

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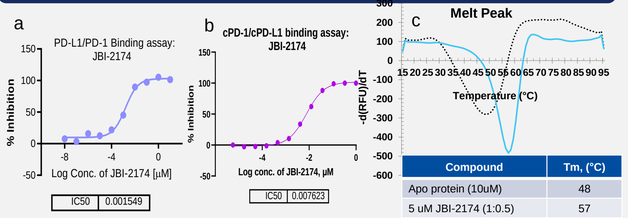
SUMMARY

One of the primary mechanisms of immune evasion deployed by cancer cells is up-regulation of PD-1/PD-L1 pathway. 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 leads to apoptosis, inhibition of proliferation and anergy of 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 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 cell based dimerization assays were used to assess the ability of the compounds to modulate PD-L1/PD-1 signaling.

Three novel chemical series as potent PD-1/PD-L1 pathway inhibitors are being developed for the treatment of cancer. JBI-2174, our lead compound, exhibited low nM potency *in vitro* and also highly selective against 27 immuno oncology targets. JBI-2174 leads to stabilization of PD-L1 as observed by a shift of Tm ~9˚C in TSA. It also induces the dimerization of the protein in cellular assays. In Hs746T cells, JBI-2174 treatment showed the inhibitor competes with anti-PD-L1 blocking antibody. Co-crystallization studies confirmed the PD-L1 dimer formation induced by JBI-2174. In Jurkat cell based PD-1-SHP1 recruitment assays, JBI-2174 effectively blocked PD-1/PD-L1 mediated signalling. In a MC-38/hPD-L1 model, JBI-2174 resulted in a strong tumor growth inhibition at as low as 3 mg/kg dose. Oral administration of JBI-2174 resulted in significant survival advantage in MC-38/hPD-L1 as well as in 005 glioma orthotopic models, with a concomitant increase in tumor infiltrating T-lymphocytes. JBI-2174 was well tolerated in toxicological studies studies and is being developed as a clinical candidate.

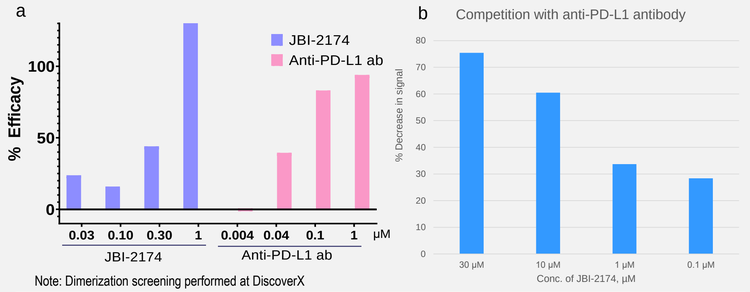
JBI-2174 is highly potent against PD-L1



Compound	Tm, (°C)
Apo protein (10uM)	48
5 uM JBI-2174 (1:0.5)	57

In vitro potency of JBI-2174 against (a) human PD-1/PD-L1 and (b) cyno PD-1/PD-L1 was assessed by HTRF; (c) Thermal shift assay with hPD-L1 protein in the presence and absence of JBI-2174

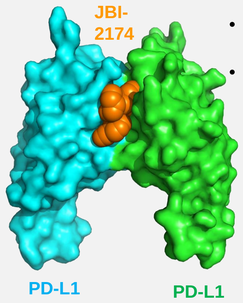
PD-L1 inhibitor induces dimerization of PD-L1 and competes with anti-PD-L1 blocking antibody



Note: Dimerization screening performed at DiscoverX

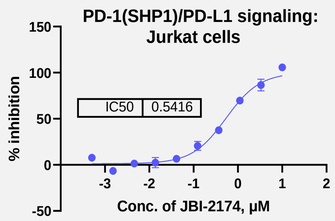
(a) U2OS cells engineered to co-express one receptor subunit fused to Enzyme Donor (ED), and a second dimer partner fused to Enzyme Acceptor (EA). Upon dimerization, functional enzyme hydrolyzes a substrate to generate a chemiluminescent signal; (b) JBI-2174 competes with anti-PD-L1 blocking antibody in a dose dependent manner as observed by FACS assay

Binding of JBI-2174 to PD-L1 is similar to anti-PD-L1 antibody



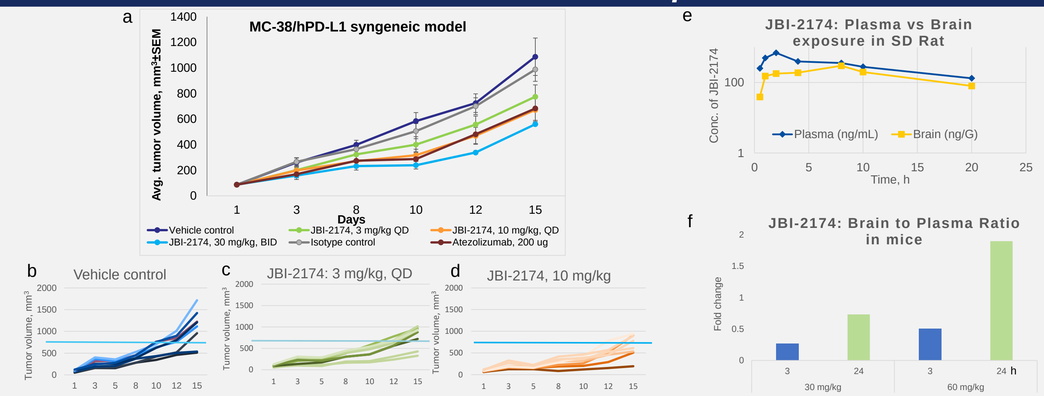
- JBI-2174 leads to dimerization of hPD-L1 as revealed by single crystal X-ray crystallography.
- Comparing **JBI-2174 bound PD-L1 crystal structure** to **PD-L1:PD1 complex crystal structure** (residues within ~4Å of interaction)
  - PD-1 has footprints of 17aa on PD-L1
  - JBI-2174 covers 65% of this footprint,
  - Known antibodies (Atezolumab, Avelumab and Durvalumab) cover 53-65% of PD-1 footprint
  - Non-specific interactions are lesser for JBI-2174 than antibodies

JBI-2174 Interrupted PD-1/PD-L1 signaling



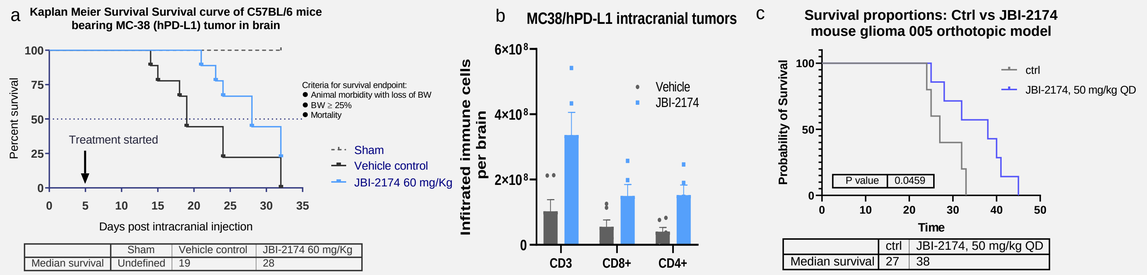
PathHunter PD-1 signaling assay in Jurkat cells (DiscoveRx)

JBI-2174 is highly efficacious in MC38/hPD-L1 syngeneic model; shows sustained brain exposure



a) JBI-2174 against is highly efficacious in MC38/hPD-L1 syngeneic model by oral administration with an ED50 of ~ 3 mg/kg; b-d) spider plots of individual animal tumor growth in 3 mg/kg and 10 mg/kg groups; e) JBI-2174 - Plasma vs Brain exposure in SD Rat at 50 mg/kg; f) JBI-2174 - Brain to plasma ratio in mice

JBI-2174 is efficacious in MC38/hPD-L1 and mouse glioma 005 orthotopic models



	Sham	Vehicle control	JBI-2174 60 mg/Kg
Median survival	Undefined	19	28

	ctrl	JBI-2174, 50 mg/kg QD
Median survival	27	38

JBI-2174 showed strong tumor growth inhibition, a) extending survival in MC38/hPD-L1 and c) 005 glioma orthotopic models ; b) enhances T-cell infiltration into the tumor

Conclusions

- Although several anti-PD-L1 mAbs have been approved for the treatment of cancers, these therapies have shown limited efficacy in CNS cancer where further efficacious treatments are the need of the hour.
- Small molecule PD-1/PD-L1 inhibitors, have the advantage of increased oral bioavailability, tumor penetrance and shorter half life for a more controllable treatment.
- In this regard, JBI-2174 is a novel, potent and selective PD-L1 inhibitor that shows strong and sustained brain exposure by oral administration. Structural and other mechanistic studies have confirmed that JBI-2174 binds and works similar to anti-PD-L1 antibodies. In vivo studies have demonstrated that JBI-2174 shows strong tumor growth inhibition and enhances tumor infiltrating T-lymphocytes.
- Exploratory toxicology studies clearly demonstrate that JBI-2174 is well tolerated; IND-enabling studies are being initiated

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