Industry Day Theme # EMERGING NANO AND ADVANCED MATERIALS

Small-Molecule Inhibitor of the Programmed Cell Death-1/ Programmed Death-Ligand 1 (PD-1/PD-L1) Interaction

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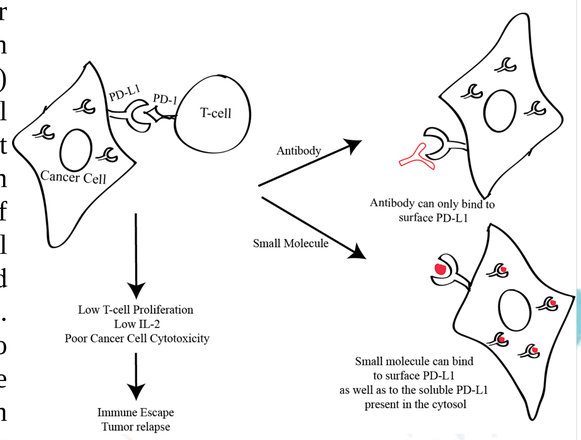
Abstract

Harnessing the immune system for cancer treatment by targeting programmed death 1/programmed death-ligand 1 (PD-1/PD-L1) pathway has made great strides as monoclonal antibodies (mAbs) against these checkpoint proteins showed durable antitumor responses in a broad spectrum of cancers. However, use of antibodies is associated with several disadvantages- expensive, immune-related adverse effects, inadequate tumor penetration. Small molecule inhibitors are the alternative to overcome these drawbacks. Here we are reporting a small molecule which can efficiently bind to PD-L1 and inhibit tumor growth by enhancing antitumor immune response.



Introduction

Blockade of immune checkpoints such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1/programmed death-ligand 1 (PD-1/PD-L1) are revolutionizing the outcome of cancer therapy. There are some anti-PD-L1 monoclonal antibodies successfully being used and approved for many cancers. However, antibodies are associated with several disadvantages which include high production cost, lower stability, poor tissue penetration and immunogenicity. To overcome these disadvantages, small molecule inhibitor development appears to be the most effective alternative. Here we report a potential small-molecule inhibitor for PD-L1 protein. When treated in mice bearing B16-F10 melanoma tumor, it demonstrated delay in tumor growth by immunomodulatory effect, such as cytotoxic T-lymphocyte (CTL) activity, T-cell proliferation and IL-2 production.

Materials and Methods

Drug and reagents. BT1 was purchased from AdooQ Bioscience, USA. Recombinant human PDL1 was purchased from Sino Biological, Antibodies and ELISA kit were purchased from Biolegend and Elabscience, USA respectively.

Mouse melanoma cell line B16-F10 was purchased from National centre for cell sciences (NCCS), India.

Animals. Male C57BL/6 J mice (5-6 weeks old) were used for all the *in vivo* experiments.

***In silico* screening of small molecule inhibitor.** Thousands of small molecules were screened for their binding efficacy with PD-L1 protein. Based on their binding affinity and stability, few molecules were shortlisted for further studies.

Cell free binding assay: Based on computational parameters, most efficient molecule BT1 was selected for cell free binding assay. Fluorescence quenching assay was performed to analyse the binding efficacy of drug with soluble PD-L1.

***In vivo* efficacy:** The *in vivo* anti-tumor efficacy and immunomodulatory effect of BT1 was evaluated in mice bearing B16-F10 melanoma with a dose of 15 mg/kg BW daily for 10 days.

Conclusion

- BT1 treatment shown significant tumor growth delay compared to vehicle control. This is also observed in their tumor weights.
- Our study shown that more CD4+ and CD8+ T cells were found in spleen of the mice treated with BT1 than the control group.
- The antitumor effect of the BT1 can be further explained by the enhanced cytotoxic activity of CTL isolated from spleen of treated mice when compared to that of vehicle treated mice.
- BT1 induced T cells to produce more IL-2 in splenocytes and in tumor compared to the vehicle control.
- Although elevated IL-2 in spleen and tumor is associated with its antitumor influence, same in serum shows the progression of disease and metastasis In this study, the BT1 treatment significantly reduced the IL-2 level in serum compared to vehicle treated mice, which indicates the slow tumor growth and metastasis.
- These results proved that BT1 blocks the PD-1/PD-L1 pathway by inhibiting PD-L1 and promote proliferation and differentiation of immune cells which leads to antitumor cytotoxic effects.
- Hence, we believe that BT1 can be used as PD-L1 inhibitor in PD-L1 overexpressed tumors instead of anti-PD-L1 antibody to avoid the drawbacks of the antibodies.

Industrial Significance

- The small molecules can be **patented** for PD-1/PD-L1 inhibition.
- The anti-tumor efficacy and the toxicity of the small molecule can be evaluated in **clinical trials**.
- If successful these molecules can be **commercialized** for the treatment of cancer.

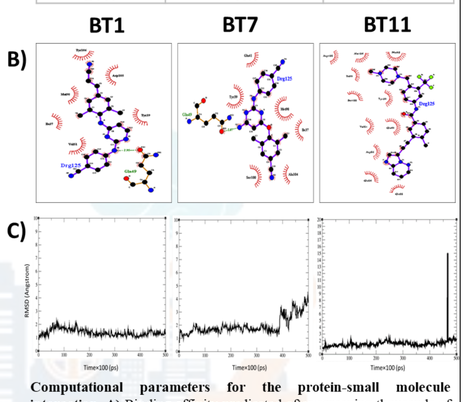
Technology Readiness Level:

This is still in development stage, the product needs to be screened for *in vivo* antitumor efficacy and toxicity in larger animals.

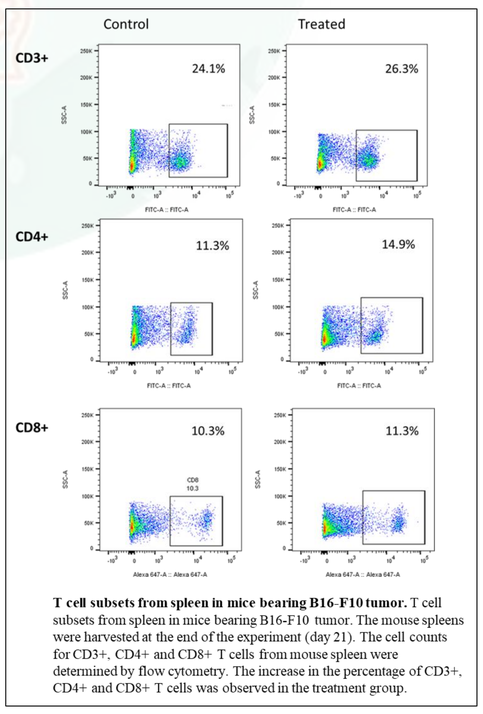
Results

A)

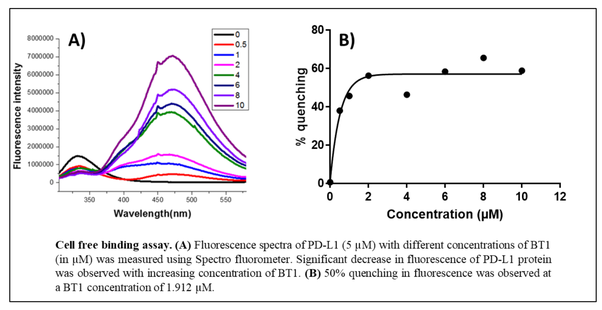
Name of the molecules	Binding Affinity (Kcal/mol)	ΔG (Kcal/mol)
BT1	-10.3	-27.8
BT2	-9.73	-22.64
BT3	-10.9	-20.99
BT4	-10.5	-21.82
BT5	-8.14	-19.28
BT6	-9.8	-8.41
BT7	-8.4	-30.7
BT8	-11.33	-19.2
BT9	-8.54	-21.65
BT10	-9.39	-23.14
BT11	-11.1	-30.7



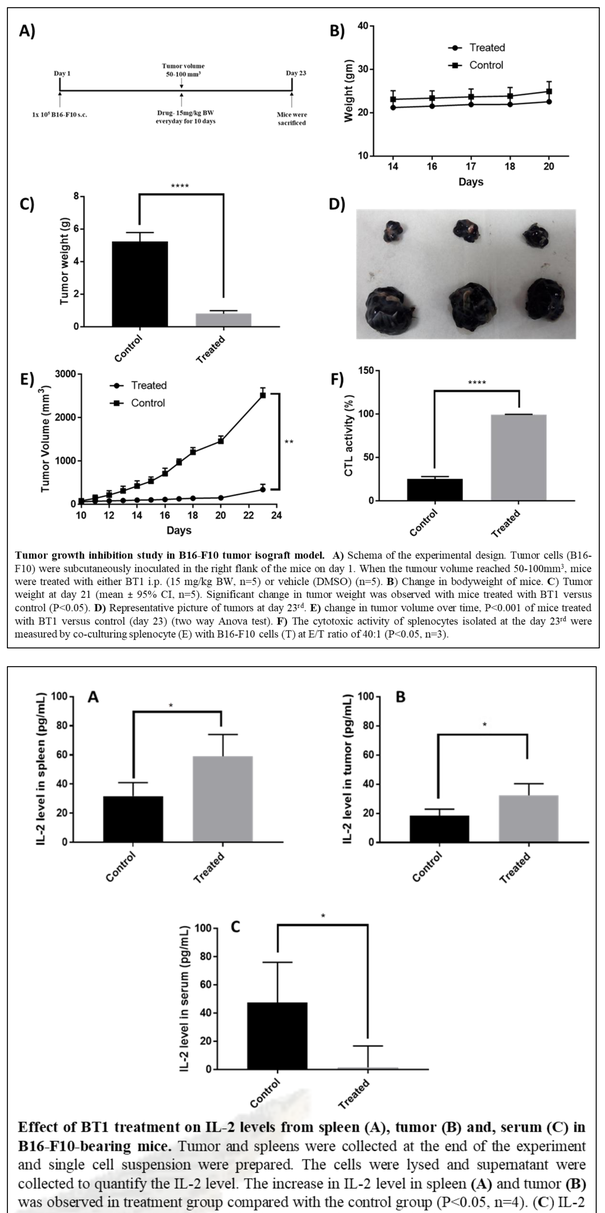
Computational parameters for the protein-small molecule interaction. A) Binding affinity predicated after screening thousands of molecules by ParDock and ΔG was calculated after MD simulation. **B)** One of the frames where the ligand was found to be stable most of the time during molecular dynamics simulations was selected for plotting the protein–small molecule drug interaction diagram. C) RMSF plots of the protein-small molecule complex were obtained from the MD simulations during 50 ns.



T cell subsets from spleen in mice bearing B16-F10 tumor. T cell subsets from spleen in mice bearing B16-F10 tumor. The mouse spleens were harvested at the end of the experiment (day 21). The cell counts for CD3+, CD4+ and CD8+ T cells from mouse spleen were determined by flow cytometry. The increase in the percentage of CD3+, CD4+ and CD8+ T cells was observed in the treatment group.



Cell free binding assay. (A) Fluorescence spectra of PD-L1 (5 μM) with different concentrations of BT1 (in μM) was measured using Spectro fluorometer. Significant decrease in fluorescence of PD-L1 protein was observed with increasing concentration of BT1. **(B)** 50% quenching in fluorescence was observed at a BT1 concentration of 1.912 μM.



Tumor growth inhibition study in B16-F10 tumor isograft model. A) Schema of the experimental design. Tumor cells (B16-F10) were subcutaneously inoculated in the right flank of the mice on day 1. When the tumour volume reached 50-100mm³, mice were treated with either BT1 i.p. (15 mg/kg BW, n=5) or vehicle (DMSO) (n=5). **B)** Change in bodyweight of mice. **C)** Tumor weight at day 21 (mean ± 95% CI, n=5). Significant change in tumor weight was observed with mice treated with BT1 versus control (P<0.05). **D)** Representative picture of tumors at day 23rd. **E)** change in tumor volume over time, P<0.001 of mice treated with BT1 versus control (day 23) (two way Anova test). **F)** The cytotoxic activity of splenocytes isolated at the day 23rd were measured by co-culturing splenocyte (E) with B16-F10 cells (T) at E/T ratio of 40:1 (P<0.05, n=3).

Effect of BT1 treatment on IL-2 levels from spleen (A), tumor (B) and, serum (C) in B16-F10-bearing mice. Tumor and spleens were collected at the end of the experiment and single cell suspension were prepared. The cells were lysed and supernatant were collected to quantify the IL-2 level. The increase in IL-2 level in spleen **(A)** and tumor **(B)** was observed in treatment group compared with the control group (P<0.05, n=4). (**C**) IL-2 level was significantly decreased in the serum of the treated mice compared to that of control group (P<0.05, n=4).

References

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Acknowledgement

- Animal facility, National Institute of Immunology, Delhi.
- Supercomputing Facility for Bioinformatics & Computational Biology, IIT Delhi.