Peptide vaccination against PD-L1 reduces tumor growth in preclinical models through stimulation of PD-L1-targeting T cells in the tumor microenvironment.

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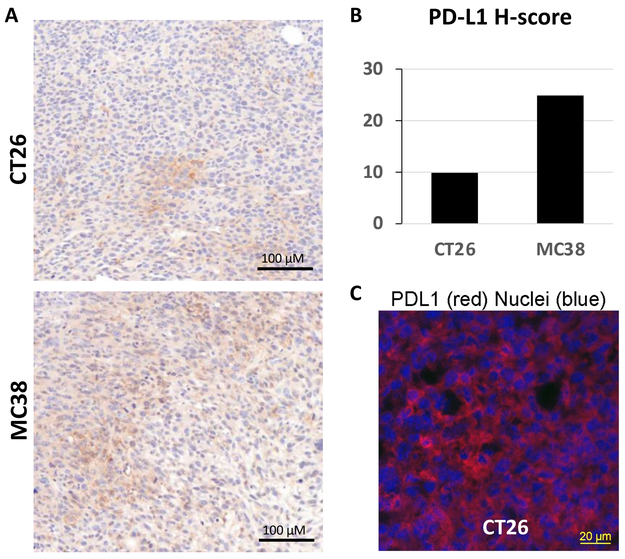
Background

Programmed Death Ligand 1 (PD-L1) is a type 1 transmembrane protein encoded by CD274 gene, its interaction with PD-1 inhibits T cell proliferation and activation to control immune response and contributes to cancer progression and immune escape. The PD-1/PD-L1 blocking antibodies have shown clinical efficacy in many cancers, however drug resistance mechanisms in patients remain a key challenge.

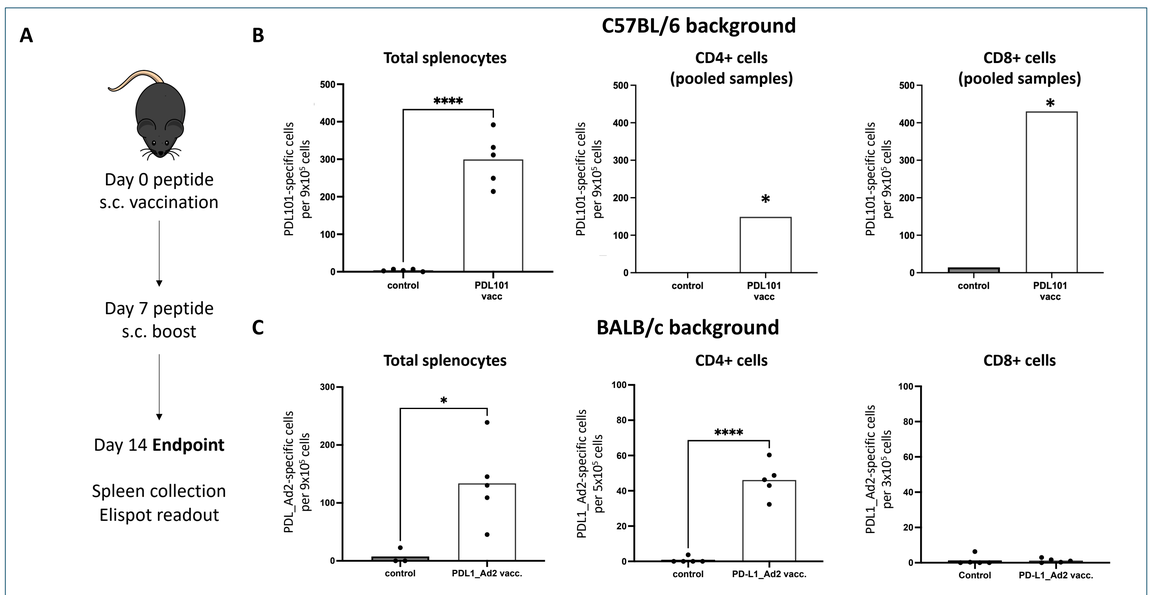
As an alternative and supplemental approach to PD-1/PD-L1 blockade, vaccination approach to promote T-cell immunity against PD-L1$^+$ target cells has been proposed after the observation that effector/cytotoxic T cells reactive against PD-L1 are found in the peripheral blood of cancer patients and healthy individuals [1,2]. Our current study aims to evaluate the efficacy and mechanism of PD-L1 peptide-vaccine using preclinical models.

PD-L1 expression in tumor models



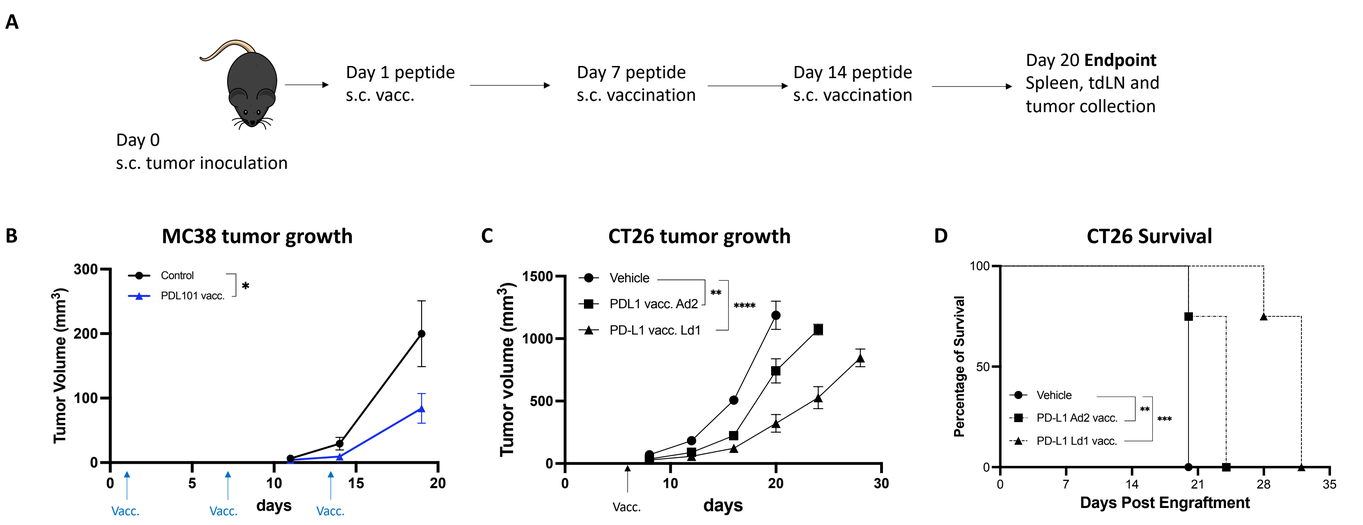
(A) PD-L1 expression was detected on resected tumors from CT26 and MC38 syngeneic tumor models and (B) PD-L1$^+$ cells were scored following intensity levels, H-score was calculated according to standard formula. (C) PD-L1 expression in CT26 tumors was further confirmed per immunofluorescence on frozen tissues sections.

PD-L1 peptide vaccination induces the expansion of PD-L1 specific T cells in mice



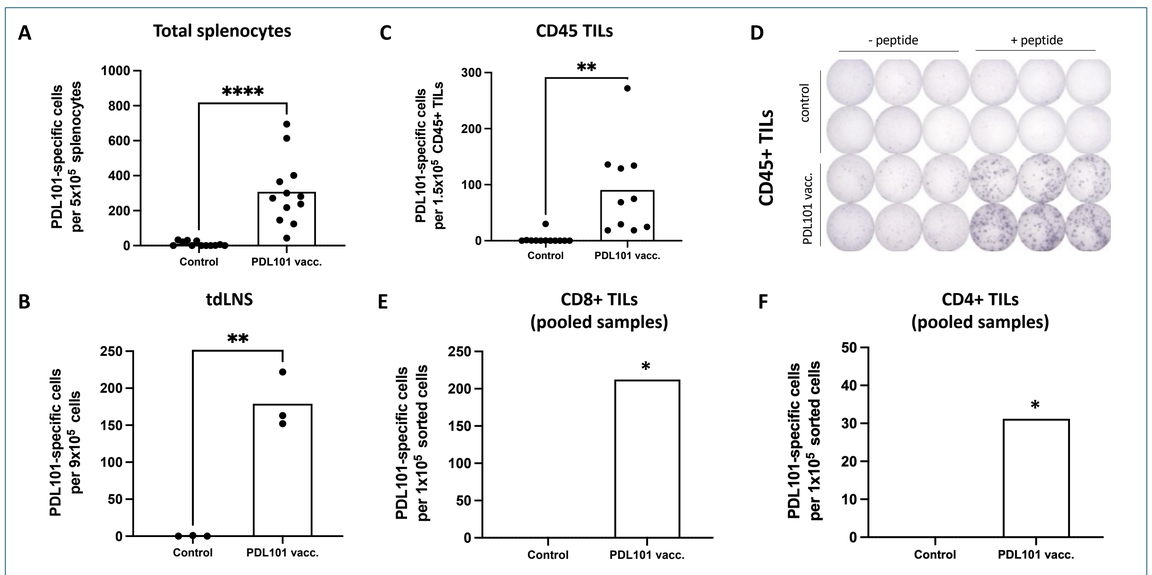
(A) C57BL/6 animals were vaccinated with an MHC-I directed peptide PDL101 and BALB/c animals were vaccinated with an MHC-II directed peptide PD-L1_Ad2. Animals received two treatments on days 0 and 7 and splenocytes were collected 7 days after the last vaccination. The presence of PD-L1 specific T cells was evaluated in an IFNγ Elispot assay after overnight stimulation of total or CD4/CD8 sorted cells. Scatter plots show number of PD-L1 specific T cells detected in cell populations from (B) C57BL6 strain and (C) BALB/c strain treated with background relevant peptides. Data shown as mean +/- SEM.

PD-L1 peptide vaccination reduces tumor growth in murine models



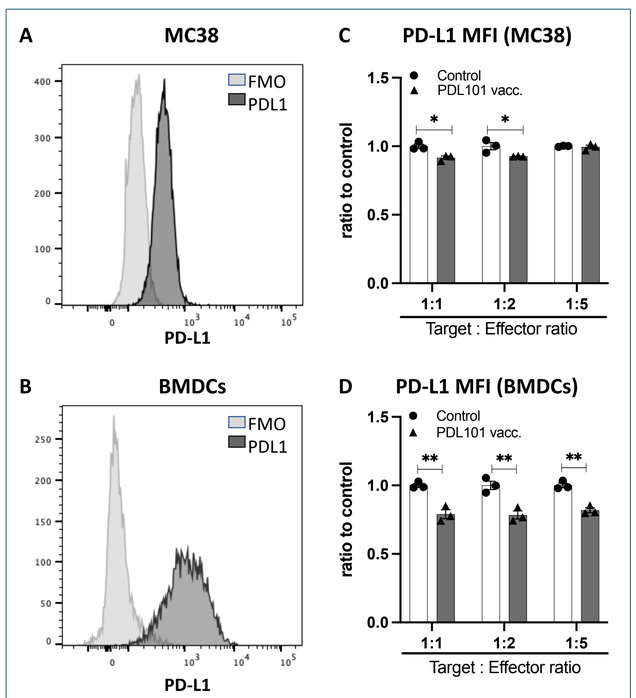
(A) Animals were inoculated with MC38 or CT26 tumor cells and vaccinated with relevant PD-L1 peptide on days 1, 7 and 14 (MC38) or on day 7 (CT26). (B-C) Tumor growth curves of MC38 and CT26 following treatment with PD-L1 peptides were established using mean tumor volume at each time point +/- SEM. (D) Kaplan-Meier survival analysis was performed in CT26 model.

PD-L1 vaccine induces PD-L1 specific T cells traffic into the tumor site



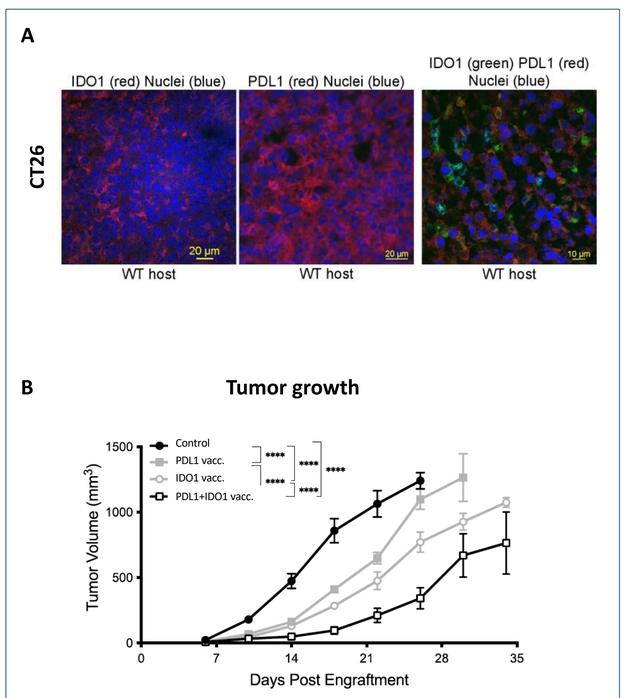
The presence of PD-L1 specific cells was verified by IFNγ Elispot assays on (A) total splenocytes, (B) tumor draining lymph node cells (tdLNs), (C-D) CD45+ TILs, (E) CD4+ TILs and (F) CD8+ TILs collected from MC38-bearing animals untreated or treated with PD-L1 peptide. Data shown as mean +/- SEM.

T cells from PD-L1 vaccinated animals target PD-L1+ cells



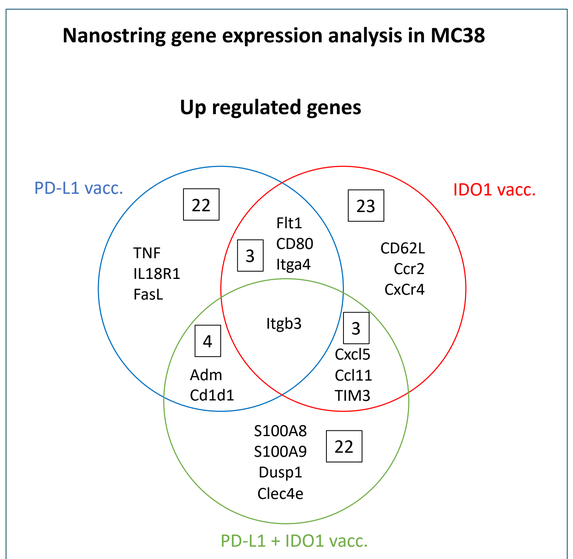
(A) PD-L1 expressing cells (MC38 cells or murine BMDCs) were labelled with cell trace (Targets) and co-cultured overnight with splenocytes from control or PD-L1 vaccinated animals (Effectors). After overnight culture, PD-L1 expression was assessed within Cell trace$^+$ target population. (C-D) % of PD-L1 positive within Cell trace$^+$ populations normalized to average control value as mean +/- SEM.

Combined PD-L1 and IDO1 vaccination enhances anti-tumor effect



(A) IDO1 and PD-L1 were found to be expressed by different cells in within CT26 tumors analyzed per immunofluorescence. (B) BALB/c animals were inoculated with CT26 tumor cells and treated with PD-L1 peptide alone or combined to IDO1 peptide and tumor growth was analyzed. Data shown as mean value +/- SEM.

PD-L1 and IDO1 vaccine induce distinct molecular changes at tumor site



Gene expression analysis was performed using Nanostring nCounter PanCancer IO360 Panel on MC38 tumors from animals treated with PD-L1, IDO1 or combined vaccine. Differentially upregulated genes were identified in the different treatment groups compared to untreated control samples. Lists of top30 differentially upregulated genes from each treatment group were then compared to identify specific and shared molecular changes.

Conclusion

The effect of a peptide-based treatment against PD-L1 was evaluated in two PD-L1+ murine tumor models. A delay in tumor growth was observed in both MC38 and CT26 tumor models upon treatment with PD-L1 peptides. We here highlight that PD-L1 vaccine-induced T cells localize to the TME where they target PD-L1 expressing cells thus reducing the immunosuppression at tumor site and leading to decreased tumor growth as observed in two different models.

A similar peptide-based vaccine against IDO1 previously showed anti-tumor activity and changes in the tumor microenvironment[3]. Combination of PD-L1 and IDO1 vaccines showed enhanced effect *in vivo* in CT26 model where IDO1 and PD-L1 were found expressed by different target populations. Molecular analysis showed distinct gene expression profiles for each monotherapy alone or combined.

Our data support further development of peptide-based vaccines as therapeutic strategies against immuno-suppressive targets in the TME for the treatment of a wide range of tumors.

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References

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