Immune modulatory therapeutic cancer vaccines against IDO1 and PD-L1 control tumor growth through target specific changes in the tumor microenvironment

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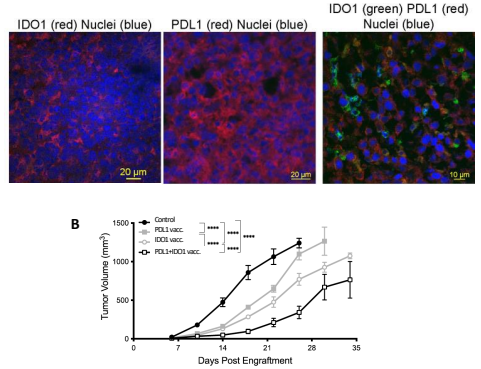
Background

Checkpoint inhibitors such as blocking antibodies against Programmed Death protein (PD)-1 and Programmed Death Ligand (PD-L)1 have demonstrated clinical efficacy, however drug resistance remains a significant obstacle in various cancer types.

IO102-IO103 is an investigational immuno-modulatory therapeutic cancer vaccine targeting IDO1+ and PD-L1+ tumor cells and immune suppressive cells. Previously, preclinical data showed that vaccination with mouse surrogate for IO102 reduces IDO1+ myeloid suppressor cells in the TME (Dey et al, Nandre et al). Furthermore, combination of IDO1 and PD-L1 vaccines showed additive therapeutic effect preclinically (AACR 2024-Abstract 4094), promising clinical results in metastatic melanoma (Kjeldsen et al); and is now being investigated in a Phase 3 clinical trial in advanced melanoma and in a Phase 2 trial in SCCHN/NSCLC.

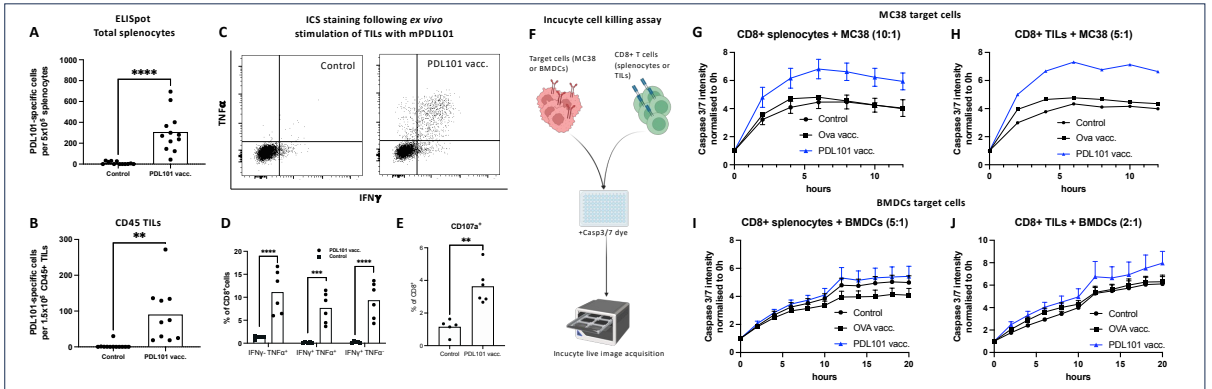
Using preclinical models, the present study aims to further characterize the mode of action of these vaccines and compare it with conventional checkpoint inhibitors.

IDO1 and PD-L1 vaccines control tumor growth and target different cells from the TME



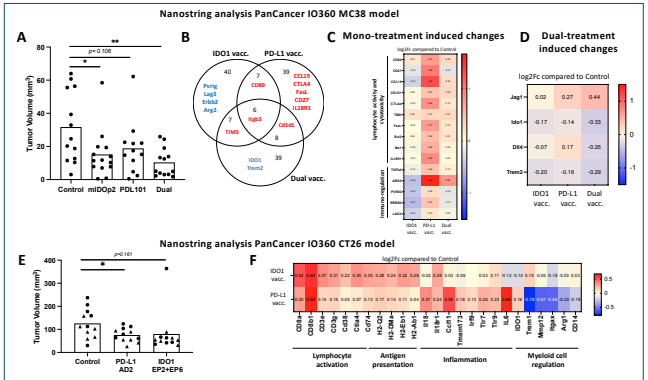
(A) IDO1 and PD-L1 were found to be expressed by different cells in within CT26 tumors analyzed per immunofluoresence. (B) BALB/c animals were inoculated with CT26 tumor cells and treated with PD-L1 peptide alone or combined to IDO1 peptide and tumor growth was analyzed. Data shown as mean value +/-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

PD-L1 vaccine induces trafficking of PD-L1 specific CD8 T cells into the TME which kill PD-L1+ tumor cells



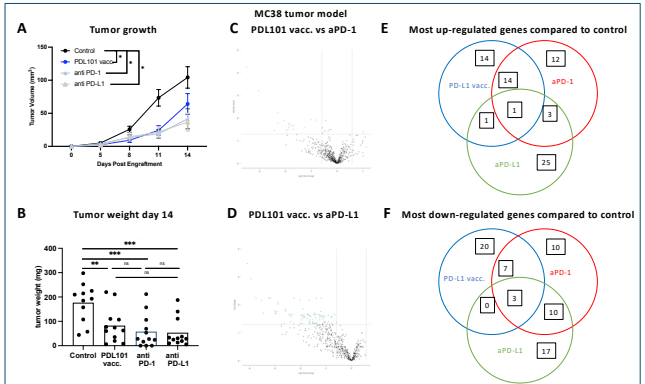
Eight weeks old C57BL/6 mice were inoculated subcutaneously with $5x10^5$ MC38 tumor cells. Animals were treated with 100 µg of PD-L1 MHC-I peptide-vaccine emulsified in Montanide adjuvant and delivered by subcutaneous injection on days 0 and 7. Control animals were treated with a peptide-free emulsion. Two weeks after inoculation, tumors and spleens were collected. The presence of PD-L1 specific cells was verified by IFNγ Elispot assays on (A) total splenocytes and (B) CD45+ TILs; and by (C-E) intracellular staining (ICS) for IFNγ, TNFα and CD107a on TILs from Control or PDL101 vaccinated animals, after four hours of in vitro stimulation with the peptide. (F-J) $CD8^+$ TILs or splenocytes from MC38 tumor bearing animals treated with PD-L1 or OVA MHC-I peptide-vaccine were enriched and co-cultured with PD-L1 expressing target cells. Caspase 3/7 green dye was added to the co-culture and images were acquired on Incucyte every two hours. Data show Caspase 3/7 intensity normalized to day 0. (G-H) MC38 target cells, previously stimulated with IFNγ overnight and expressing PD-L1, were co-cultured with a pool of sorted $CD8^+$ splenocytes or TILs at a 10:1 and 5:1 ratio respectively. (I-J) Bone marrow derived cells (BMDCs) expressing PD-L1, were co-cultured with sorted $CD8^+$ splenocytes or TILs at a 5:1 and 2:1 ratio respectively..

IDO1 and PD-L1 peptide vaccines reduce tumor growth through distinct molecular changes in MC38 and CT26 models

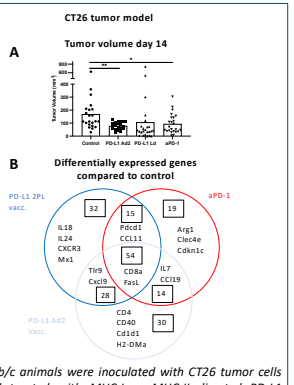


Gene expression analysis was performed using Nanostring nCounter PanCancer IO360 Panel, on tumor samples collected from (A-D) MC38 tumor bearing animals vaccinated with MHC-I directed peptides against IDO1 (mIDOp2) and/or PD-L1 (PDL101), or (E-F) CT26 tumor bearing animals treated with MHC-I and MHC-II directed IDO1 (EP2, EP6) peptides or MHC-II PD-L1 (PD-L1 Ad2) directed peptide. (B) Heatmap and (C) Venn diagram show distinct molecular changes induced by treatments against IDO1 or PD-L1 in MC38 model and (D) shows additional molecular changes identified upon dual treatment compared to monotreatment. (F) Heatmap shows distinct molecular changes induced by treatments against IDO1 or PD-L1 in CT26 model

PD-L1 vaccine induces gene expression changes distinct from aPD1 or aPD-L1 treatments



(A-B) C57BL/6 animals were inoculated with MC38 tumor cells and vaccinated with MHC-I directed peptide against PD-L1 (PDL101) every 7 days from day 0 or treated with aPD1 or aPD-L1 bi-weekly form day 7. Samples were collected two weeks after tumor inoculation and gene expression analysis was performed using Nanostring nCounter PanCancer IO360 Panel on bulk tumor samples. Volcano plots show differential gene expression upon (C) PD-L1 vacc. vs aPD-1 and (D) PD-L1 vacc. vs aPD-L1. Lists of top30 differentially (F) upregulated and (F) down-regulated genes from each treatment group were compared to identify treatment-specific changes.



Balb/c animals were inoculated with CT26 tumor cells and treated with MHC-I or MHC-II directed PD-L1 peptides (PD-L1 Ld and Ad2 respectively) or with aPD1. Tumors were collected on day 14 and gene expression analysis was performed using Nanostring nCounter PanCancer IO360 Panel. Differentially expressed genes within responder animals were identified compared to control and across treatment groups.

Conclusion

- Vaccines targeting IDO1 and PD-L1 target different cells in the TME and cooperatively reduced tumor outgrowth
- We previously reported that IDO1 peptide vaccine reduces IDO1+ myeloid suppressor cells in the TME, and we here show that PD-L1 peptide vaccine expands target specific T cells that localize to tumor site where they can eliminate PD-L1 expressing cells.
- Vaccines targeting IDO1 and PD-L1 contribute to the anti-tumor effect through distinct molecular programs.
  - In MC38 model, IDO1 vaccine appears to impact predominantly by reduction of myeloid-derived immune suppression whilst PD-L1 vaccine enhances the anti-tumor T-effector functions.
  - In CT26 model , a clear increase in T cell infiltration and activation is evident by IDO1 vaccine, while myeloid compartment is impacted by PD-L1 vaccine.
- PD-L1 peptide vaccine induces different gene expression changes from the signatures of anti-PD-L1 and anti-PD1, suggesting a complementary mode of action to those therapeutics.
- Altogether, our data support the clinical potential of IDO1 and PD-L1 immune-modulatory vaccines to improve outcomes with novel therapeutic combination in patients with cancer.

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References

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