Immune modulatory cancer vaccines against IDO1 and PD-L1 trigger distinct pathways and cooperatively reduce tumor growth in preclinical models

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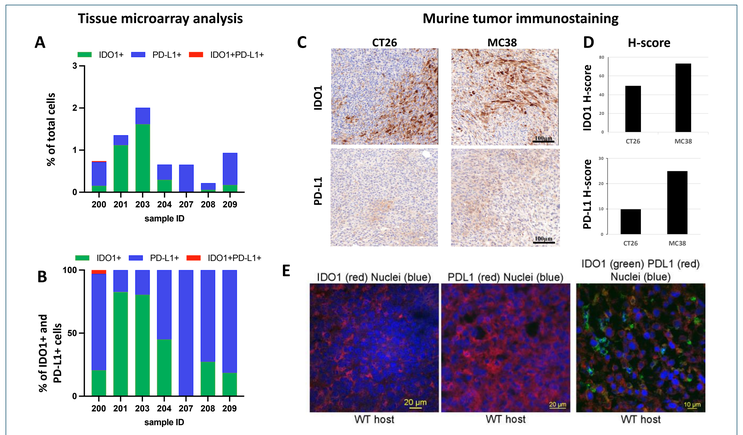
Background

While immune checkpoint inhibitors have shown clinical efficacy in many cancers, drug and immune resistance remain challenging. Indoleamine 2,3-deoxygenase (IDO1) and Programmed Death Ligand 1 (PD-L1) both contribute to immuno-suppression, leading to immune escape and cancer progression.

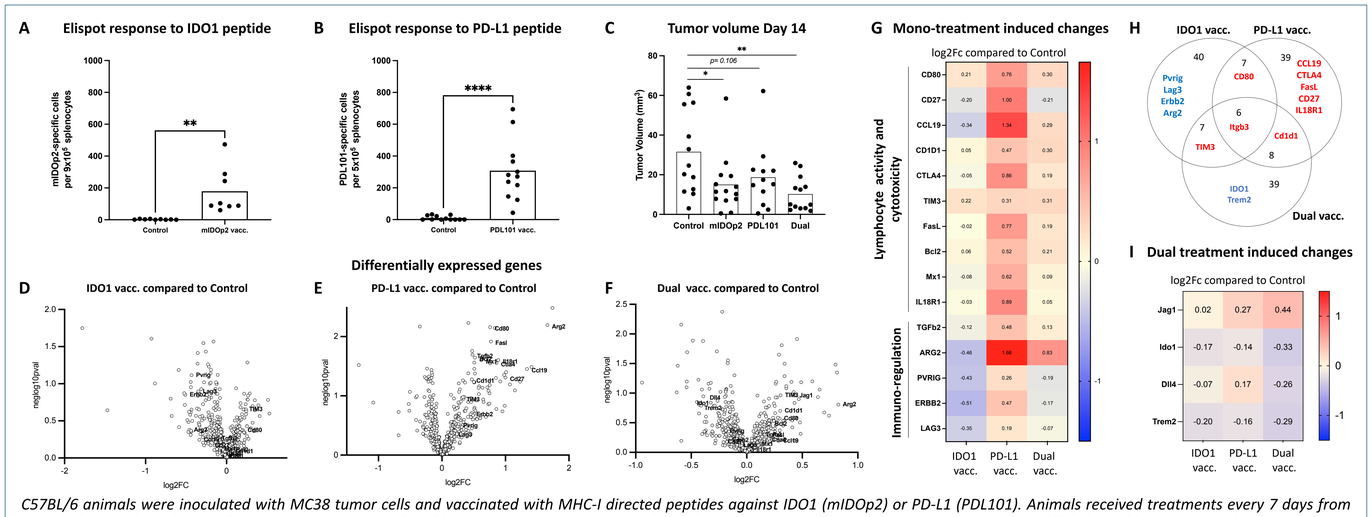
In this context, therapeutic vaccination to promote T-cell immunity against IDO1$^+$ and PD-L1$^+$ cells is an attractive strategy that demonstrated encouraging clinical results in melanoma (Kjeldsen et al. Nat Med. 2021). Our study aims to further evaluate the efficacy and mode of action of combined IDO1 and PD-L1 peptide vaccines.

IDO1 and PD-L1 are differentially expressed in the TME of melanoma patients and murine tumor models



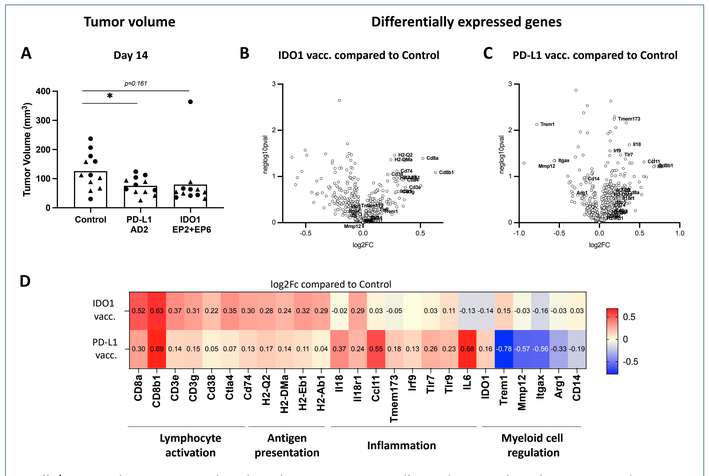
IDO1 and PD-L1 expression was assessed in Tissue Microarray slides of melanoma patients using Neogenomics MultiOmyx™ technology. Data show (A) the frequency of cells expressing IDO1, PD-L1 or both markers per independent patient sample ('sample ID') normalized to the total number of cells per ROI and (B) the distribution of IDO1$^+$ and/or PD-L1$^+$ cells within total cells expressing IDO and/or PDL1. IDO1 and PD-L1 expression was detected on resected tumors from (C) CT26 and MC38 syngeneic tumor models and (D) IDO1$^+$ and PD-L1$^+$ cells were scored following intensity levels, H-score was calculated according to standard formula. (E) IDO1 and PD-L1 were found to be expressed by different cells within CT26 tumors analyzed per immunofluorescence.

IDO1 and PD-L1 peptide vaccines result in target-specific T cell expansion and control tumor growth through distinct molecular pathways in MC38 model



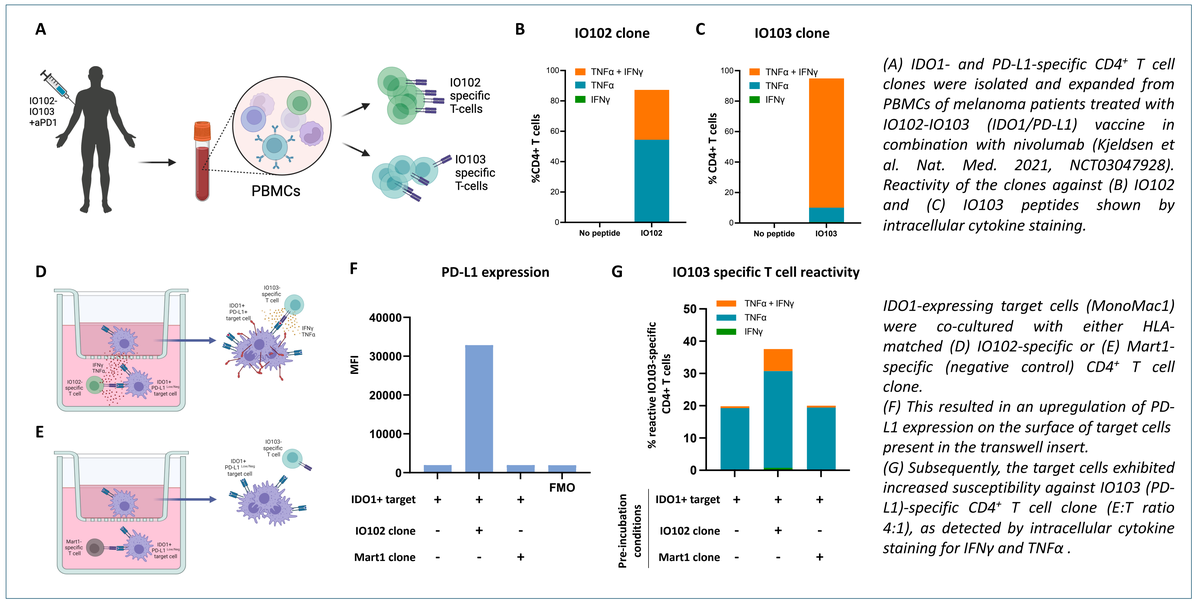
C57BL/6 animals were inoculated with MC38 tumor cells and vaccinated with MHC-I directed peptides against IDO1 (mIDOp2) or PD-L1 (PDL101). Animals received treatments every 7 days from day 0. (A-B) Vaccine-induced T cells were detected in IFNγ Elispot assays after overnight stimulation of total spleen cells. Data shown as mean +/- SEM. (C) MC38 tumor-bearing animals were treated with mIDOp2 or PDL101 alone or combined (Dual) on days 0 and 7 and tumors were collected on day 14. Gene expression analysis was performed using Nanostring nCounter PanCancer IO360 Panel, and differentially expressed genes were identified upon each treatment compared to control as illustrated in volcano plots (D-F). (G) Heatmap and (H) Venn diagram showing distinct molecular changes induced by treatments against IDO1 or PD-L1. (I) Additional molecular changes were identified upon dual treatment against IDO1 and PD-L1 compared to monotreatment.

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



Balb/c animals were inoculated with CT26 tumor cells and treated with MHC-I and MHC-II directed IDO1 (EP2, EP6) peptides or MHC-II PD-L1 (PD-L1 Ad2) directed peptide. (A) Tumors were collected on day 14 and gene expression analysis was performed using Nanostring nCounter PanCancer IO360 Panel. Volcano plots show differential gene expression upon (B) IDO1 Ep2+Ep6 and (C) PD-L1 Ad2 treatments compared to control. (D) Heatmap displaying key differentially expressed genes upon IDO1 or PD-L1 vaccination.

IDO1-specific T cells from IO102-IO103 treated patient converts PD-L1$^{neg/low}$ cells to PD-L1^{high}, making them susceptible to subsequent attack by PD-L1-specific T cells.



(A) IDO1- and PD-L1-specific CD4$^+$ T cell clones were isolated and expanded from PBMCs of melanoma patients treated with IO102-IO103 (IDO1/PD-L1) vaccine in combination with nivolumab (Kjeldsen et al. Nat. Med. 2021, NCT03047928). Reactivity of the clones against (B) IO102 and (C) IO103 peptides shown by intracellular cytokine staining.

IDO1-expressing target cells (MonoMac1) were co-cultured with either HLA-matched (D) IO102-specific or (E) Mart1-specific (negative control) CD4$^+$ T cell clone.
(F) This resulted in an upregulation of PD-L1 expression on the surface of target cells present in the transwell insert.
(G) Subsequently, the target cells exhibited increased susceptibility against IO103 (PD-L1)-specific CD4$^+$ T cell clone (E:T ratio 4:1), as detected by intracellular cytokine staining for IFNγ and TNFα .

Conclusion

- Our data collectively show that cells expressing IDO1 and PD-L1 represent distinct populations in the TME of patients and in murine models thus targetable by the IDO1-PD-L1 vaccination approach.
- Vaccines targeting IDO1 and PD-L1 induced specific T cell expansion and cooperatively reduced tumor outgrowth in different murine models and each contributes to the anti-tumor effect through distinct molecular programs.
- In MC38 model with high levels of IDO1 and PD-L1 expression in the TME, 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 contrast, in CT26 model where IDO1/PD-L1 expression is comparatively low, a clear increase in T cell infiltration and activation is evident by IDO1 vaccine, while myeloid compartment is impacted by PD-L1 vaccine.
- *Ex vivo* functional study using IDO1-specific T cells isolated from patients treated with IO102-IO103 support that IO102 vaccine can directly lead to upregulation of PD-L1 expression in neighboring cells thereby enhancing the effect of IO103 treatment.
- While further studies are needed to fully discern the relationship between IDO1$^+$/PD-L1$^+$ target populations within the TME and the impact of IDO1/PD-L1 targeted vaccination, our data support the use of a dual antigen approach to reduce the immunosuppression and enhance anti-tumor effect.

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References

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