Vaccination against arginase 1 controls tumor growth via modulation of tumor-associated macrophages

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Background

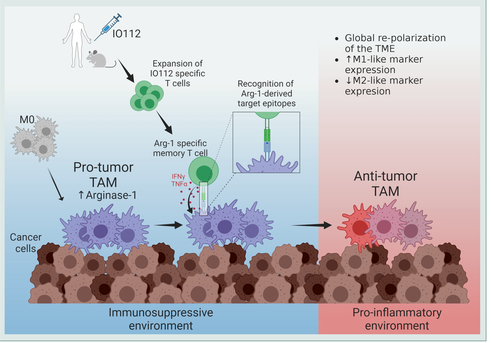
Arginase-1 (Arg1) is a metalloenzyme that plays a central immune suppressive role via regulating the availability of L-arginine for activated T cells and its overexpression has been reported in several cancers. Arg1 is produced by broad immune suppressive cell types including myeloid-derived suppressor cells and tumor-associated macrophages (TAMs).

IO112 is an IND-ready investigational therapeutic vaccine candidate from IO Biotech's T-win® platform*, encoding Arg1 peptide, designed to activate intrinsic immunity against Arg1+ cells[1]. We previously reported that murine IO112 (mIO112) controls tumor growth, which was associated with the elevation of M1/M2 macrophage ratio and synergizes with anti-PD1 treatment in mouse cancer models[2]. In the present study, we sought to test the hypothesis that TAMs are the primary and direct targets of Arg1-specific T cells induced by IO112 treatment.

***T-win® platform**: IO Biotech's dual-action immune modulating cancer vaccines targeting both immune suppressive cells and the tumor cells in the TME. The first T-win® clinical program, IO102-IO103 against IDO1 and PD-L1, has shown activity in three types of cancer and is now in Phase 3 in advanced melanoma.*

Conclusions

- IO112 presents a unique immunomodulatory approach, whereby Arg1+ immunosuppressive TAMs are targeted via vaccination to boost T cell immunity.
- Our data demonstrate that the T cells induced by IO112 directly impact TAMs, skewing the balance from an immunosuppressive to a pro-inflammatory microenvironment, leading to effective anti-tumor responses.
- The data strongly support the foundation for an IO112 IND submission planned for 2025 and present a potential synergistic and/or alternative approach to other strategies to treat a wide range of cancer indications.



References

1. Martinenaite E et al. *Frequent adaptive immune responses against arginase-1.* Oncoimmunology. 2017 Dec 26;7(3)

2. Jørgensen M et al. *Arginase 1-based immune modulatory vaccines induce anticancer immunity and synergize with anti-PD-1 checkpoint blockade.* Cancer Immunol Res. 2021 Nov;9(11):1316-1326.

mIO112 vaccination induces strong T cell responses and inhibits tumor growth by modulating the TAMs in the tumor TME

mIO112 vaccination leads to expansion of T cells and reduction of the tumor growth by modulating gene expression in the immune suppressive TAMs in the TME.

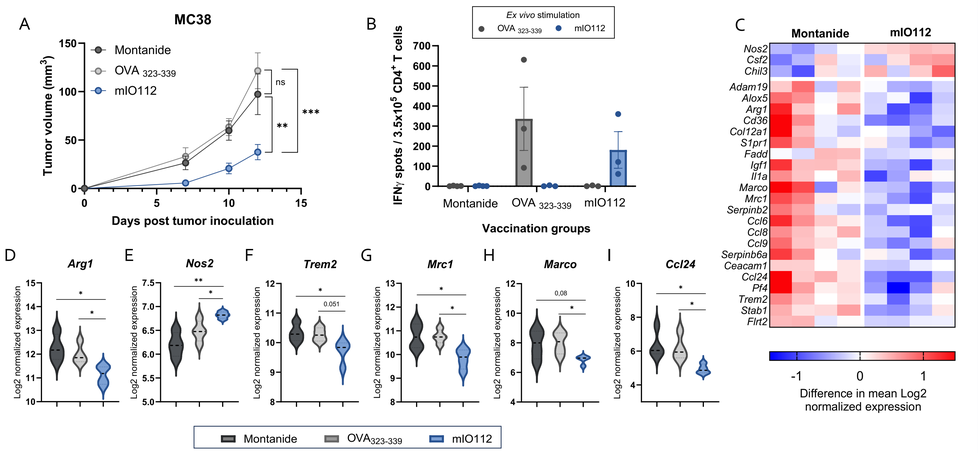


Figure 1. mIO112 treatment-induced MC38 tumor growth delay is associated with changes in TAM phenotype *in vivo*. (A) MC38 tumor growth curves of mice treated with mIO112 (n=12), OVA (n=9) peptide emulsion in Montanide or peptide-free Montanide emulsion (n=12) on days 0 and 7 post tumor inoculation. Statistical differences were identified by applying a mixed model. (B) mIO112 and OVA $_{323-339}$ peptide-specific responses quantified by IFNγ ELISPOT in CD4+ T cells isolated from splenocytes of treated tumor bearing animals. (C) Heat map of differentially expressed genes in TAMs purified from mIO112 treated mice, in comparison with gene signature of Montanide control treated animals. (D-I) Differences in gene expression for TAMs sorted from peptide-free Montanide control, OVA or mIO112 vaccinated animals. Differences in *Arg1* (D), *Nos2* (E), *Trem2* (F), *Mrc1* (G), *Marco* (H) and *Ccl24* (I) were evaluated by applying an unpaired T test

mIO112 treatment-induced CD4+ T cells target and promote proinflammatory phenotype of Arg1+ TAMs *ex vivo*

mIO112-specific T cells recognize Arg1-expressing macrophages and modulate their phenotype by secreting pro-inflammatory cytokines.

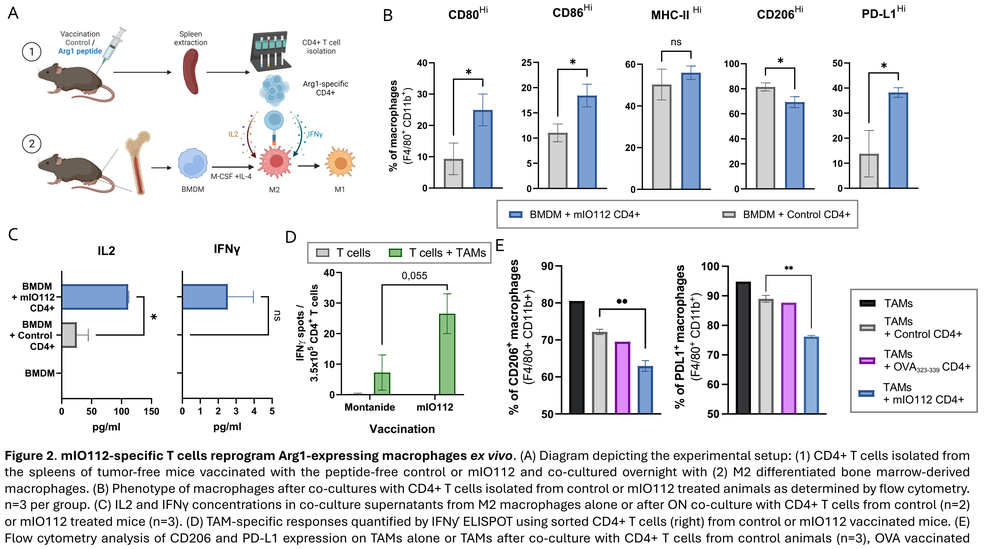


Figure 2. mIO112-specific T cells reprogram Arg1-expressing macrophages *ex vivo*. (A) Diagram depicting the experimental setup: (1) CD4+ T cells isolated from the spleens of tumor-free mice vaccinated with the peptide-free control or mIO112 and co-cultured overnight with (2) M2 differentiated bone marrow-derived macrophages. (B) Phenotype of macrophages after co-cultures with CD4+ T cells isolated from control or mIO112 treated animals as determined by flow cytometry. n=3 per group. (C) IL2 and IFNγ concentrations in co-culture supernatants from M2 macrophages alone or after ON co-culture with CD4+ T cells from control (n=2) or mIO112 treated mice (n=3). (D) TAM-specific responses quantified by IFNγ ELISPOT using sorted CD4+ T cells (right) from control or mIO112 vaccinated mice. (E) Flow cytometry analysis of CD206 and PD-L1 expression on TAMs alone or TAMs after co-culture with CD4+ T cells from control animals (n=3), OVA vaccinated animals (n=1) or mIO112 vaccinated mice (blue) (n=2).

IO112-specific T cells target and drive proinflammatory polarization of Arg1+ myeloid cells *in vitro*

Arg1-derived peptides are presented by Arg1+ myeloid cells, allowing recognition by human IO112-specific CD4+ T cells. The interaction induces pro-inflammatory polarization of targeted myeloid cells

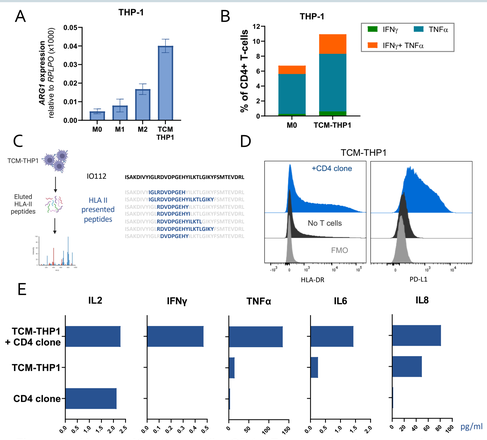


Figure 3. Human IO112-specific CD4+ T cells directly recognize Arg1+ myeloid cells and drive proinflammatory polarization. (A) Arg1 expression in THP1 either undifferentiated (M0), treated with 200 U/ml IFNγ (M1-like), IL-13 20U/ml (M2-like), or tumor conditioned media (TCM-THP1) for 48h prior to RT-qPCR. Average values ± SEM. (B) Intracellular IFNγ and TNFα production by IO112-specific CD4+ T cell clone upon co-culture with HLA-matched myeloid cells (THP1) undifferentiated (M0) or differentiated with TCM (TCM-THP1) for 48h. E:T ratio 4:1. (C) Arg1-derived peptide sequences presented by the TCM-THP1 cells (blue) aligned with the IO112 peptide (black) as identified by mass spectrometry based immunopeptidome profiling. (D) Changes in HLA-DR and PD-L1 on TCM-THP1 after co-culture with IO112-specific CD4+ T cell clone. (E) Secreted cytokines in culture supernatants of either an IO112-specific T cell clone co-cultured with HLA-matched TCM-THP1, TCM-THP1 alone, or the IO112-specific CD4+ T cell clone alone.

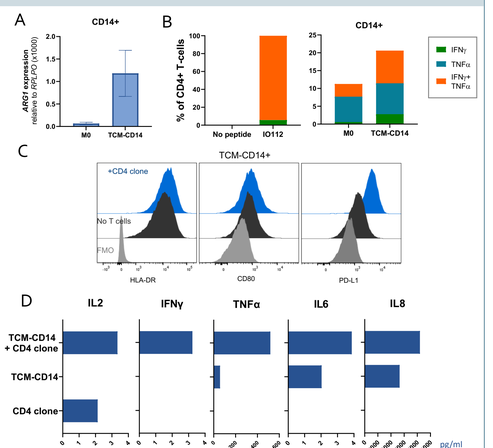


Figure 4. Human IO112-specific CD4+ T cell clones recognize and polarize Arg1+ CD14+ monocyte derived myeloid cells towards a proinflammatory phenotype. (A) Arg1 expression as determined by RT-qPCR in undifferentiated human CD14+ cells or CD14+ cells differentiated to a TAM-like phenotype by culturing with tumor-conditioned media (TCM-CD14) for 48h. Average values ± SEM. (B) Intracellular IFNγ and TNFα production by IO112-specific CD4+ T cell clone against the IO112-derived epitope (Left) or autologous CD14 myeloid cells (right), either undifferentiated (M0) or differentiated with TCM (TCM-CD14) for 48h. E:T ratio 4:1. (C) Changes in expression of HLA-DR, CD80 and PD-L1 on TCM-CD14 cells after co-culture with autologous IO112-specific CD4+ T cell clone. (D) Secreted cytokines in supernatants of IO112-specific T cell clone co-cultured with autologous TCM-CD14, TCM-CD14 alone or the IO112-specific CD4+ T cell clone alone.

IO112-specific CD4+ T cells induce reprogramming of directly targeted and bystander macrophages *in vitro*

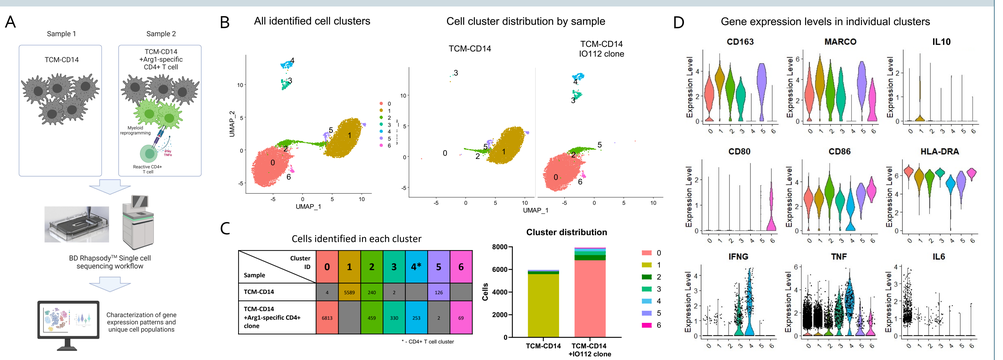


Figure 5. IO112-specific CD4+ T cells induce a reprogramming of directly targeted and bystander macrophages *in vitro*. (A) Experimental set up of single cell sequencing experiment. E:T ratios of 1:10. (B) Left – UMAP of all unique cell clusters identified in the control and TCM-CD14 co-culture with Arg1-specific CD4+ T cell clone. Right – Segregated UMAPs depicting clusters per sample. (C) Left – table summarizing cell distribution in individual clusters for each sample. *- Cluster 4 represents CD4+ T cells. Right – cell cluster distribution per sample. (D) Gene expression levels of M2-macrophage associated genes (CD163, MARCO, IL10) and M1-macrophage associated genes (CD80, CD86, HLA-DRA, IFNG, TNF, IL6) across cell clusters.

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