Induction of T cell immunity against arginase 1+ myeloid cells is a unique feature that differentiates tumor growth suppression of Arg1 immune-modulatory vaccines from that of Arg1 inhibitors.

Inés Lecoq[1], Evelina Martinenaite[1,2], Matteo Bocci[1], Marion Chapellier[1], Rasmus Agerholm-Nielsen[1], Marco Carretta[1], Aimilia Schina[2,3], Sarah Rosemberg Asmussen[2,4], Eszter Virág Toldi[4], Lea Svendsen[1], Lucía Lara de la Torre[2,3], Mads Hald Andersen[2,3] and Ayako Wakatsuki Pedersen[1]

1 – IO Biotech ApS. 2 – National Center for Cancer Immune Therapy (CCIT-DK). 3 – University of Copenhagen. 4 – Technical University of Denmark.

IO BIOTECH
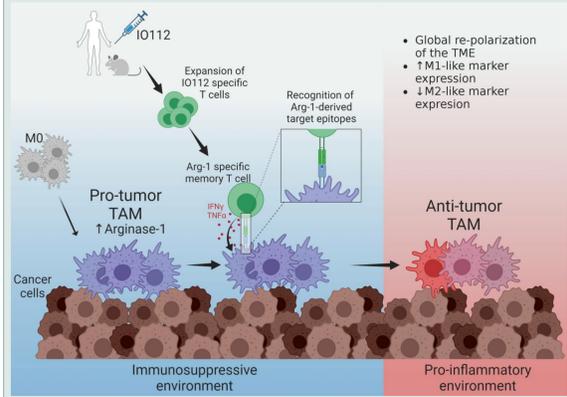
Poster #932

Background

Arginase-1 (Arg1) is a metalloenzyme that contributes to an immuno-suppressive tumor microenvironment by limiting the availability of l-arginine and hence, T cell function within the tumor. The overexpression of Arg1 has been reported in several cancers and constitutes a challenge for the success of cancer treatment. However, clinical interventions to inhibit Arg1 function have shown limited success.

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 vaccination against Arg1 enhances anti-tumor activity in murine models[2] via reducing the immunosuppressive function of Arg1+ tumor-associated macrophages (TAMs)[3]. In the present study, we sought to further investigate the mechanism behind Arg1-based peptide vaccination and compare its therapeutic effect to that of the Arg1 inhibitor CB-1158.

*T-win® platform: IO Biotech's dual-action immune modulating cancer vaccines targeting both immune suppressive cells and tumor cells. The first T-win® clinical program, IO102-IO103 against IDO1 and PD-L1, has shown clinical activity and favorable safety profile across various tumor types and is currently being tested in phase 3 for advanced melanoma.

Conclusions

- IO112 presents a unique immunomodulatory approach, whereby Arg1+ immunosuppressive myeloid cells 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 2026 and present a potential synergistic and/or alternative approach to other strategies to treat a wide range of cancer indications.

References

1   2   3

Contact:
Inés Lecoq
Senior Scientist
ilm@iobiotech.com

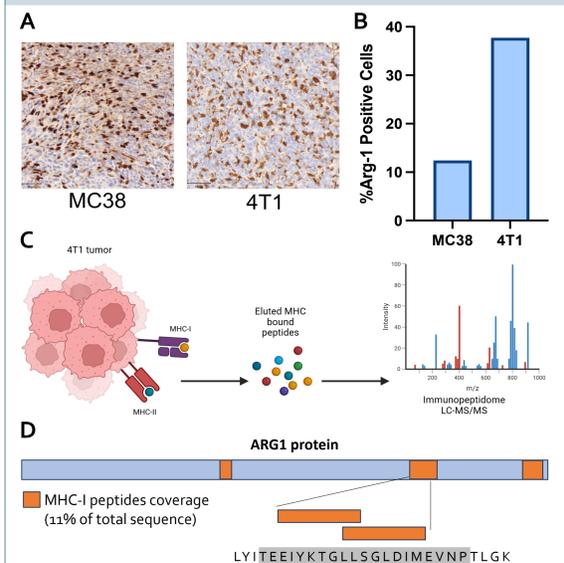## Arg1-derived peptides are presented in MHC molecules

Figure 1. Arg1 is highly expressed in 4T1 tumors and Arg1-derived peptides are presented in MHC molecules. (A) Arg1 IHC on tumor sections from MC38 and 4T1 models. (B) Percentage of Arg1-positive cells in the tumor section. IHC score calculated as the ratio of positive cells to total cell count. (C) Diagram of the immunopeptidome profile of 4T1 tumors. (D) Arg1-derived peptides are presented in MHC-I molecules. The immunopeptides YKTGLLSGL and ITEEIYKTGL are contained within the immunogenic peptide Arg1_191-210.

mIO112 elicits Arg1-specific T cell expansion

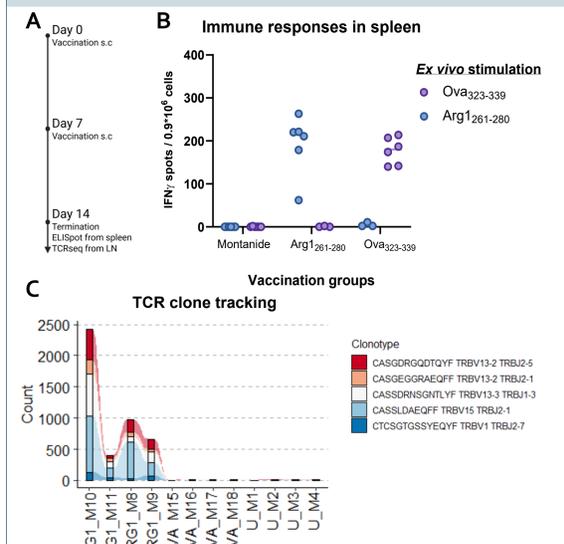Vaccination expanded Arg1-specific T cells, detectable through IFNγ and TCR sequencing

Figure 2. Vaccination with Arg1-derived peptides elicits Arg1-specific T cell responses. (A) Diagram of vaccination schedule. (B) IFNγ ELISpot responses detectable in spleen of mice vaccinated with OVA_323-339 and Arg1_261-280 peptides. (C) TCR sequences identified in lymph node of Arg1_261-280 vaccinated mice.

mIO112 vaccination induces antitumor immunity by targeting the myeloid compartment of 4T1 tumors

Vaccination with Arg1_191-210 peptide led to a downregulation of pathways associated with the myeloid compartment in the tumor and their functions within the tumor microenvironment

Figure 3. Therapeutic vaccination with Arg1-derived peptides delays the growth of Arg1-expressing 4T1 tumors. (A) Tumor growth curve ±standard deviation of the mean (SEM) of 4T1-bearing mice treated with the control vaccine (n=19) or the Arg1_191-210 containing vaccine (n=20). (B) Immune responses in the spleen of control (montanide) and Arg1_191-210 vaccinated mice. (C) Ly6G staining of lungs of 4T1-bearing mice 14 days after tumor inoculation for control (montanide) and Arg1_191-210 vaccinated mice. (D) Gene set enrichment of pathways up or downregulated in tumor microenvironment of 4T1-bearing mice vaccinated with Arg1_191-210 in comparison with gene signature from montanide control treated group. (E-H) Differences in expression of genes associated with tumor-infiltrating myeloid cells in mice treated with an Arg1_191-210 peptide vaccine (n=12) or a peptide-free control vaccine (montanide, n=12).

mIO112 vaccination, but not Arg1 inhibition with CB-1158, reprograms Arg1-expressing macrophages

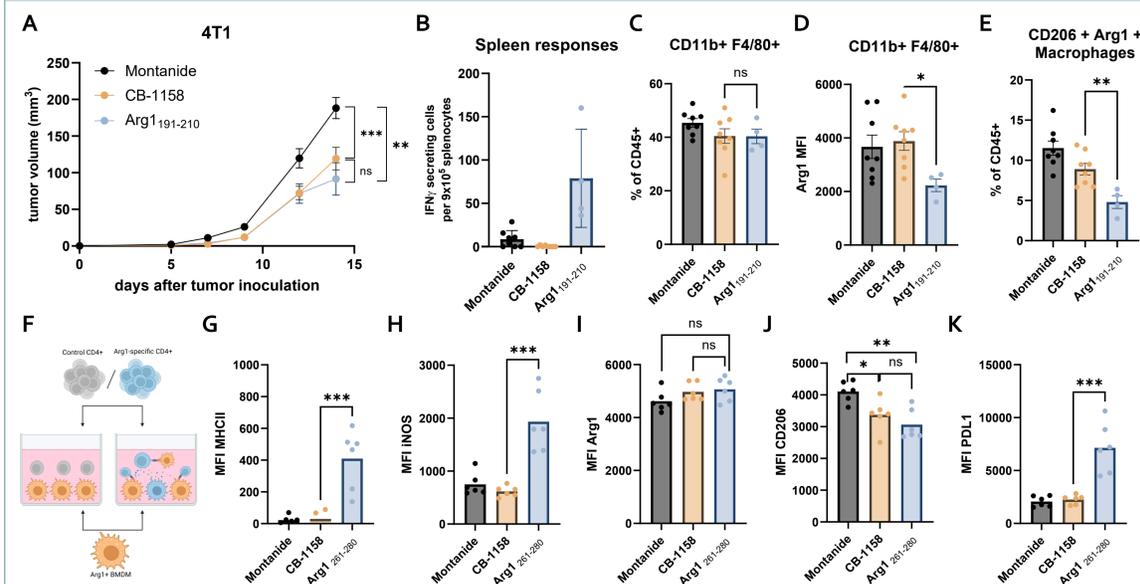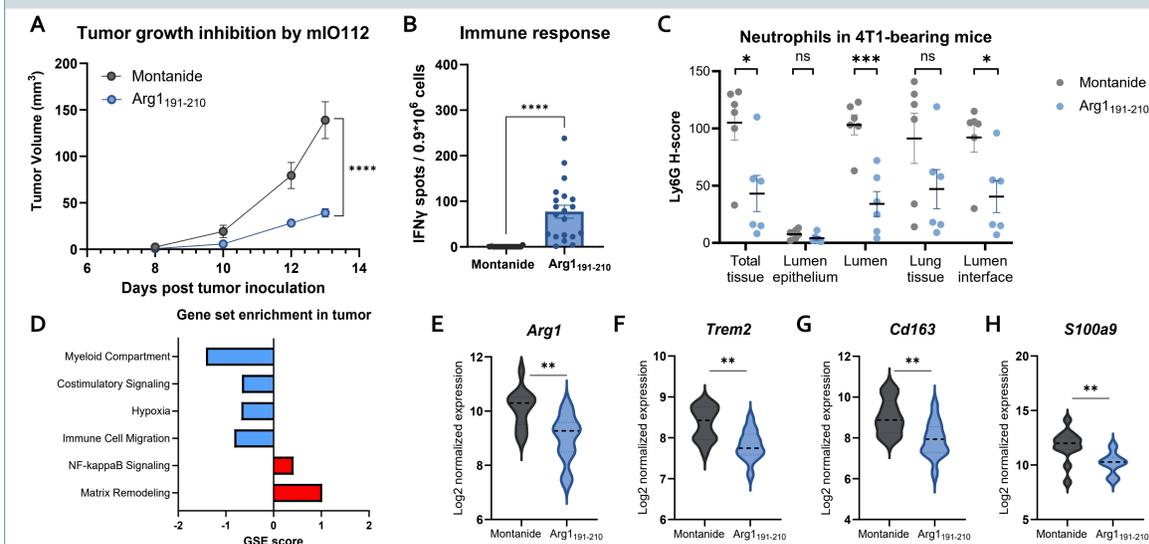mIO112-specific T cells reduce Arg1+ TAMs in vivo and reprogram macrophages ex vivo

Figure 4. mIO112 vaccination has a distinct mode of action to the Arg1 inhibitor CB-1158 by modulating Arg1+ TAMs. (A) Tumor growth curve ±standard deviation of the mean (SEM) of 4T1-bearing mice treated with the control montanide vaccine (n=12), Arg1 inhibitor CB-1158 (n=12) or the Arg1_191-210 containing vaccine (n=4). (B) IFNγ ELISpot responses detectable in the spleen against Arg1_191-210 peptide. (C-E) Macrophages, Arg1 MFI of macrophages and (E) CD206+ Arg1+ macrophages in 4T1 tumors of control, Arg1 and CB-1158 treated mice (F) Diagram of experimental setup for ex vivo modulation of Arg1-specific T cells and Arg1 inhibitor (CB-1158) (G-K) Phenotype of macrophages after co-cultures with CD4+ T cells isolated from control or mIO112 treated animals as determined by flow cytometry. n=6 per group

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

Immune targeting of Arg1+ immunosuppressive myeloid cells by IO112-specific CD4+ T cells drives differentiation towards a pro-inflammatory phenotype with improved capacity for antigen presentation
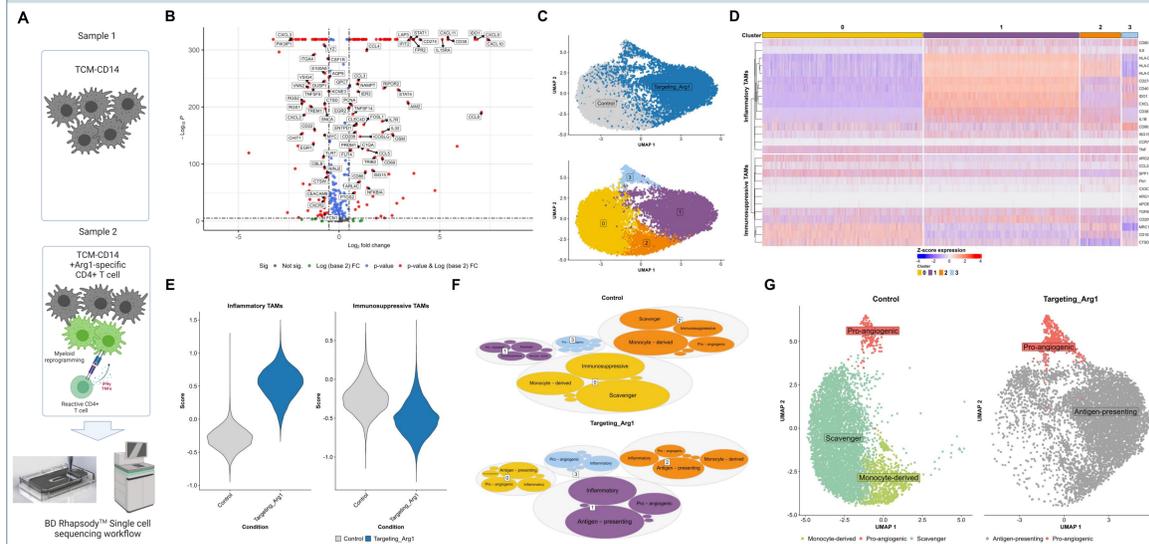

Figure 5. IO112-specific CD4+ T cells induce a reprogramming of macrophages in vitro. (A) Experimental set up of single cell sequencing experiment. E:T ratios of 1:10. CD14 cells were polarized toward an immunosuppressive phenotype by incubation with tumor-conditioned medium (TCM) from MDA-MB-231 cells prior to the co-culture (B) Volcano plot of differentially expressed genes between CD14 population of Arg1-culture and TCM-treated CD14 control (C) UMAP of all cell clusters identified in the co-culture and TCM-CD14 co-culture with Arg1-specific CD4+ T cell clone. (D) Heat map of inflammatory and immunosuppressive gene signatures in the different clusters identified. (E) Violin plot of inflammatory and immunosuppressive signatures for the control TCM-CD14 and co-culture with Arg1-specific CD4+ T cell clone. (F) Functional myeloid/TAM signatures associated with the different clusters per condition. (G) UMAP of Dominant functional TAM signature per cluster