Arginase-1 vaccine promotes T cell immunity against arginase-1+ cells, controls tumor growth via immune modulation of tumor microenvironment

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Background

Arginase-1 (ARG1) is involved in various processes that allow tumor cells to evade immune responses within the tumor microenvironment (TME). Numerous attempts to target ARG1 in clinical settings have been made thus far, though with limited success. Immunization against TME-related targets has emerged as a promising treatment approach. We previously demonstrated that vaccination against Arg1 enhances anti-tumor activity in murine models (1). This study aims to extend our earlier observations and clarify the underlying mechanism of the anti-tumor activity of the Arg1 vaccine.

ARG1 is expressed by different cell populations in TME across several cancer indications

Tumor tissue microarray analysis reveals that ARG1 expression is predominantly observed in myeloid-derived suppressor cells (MDSCs) across various cancer types

CRC	Colorectal Cancer
ESOPHAGEAL	Esophageal squamous cell carcinoma
GASTRIC	Gastric Cancer
HNN	Head and Neck Cancer
NSCLC	Non-Small Cell Lung Cancer (Adenocarcinoma)
OVARIAN	Ovarian Cancer (Serous Papillary Adenocarcinoma)
UROTHELIAL	Urothelial Carcinoma (Bladder Cancer)

Table 1. Cancer types analyzed

Label	Description	Markers
Tumor cell	Tumor cell	PanCk, Sox10 (melanoma)
Fibroblast	Fibroblast	FAP
CD8	CD8+ T cell	CD3+,CD8+
CD4	CD4+ T cell	CD3+,CD4+
Treg	Regulatory CD4+ T cell	CD4+,FoxP3+
exCD8	Exhausted CD8+ T cell	CD3+, CD8+, PD-1+
M1_TAM	M1 tumor associated macrophage	CD68+HLADR+CD163-
M2_TAM	M2 tumor associated macrophage	CD68+HLADR-CD163+
APCmye	Myeloid Antigen Presenting Cell	CD11b+HLADR+
MDSCs	Myeloid-derived suppressor cells	CD33+CD11b+HLDR-

Table 2. Markers and cell types definition

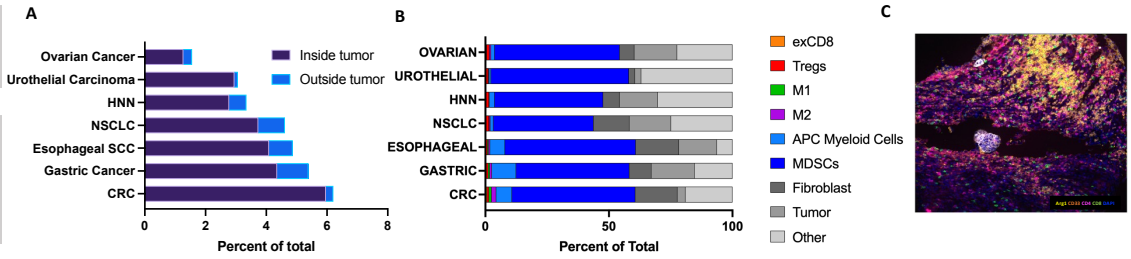

Fig 1. ARG1 is expressed by different cell populations in TME from several cancer indications. ARG1 expression in the tumor microenvironment (TME), was assessed with Neogenomics MultiOmyx™. Samples from 7 cancer types were stained with 18 markers for cell identification (Tables 1-2). Deep learning algorithms classified cells based on marker positivity, and 32 regions of interest (ROI) were analyzed per cancer type. (A) Stacked bars diagram showing the fraction of cells expressing ARG1 for each cancer indication analyzed, within or outside the tumor margins. (B) Stacked bars diagram showing the different cell types expressing ARG1 in the TME for each indicated cancer type analyzed (C) Examples of multiplex images of CRC samples. Arg1 positive cells in yellow, CD33 in orange, CD4 in magenta, CD8 in green and DAPI in blue.

ARG1 expression in murine tumor models

In MC38 and 4T1 murine tumors, ARG1 expression is mostly found within CD45+ and CD11b+ cell populations

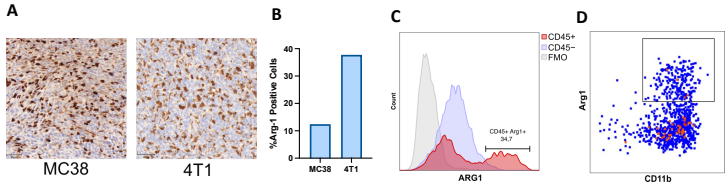

Fig 2. ARG1 expression in murine tumor models MC38 and 4T1. (A) IHC staining for ARG1 performed on tumor sections from MC38 colon adenocarcinoma and 4T1 mammary carcinoma syngeneic murine models. (B) Percentage of ARG1-positive cells in the whole section, excluding large necrotic and stroma areas. IHC score calculated as the ratio of positive cells to total cell count. (C) Representative example of ARG1 expression levels from flow cytometry analysis on untreated 4T1 tumor. (D) FACS plot highlighting ARG1 expression in CD11b+ cells in 4T1 tumor

Immunopeptidome profiling confirms MHC antigen presentation of ARG1-derived peptides in 4T1 model

In the tumor sample 4T1, multiple ARG1-derived peptides are presented on MHC molecules. Two of the MHC-I immunopeptides are encoded within ARG1 peptide vaccine sequence (Arg1$_{191-210}$) identified earlier (1).

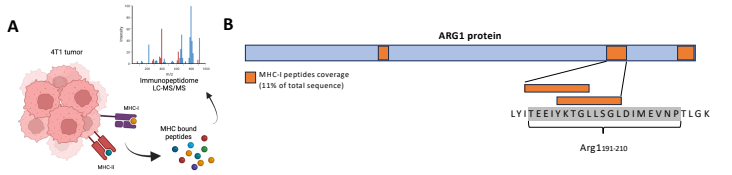

Fig 3. The immunopeptides YKTGLLSGL and ITEEIYKTGL were found to correspond to the immunogenic peptide sequence (ARG1$_{191-210}$). (A) (B) Mouse MHC class I and II complexes with associated peptides were captured using antibodies. After immunoprecipitation, peptides were eluted, pooled, and fractionated by high pH reversed-phase chromatography. Isolated peptides were then desalted and analyzed by HPLC LC-MS/MS.

ARG1 vaccination controls tumor growth by modulating the TME

ARG1 vaccination prompts strong T cell responses and reduces 4T1 tumor growth, observed both with ARG1 alone and in combination with aPD1. It achieves this by altering the cellular makeup of the TME and influencing cell infiltration in the tumor-draining lymph node

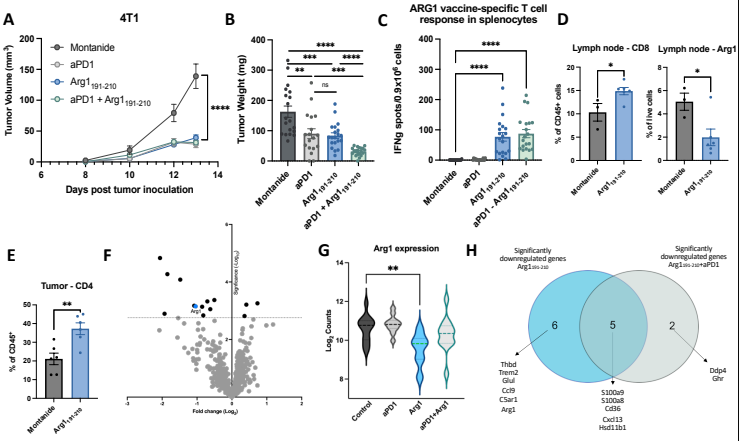

Fig. 4: Effects of ARG1$_{191-210}$ vaccination on tumor microenvironment modulation. (A) Balb/c mice (n=20 per group) treated with Montanide, antiPD1, ARG1$_{191-210}$ vaccination or the combination of ARG1$_{191-210}$ and antiPD1. ARG1 vaccination was performed at day 0 and 7 after the orthotopic inoculation of the tumor. antiPD1 treatment was performed biweekly after day 5 (B) Tumor weights measured on day 13 post-inoculation. (C) ARG1$_{191-210}$ peptide-induced IFNγ ELISpot responses in bulk splenocytes from treated tumor-bearing animals. (D) Vaccine-induced changes assessed by flow cytometry: Proportions of CD8 T cells and ARG1+ cells in the tumor-draining lymph node. (E) Percentage of CD4+ T cells in tumors. (F) Analysis of vaccine-induced changes using the Nanostring PanCancer IO 360 panel. Volcano plot showcasing genes with a log2 fold change greater than 0,5 in the ARG1 treated group versus control group. (G) Log2 transformed expression levels of the ARG1 gene. (H) Venn diagram showing genes significantly downregulated compared to control in both ARG1 treated and ARG1+antiPD1 treated groups. Statistical significance markers: (ns – not significant; * - p ≤ 0.05; ** - p ≤ 0.01; *** - p ≤ 0.001).

ARG1 vaccine directly alters TAM phenotype

ARG1 vaccination reduces MC38 tumor growth and diminishes ARG1 expression in tumor-associated macrophages (TAMs) *in vivo. Ex vivo* isolated CD4+ T cells from ARG1-vaccinated mice target and alter the phenotype of ARG1+ TAMs.

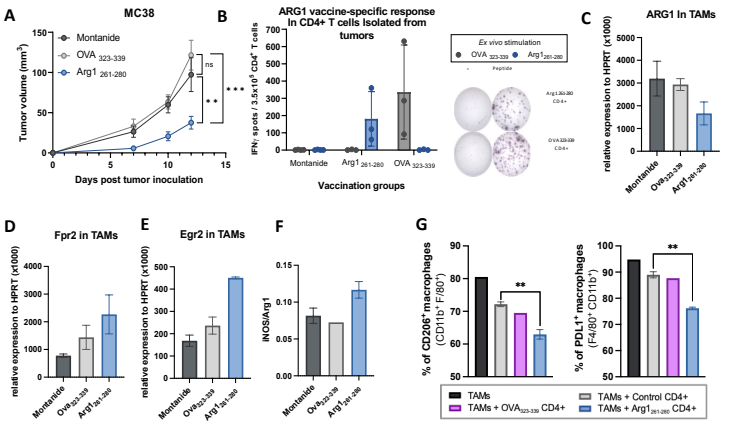

Fig.5. ARG1$_{261-280}$ vaccination induce MC38 tumor growth delay and is associated with changes in TAM phenotype *in vivo*. (A) C57Bl/6 mice with MC38 tumors received treatments of ARG1$_{261-280}$ (n=12), OVA (n=9), or montanide (n=12) on days 0 and 7 post-inoculation. (B) Left: Quantification of ARG1$_{261-280}$ and Ova$_{323-339}$ peptide-specific responses via IFNγ ELISpot in CD4+ T cells from treated tumor-bearing animals. Right: Representative ELISpot wells for ARG1$_{261-280}$ and OVA$_{323-339}$ peptide responses. (C) Relative expression of ARG1 in F4/80+ macrophages post-inoculation. (D) Relative expression of FPr2 in the same macrophage population. (E) Egr2 expression levels in the macrophages. (F) Ratio of iNOS to ARG1 in the F4/80+ macrophages as determined by RT-qPCR. (G) Flow cytometry analysis of CD206 and PD-L1 expression on TAMs in comparison to TAMs after co-culturing with CD4+ T cells from control, Ova-vaccinated, or ARG1$_{261-280}$ vaccinated animals. Statistical significance markers: (ns - not significant; ** - P ≤ 0.01; *** - P ≤ 0.001).

Conclusions

ARG1 presents a compelling target within the tumor microenvironment (TME) for immunotherapy. Vaccination targeting ARG1 to bolster T cell immunity results in anti-tumor activity by modulation of TME. Our data support that vaccination directly reduces Arg1+ cells, both in the TME and in the draining lymph nodes. Further, vaccination directly impacts on TAMs, skewing the balance from an immunosuppressive to a proinflammatory microenvironment. Such a strategy offers an appealing alternative to other approaches currently undergoing nonclinical and clinical evaluations. Our findings reinforce the potential of an ARG1 vaccine as a therapeutic strategy for various Arg1+ tumors.

Reference

1. Jørgensen et al, Immunol. Res. 2021

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