An Arginase-1 peptide-based vaccine is an exciting approach to modulate the tumor microenvironment and drive efficacy in preclinical tumor models

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Introduction

Arginase-1 (ARG1) regulates tumor cells immune escape through various mechanisms in the tumor microenvironment (TME) and is being targeted experimentally in the clinic. However, selective and efficacious inhibitors of ARG1 remain elusive. Vaccination against TME targets have shown to be an exciting therapeutic approach where an IDO1/PD-L1 dual vaccine has demonstrated substantial activity in early trials in melanoma (Kjeldsen et al, Nat. Med. 2021 and a confirmatory Ph3 trial underway NCT05155254). Vaccination against ARG-1 increases anti-tumor activity in murine mouse models (Jørgensen et al, Immunol. Res. 2021). We set out to establish a biological rationale for an ARG1 vaccine as a monotherapy or in combination with other immune therapies in cancer indications, in addition to shedding the light the underlying mechanism of act for such an approach. of the treatment.

Here we evaluate ARG1 expression and the identities of the cells expressing it in the TME of tumor biopsies from multiple solid tumor indications. Furthermore, we show that ARG1 peptides are capable of eliciting immune responses and demonstrate *in vivo* functional activity in murine tumor models. We then undertook detailed cellular and molecular analysis to elucidate the mechanism(s) of this functional activity of the ARG1 vaccine in these mouse tumor models.

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

Methods

To determine if ARG1 is expressed in the TME, Neogenomics MultiOmyx™ technology was used. Samples from 7 cancer indications were stained for a panel of 18 markers defining key cell types (Tables 1-2). Proprietary deep learning algorithms were used to classify positive cells for each marker. 32 regions of interest (ROI) were analyzed for each cancer type.

Results

Samples from all indications tested showed various level of expression of ARG1 in the TME. Immune suppressive cells in the TME expressing ARG1 were for the most part distinct from other cell populations expressing other immune suppressive antigens (e.g. IDO1 and PD-L1) suggesting a rationale for combining such vaccines in a single treatment. Positive cells for ARG1 were found across various lineages including immune cells, stromal cells and tumor cells themselves.

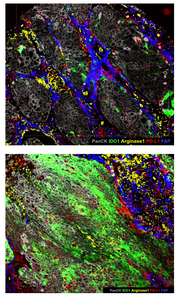
Table 1 Cancer types analysed

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

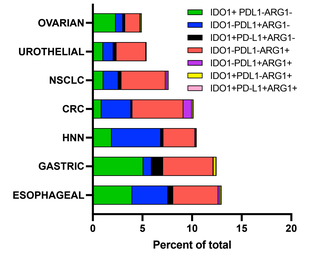
Table 2 Markers and cell type definitions

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/TIGIT/LAG-3+
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+

ARG1 costaining with other markers in TME

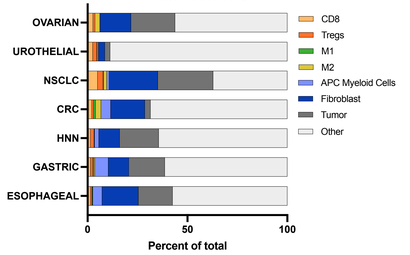


ARG1 is expressed in the TME of several cancer types



Stacked bars diagram showing the fraction of cells expressing one or several of the indicated antigens (IDO1, PD-L1, ARG1) for each cancer indication analyzed.

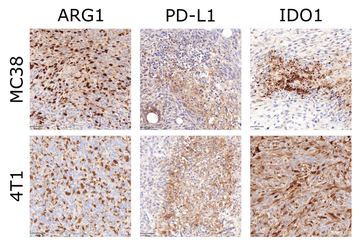
ARG1 is expressed by several cell types within the TME



Stacked bars diagram showing the different cell types expressing ARG1 in the TME for each indicated cancer type analyzed

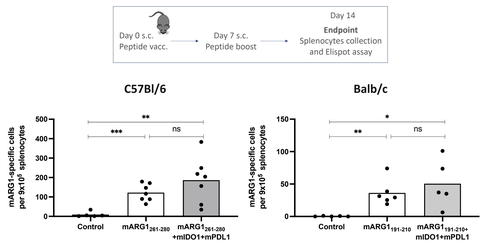
Combination of an ARG1 with an IDO1/PD-L1 vaccine enhances anti-tumor effect *in vivo* in MC38 and 4T1 tumor models

ARG1 is expresed in MC38 and 4T1 tumor models



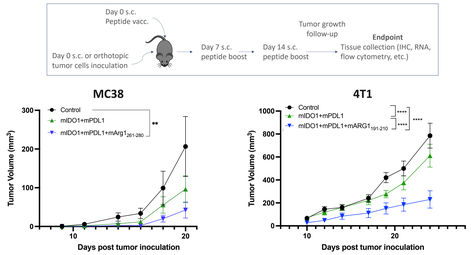
IHC staining for ARG1, IDO1 and PD-L1 performed on tumor sections from MC38 colon adenocarcinoma and 4T1 mammary carcinoma syngeneic murine models.

ARG1 peptide vaccination induces ARG1 specific responses in mice



Scatter plots showing ARG1 specific cells detected in IFN$_\gamma$ Elispot assays performed following overnight stimulation of splenocytes collected from mice treated twice with relevant mARG1 peptides. Data shown as mean ± SEM.

ARG1 vaccination suppresses MC38 and 4T1 tumor growth



Tumor growth curves of MC38 and 4T1 following treatment with mARG1 peptides combined to mIDO1 and mPDL1. Animals were treated on Days 0, 7 and 14. Control animals received peptide-free montanide emulsion. Data shown as as mean ± SEM.

Methods

- To select relevant animal models, tumor samples were collected from different models and the expression of ARG1 and other relevant targets were evaluated per IHC.
- Two ARG1 peptides were designed; mARG1 $_{191-210}$ for Balb/c background and mARG1 $_{261-280}$ for C57Bl/6 mice. To test their ability to generate *in vivo* responses, animals were treated on days 0 and 7 with mARG1 peptides, splenocytes were collected and specific responses were evaluated in an IFN$_\gamma$ Elispot assay.
- To evaluate the effect of mARG1 peptides in a tumor context, animals were inoculated with MC38 (s.c.) or 4T1 (orthotopic) tumor cells and treated with mARG1 combined to mIDO1 and mPD-L1 peptides, mixed with montanide adjuvant and delivered per subcutaneous injection (s.c.). Animals were monitored for tumor growth.

Results

Two relevant murine tumor models were selected based on the expression of ARG1 and treated with mARG1 peptides shown to induced ARG1 specific T-cell responses. Combined treatment with mARG1 and mIDO1/mPD-L1 peptides induced significant anti-tumor effect in both MC38 and 4T1 models.

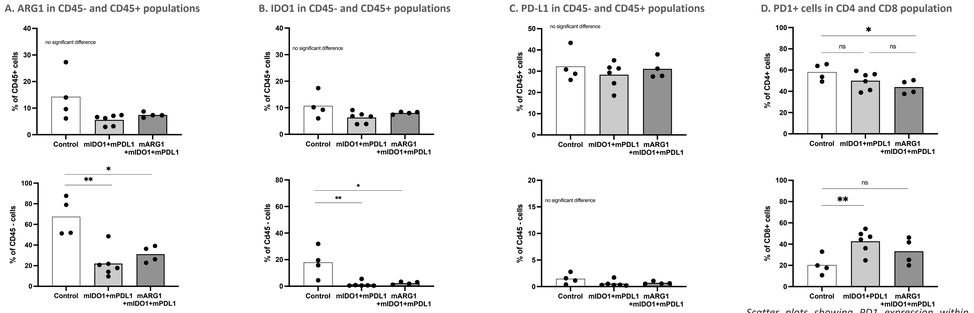
Cellular and molecular analysis of the TME following vaccination with an ARG1 peptide in a mouse model

Methods

To gain insights into the mode of action of mARG1 treatment, tumor samples were collected from mice inoculated with MC38 cells and treated as described above. Tumor samples were collected on day 18 and enzymatically dissociated. Isolated cells were then stained for cytoplasmic and intracellular markers including CD45, CD3, CD4, CD8, IDO1, ARG1, PD-1 and PD-L1. Graphs show flow cytometry analysis results for the expression of (A) ARG1, (B) IDO1 and (C) PD-L1 within CD45+ and CD45- cells and for (D) PD1 expression within CD4 and CD8 compartments.

Results

Flow cytometry analysis showed reduction in ARG1 and IDO1 expression particularly in CD45- compartment that contains tumor and stromal cells upon treatment. In addition, a significant decrease in PD1+CD4+ T cells was detected in tumor samples from animals treated with mARG1 combined to mIDO1 and mPDL1. RNAseq to decipher molecular changes in the TME is underway.



Scatter plots showing expression of ARG1, IDO1 and PD-L1 among CD45+ and CD45- cellular fractions isolated from MC38 tumor samples

Scatter plots showing PD1 expression within CD4+ and CD8+ fractions

Conclusions

- ARG1 expression marks a diverse set of immune suppressive cells in the tumor microenvironment that are mostly independent of IDO1 and PD-L1 expressing cells (currently targeted in the clinic) in multiple cancer indications
- Elimination of ARG1 expressing cells in the TME via a vaccine approach presents an attractive and a novel strategy to tackle cells expressing the antigen and drive therapeutic benefit in combination with an IDO1/PD-L1 vaccine
- ARG1 peptide vaccines are capable of inducing strong targeted immune responses and in combination with an IDO1/PD-L1 vaccine significantly reduces tumor growth in both MC38 (colon cancer) and 4T1 (breast cancer) mouse tumor models
- Cellular and molecular analysis are underway to elucidate the mechanisms by which the vaccination with ARG1 peptides modulates the TME to drive an anti-tumor effect in these mouse models
- The combined data supports further preclinical development of an ARG1 vaccine in combination with an IDO1/PD-L1 vaccine to modulate the TME for therapeutic benefit in a wide range of cancer indications

References

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A phase 1/2 trial of an immune-modulatory vaccine against IDO/PD-L1 in combination with nivolumab in metastatic melanoma. Nat Med. 2021 Dec;27(12):2212-2223.

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