Poster no. 124P
Abstract no. 566
ESMO IO 2024

AI-designed cancer vaccines: antigens from the dark genome are promising cancer vaccine targets

EVAXION

Daniela Kleine-Kohlbrecher, S. Vester Kofoed, R. O. Andersen, M. Barrio-Calvo, S. Friis, R. Villebro, M. S. Klausen, J. Kringelum, S. F. Thorsen, B. Rønø;
Evaxion Biotech A/S, Hørsholm, Denmark

Introduction

Personalized cancer vaccines targeting patients' tumor mutations, so-called neoantigens, have significantly advanced cancer treatment. However, the use of neoantigens is restricted to tumors with sufficiently high mutational burden, limiting their use to selected clinical indications. The discovery of endogenous retroviruses (ERVs) as relevant immunologic features contained in the dark genome and the research demonstrating their dynamic role in cancer development and progression provides potential prospects for therapeutic use. Evaxion Biotech's AI-Immunology™ platform allows for the identification and selection of ERVs as a new antigen source for designing personalized and precision therapeutic cancer vaccines.

In the presented work, we explore the efficacy of AI-Immunology™ identified ERVs as alternative antigens for DNA cancer vaccines in preclinical mouse and human cell models. The precision vaccine designs contain multiple ERV protein fragments, defined as ERV hotspot, consisting of one or more MHC allele ligands. This vaccines have the potential to be effective across a wide patient population within the same cancer indication, moving from a personalized to a more precision vaccine approach.

Results and Conclusions

AI-Immunology™ identified hotspot designs were tested in mice and human PBMCs, demonstrating:

- immunization with murine ERV antigenic hotspots lead to tumor growth inhibition in two syngeneic mouse models
- induction of antigen-specific T-cell responses in mice is mediated by activated CD8+ and CD4+ T-cells
- human ERV antigenic hotspots induce significant antigen-specific T-cell responses in six human donor PBMC samples

The obtained results prove that the AI-Immunology™ platform can identify functional and potent ERV antigenic hotspots. This warrants for further development towards clinical application.

1. ERV antigenic hotspot concept

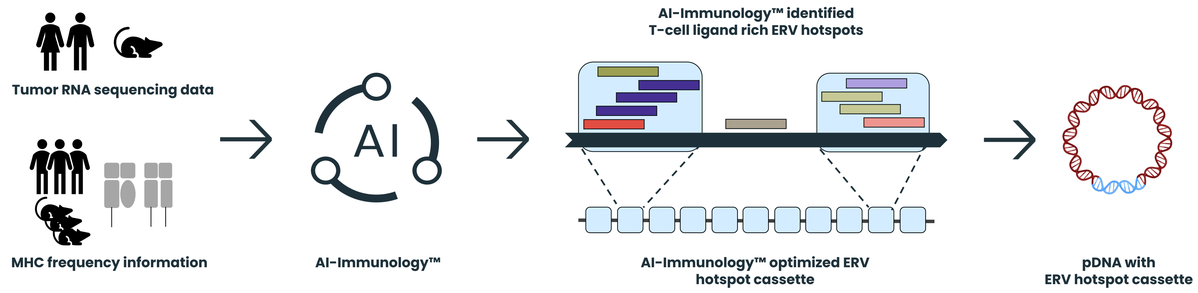
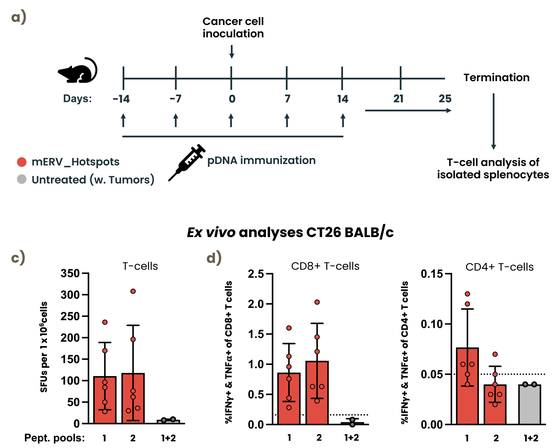


Figure 1. Schematic showing the hotspot identification, selection and DNA vaccine design concept
AI-Immunology™ can generate an ERV based vaccine from tumor RNA sequencing data and MHC frequency information. To quantify expressed ERVs, tumor RNA sequencing data are mapped to a custom reference genome that includes ERV information. ERV sequences rich in T-cell ligands, so-called ERV T-cell ligand hotspots, are identified by predicting ERV specific T-cell ligands for all MHC alleles with frequencies above a certain threshold. A cassette of T-cell ligand hotspots connected by short linkers is inserted into a plasmid DNA (pDNA) for a precision vaccine design. The ERV hotspot cassette is optimized using an algorithm that simulates population coverage based on ERV expression and MHC allele frequencies.

2. Immunization with mERV hotspots leads to tumor growth control and immunogenicity in mice



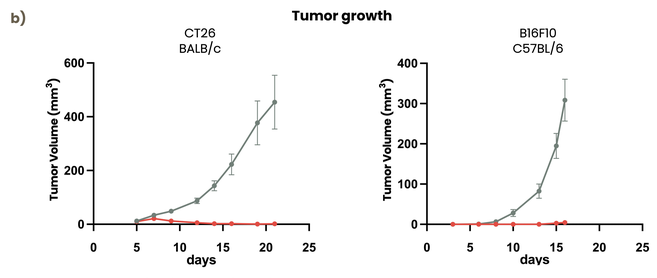
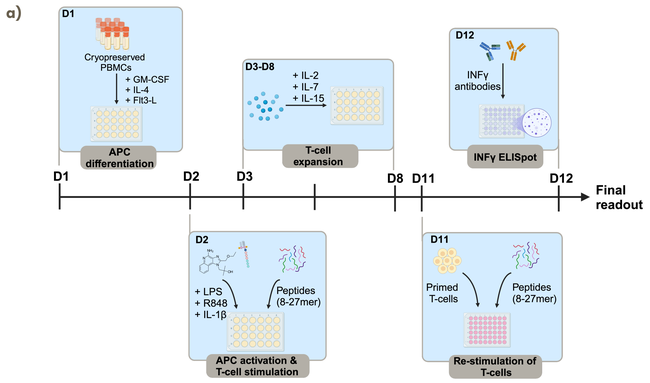
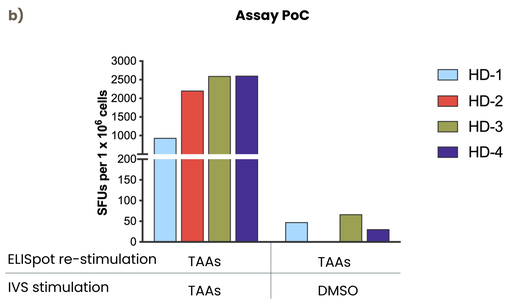


Figure 2. Immunization of mice with mERV hotspots leads to tumor growth control and induction of specific T-cell responses
a) *In vivo* experimental timeline. Groups of n=13 mice (BALB/c or C57BL/6) were immunized prophylactically with 25 µg pDNA_mERV_Hotspots before subcutaneous inoculation with CT26 (BALB/c) and B16F10 (C57BL/6) tumor cells. After euthanization, splenocytes were isolated for T-cell analyses. **b)** Tumor growth over time (Mean +/- SEM). **c)** T-cell immunogenicity measured by IFNγ ELISpot upon splenocyte re-stimulation with vaccine ERV peptide pools (Mean +/- SD). **d)** Splenic CD8+ (left) and CD4+ (right) T-cell reactivity upon re-stimulation with vaccine ERV peptide pools measured by intracellular cytokine staining (Mean +/- SD). The dotted lines indicate the background level.

3. *In vitro* T-cell priming in human PBMCs shows specific T-cell activation





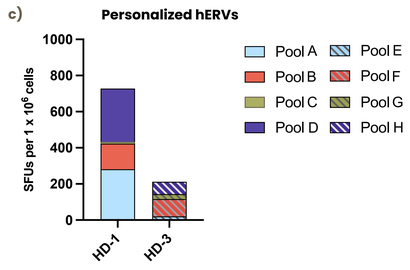
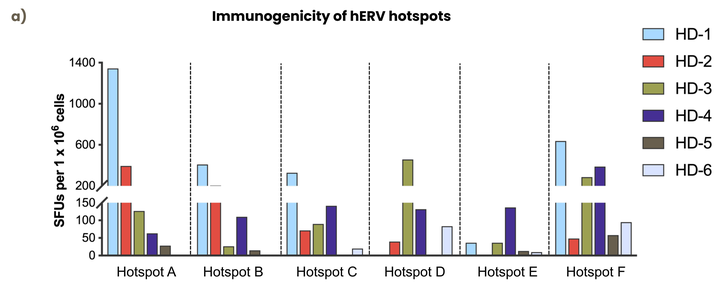


Figure 3. *In vitro* T-cell priming in human PBMCs
a) *In vitro* stimulation (IVS) of human peripheral blood mononuclear cells (PBMCs) to prime antigen-specific T-cell responses (adapted from Bozkus et al. 2021). First, PMBCs were cultured for 24 hours with cytokines to induce antigen-presenting cell (APC) differentiation and maturation. Subsequently, the PBMCs were incubated for 24 hours with the peptides of interest and adjuvants to promote antigen presentation and T-cell priming. During the following 10 days, the cells received cytokines to enhance expansion of the primed T-cells. Finally, immunogenicity was evaluated using the IFNγ ELISpot assay. **b)** Assay Proof-of-Concept using tumor associated antigens (TAAs). PBMCs from four healthy donors (HDs) were primed and re-stimulated with TAA peptides matching the HLA alleles of the respective HDs. The graph depicts the spot forming units (SFUs) per 1 million cells, obtained by ELISpot analysis. DMSO served as negative control. Values are presented as the means of technical replicates, with background subtraction. **c)** PBMCs from two different HDs were primed and re-stimulated with four pools of hERV antigens matching their HLA alleles. The graph shows the SFUs per 1 million cells obtained by ELISpot analysis. Values are presented as the means of technical replicates, with background subtraction.

4. Human ERV hotspots induce specific T-cell responses in vitro



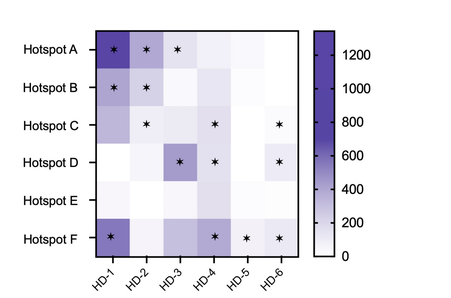


Figure 4. IFNγ response of *in vitro* primed human PBMCs towards human ERV hotspot antigens
a) Immunogenicity of AI-Immunology™ predicted hERV hotspots in human PBMCs. PBMCs from six HDs were primed and re-stimulated with peptides representing six different AI-Immunology™ predicted hERV hotspots (A-F). The bar plot shows the SFUs per 1 million cells, obtained by ELISpot analysis. Values are presented as the means of technical replicates, with background subtraction. Hotspots are predicted by AI-Immunology™ based on RNA sequencing data of 150 Acute Myeloid Leukemia patients from the Cancer Genome Atlas and HLA frequencies of the general worldwide population scraped from allelefrequencies.net. **b)** Summary of the in a) presented T-cell responses, depicted per hotspot pool and healthy donor. The stars (*) indicate an immunogenic response according to the defined cut-off criteria: [Mean $SFU_{ANTIGEN\ STIMULATED}$] ≥ 2 x [Mean $SFU_{UNSTIMULATED}$]+ 10 SFUs.

Acknowledgment and Contact

We wish to thank Anne Lund, Heidii Mazur and Rikke Sølberg for technical assistance in the laboratory and animal facility.

Contact Evaxion Biotech
Daniela Kleine-Kohlbrecher
Senior Project Manager
dkk@evaxion-biotech.com

Contact Download