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T-cell immunogenicity and biomarker profiling in advanced melanoma patients receiving the personalized vaccine EVX-01 in combination with pembrolizumab

EVAXION

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Introduction

Patients with advanced melanoma were treated with a personalized neoantigen (NeoAg) vaccine, EVX-01, in combination with pembrolizumab in a phase 2 clinical study (NCT05309421). The peptide-based EVX-01 vaccine consists of multiple tumor-specific NeoAgs identified by Evaxion Biotech's target discovery AI-Immunology™ platform. As reported in September 2024 at the one-year follow-up, the treatment was well tolerated, and the objective response rate was 69% in the overall cohort with 11 out of 16 patients responding to study therapy. Longitudinal blood samples were collected before, during and after EVX-01 treatment to monitor circulating vaccine specific T-cell responses. Baseline serum samples were analyzed by the Olink Target96 Immuno-oncology panel to identify potential predictive biomarkers of clinical response to treatment. Finally, baseline tumor biopsies were subjected to genomic and transcriptomic analyses, immunohistochemistry, and AI-assisted digital pathology to understand characteristics of the tumor and the immunological landscape of the tumor microenvironment (TME).

Conclusions

- EVX-01 induced a vaccine-specific immune response in all assayed patients (n=15) after EVX-01 priming
- Booster immunizations increased and maintained the broadness of NeoAg-specific T-cell responses
- 84 out of 105 (80%) of administered NeoAgs induced specific T-cell responses
- Immune responses were mediated by both CD4+ and CD8+ T cells
- Digital pathology analysis revealed a lower neutrophils-to-lymphocytes ratio and a less fibrotic TME in responders vs non-responders
- In this small cohort, serum proteome profile and tumor genomic/transcriptional profile did not correlate with clinical response
- Findings validate the precision and predictive power of the proprietary AI -Immunology™ platform

1. Trial design and clinical responses

Patient population: Unresectable stage III or stage IV melanoma patients naïve to checkpoint inhibitor (CPI) treatment were enrolled in this clinical Phase 2 study.

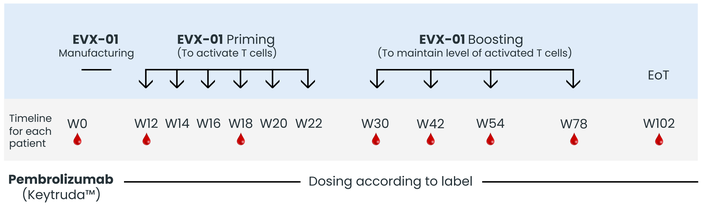

Figure 1. EVX-01 trial design and blood sampling. The combination treatment consisted of anti-PD1 **pembrolizumab** (Keytruda™, 400 mg Q6W) solution for infusion (IV) plus peptide NeoAg vaccine **EVX-01** (2 mg peptide), adjuvanted with CAF09b® (IM). EVX-01 was administered Q2W during priming vaccination followed by four booster vaccinations given at weeks 30, 42, 54 and 78. The last blood sample for immunogenicity assessment was collected 24 weeks after the last booster dose at the end of treatment (EoT) scheduled week 102.

Table 1. Overview of clinical responses at week 12 compared to best overall response

Clinical response per RECIST 1.1.	Week 12 response, n	Best overall response, n
Complete response	1 (6.2%)	3 (18.7%)
Partial response	8 (50.0%)	8 (50.0%)
Stable disease	5 (31.3%)	3 (18.8%)
Progressive disease	2 (12.5%)	2 (12.5%)
No assessment, not included	1	1

All responses have been confirmed by follow-up assessments conducted in accordance with RECIST 1.1 criteria.

EVX-01 Safety (n=16)

For the 16 patients treated with EVX-01, 159 treatment-emergent adverse events (AEs) were reported, mostly Grade 1 and 2. These included 8 injection site reactions (5.0%), 5 cases of diarrhea (3.1%), 2 of fatigue (1.3%), and 3 rashes (1.9%). In the EVX-01 plus pembrolizumab combination, reported AEs included:

- One Grade 3 immune-related type 1 diabetes
- One Grade 4 immune-related type 1 diabetes
- Two serious AEs: fatigue and anorexia
- One unrelated death

Data cut-off: July 27, 2024.

2. EVX-01 induces strong, broad and durable NeoAg specific T-cell responses

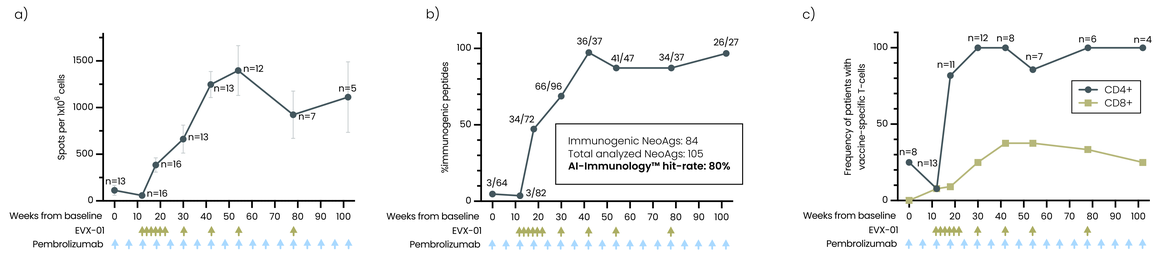

Figure 2. NeoAg-specific T-cell responses after EVX-01 prime and boost. Peripheral blood mononuclear cells (PBMCs) from patients were expanded *in vitro* against the EVX-01 peptide pool for 10 days and re-stimulated with the EVX-01 peptide pool in an IFNγ ELISpot assay or intracellular cytokine staining (ICS) and flow cytometry assay. **a)** Vaccine pool responses over time measured by IFNγ ELISpot. Data is shown as mean spots per 10^6 cells + standard error of mean (SEM). The green arrows indicate time points of vaccine administrations and blue arrows indicate time points of pembrolizumab administration. All SFUs have been background subtracted, and n indicates number of assessed patient samples per time point. **b)** Percentage of immunogenic NeoAgs per time point. The numbers on top of the bars indicate the absolute numbers per timepoint (immunogenic NeoAg/tested NeoAg). **c)** Vaccine-specific CD4+ (dark blue) and CD8+ T cells (green) were analyzed by ICS and flow cytometry after *in vitro* expansion. T-cell responses were defined as 1) %cytokine-positive$_{\text{vaccine_pool STIMULATED}}$ > 2.5 x %cytokine-positive$_{\text{UNSTIMULATED}}$ AND 2) at least 0.1% of CD4 or CD8. n indicates number of assessed patient samples per time point.

3. Baseline TME features are linked to clinical response in patients treated with EVX-01 and pembrolizumab

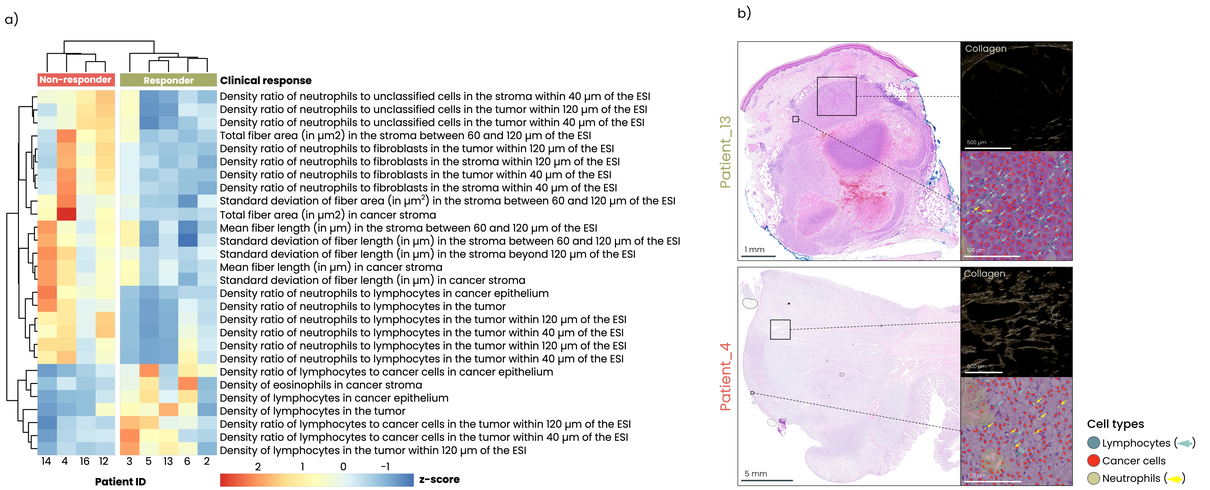

Figure 3. PathExplore analysis of hematoxylin and eosin (H&E) stained baseline tumor specimens. Baseline H&E stained tumor specimens from nine patients were analyzed by AI-assisted digital pathology using the PathExplore platform. Whole slide images (WSI) were analyzed by the PathExplore Melanoma V1.1 and the PathExplore Fibrosis V1.0 models. **a)** PathExplore human interpretable features (HIFs) were normalized, subjected to unsupervised clustering and plotted in a heatmap. Responders demonstrated a TME signature with increased densities of lymphocytes, lower ratios of neutrophils-to-other cells and a less fibrotic TME (reduced length and area of collagen fibers) compared to non-responding patients. **b)** Representative examples of WSI annotated by PathExplore overlays. ESI: Epithelial stromal interface

4. Tumor transcriptome and baseline serum proteome do not correlate with clinical response

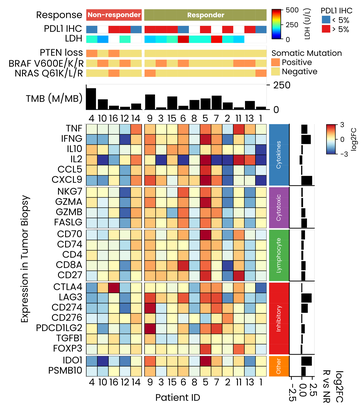

Figure 4. Tumor and TME characteristics. Summary of tumor and TME characteristics per individual patient. Shown are the clinical response (cut-off 27-Jul-24) and according RECIST status. Tumor PD-L1 expression was analyzed in a four-plex fluorescence assay using the PD-L1 specific antibody clone 28-8. PD-L1 positivity was defined as 5/100 cells (immune & tumor cells) showing significant membrane staining (>5%). LDH levels were assessed in plasma by routine assay. Melanoma associated driver mutations, and tumor mutational burden (TMB) were identified by whole exome sequencing (WES) of tumor biopsies. Gene expression signature analysis was performed using transcriptome data obtained from next generation sequencing (NGS) of tumor-derived RNA. Matching blood samples served as normal tissue control in WES and NGS. Differential expression is reported as log2 fold change between responder versus non-responder patients.

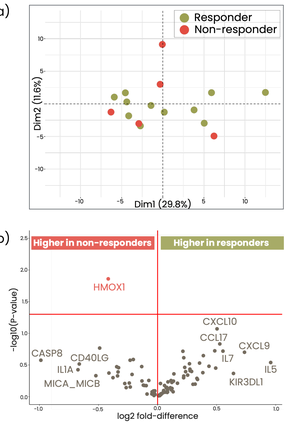

Figure 5. Olink proteomic analysis of baseline serum samples. Serum samples were collected for all 16 patients at baseline and analyzed by a proximity extension assay (PEA). In total, 92 proteins were quantified using the Olink Target96 Immuno-Oncology panel. **a)** Principal component analysis (PCA) demonstrated no overall differences in serum protein profile between responders and non-responders. **b)** Volcano plot demonstrating the difference in serum protein levels between responders and non-responders. For each protein the Log2 fold-difference is plotted against the unadjusted P-value from multiple Mann-Whitney U tests. Protein names are shown for proteins with log2FC > 0.5, log2FC < -0.5 or unadjusted p < 0.05.

Acknowledgment and Contact

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