EVAXION AI-Immunology™ Powered Vaccines

Immunogenicity of an AI-designed personalized neoantigen vaccine, EVX-01, in combination with anti-PD1 therapy in patients with metastatic melanoma

Mads Lausen[1], Nadia Viborg[1], Georgina V Long[2], Muhammad A Khattak[3], Paolo A Ascierto[4], Paola Queirolo[5], Michail A Pavlidis[1], Stine F Thorsen[1], Michael Chisamore[6], Daniela Kleine-Kohlbrecher[1], Thomas Trolle[1], Birgitte Rønø[1]

[1]Evaxion-Biotech A/S, Horsholm, Denmark, [2]Melanoma institute Australia, The University of Sydney, and Royal North Shore and Mater Hospitals [3]Oncology, One Clinical Research, Hollywood Private Hospital, Nedlands, WA, Australia, [4]Melanoma, Cancer Immunotherapy & Developmental Therapeutics, Istituto Nazionale Tumori - IRCCS - Fondazione Pascale, Napoli, Italy, [5]Divisione di Oncologia Medica del Melanoma, Sarcoma e Tumori Rari, IEO - Istituto Europeo di Oncologia IRCCS, Milan, Italy, [6]Oncology Early Clinical Development, Merck & Co., Inc., Rahway, NJ, United States of America,

Introduction

Despite the advances made by the introduction of immune-checkpoint inhibitor therapy there is still an unmet medical need for patients with metastatic melanoma. Personalized vaccines, targeting mutation-derived neoantigens (neoAgs), represent a promising frontier in cancer immunotherapy that can further boost the induction of tumor specific T-cells. In this study, we identified and selected tumor-specific neoAgs using the proprietary vaccine target discovery AI-Immunology™ platform based on tumor DNA- and RNA-sequencing data. The top-ranked neoAgs (max. 10 neoAgs) for each patient were manufactured as synthetic long peptides and formulated with an liposomal adjuvant, creating the personalized cancer vaccine, EVX-01, tailored to the individual tumor and immune system characteristics. Here we report on vaccine-induced immune responses in 12 metastatic melanoma patients treated with the AI-designed personalized cancer vaccine, EVX-01, in the ongoing single arm multicenter Phase 2 trial (NCT05309421).

Highlights

- ✓ **EVX-01 induced a vaccine-specific immune response in all assayed patients (n=12) after EVX-01 priming**
- ✓ **Immune responses were mediated by both CD4+ (11/11) and CD8+ T-cells (5/11) in patients receiving at least 6x EVX-01**
- ✓ **The first booster immunization tend to increase the neoAg-specific T-cell response**
- ✓ **64/90 (71%) of administered neoAgs induced T-cell responses**
- ✓ **AI-Immunology™ quality score correlates significantly with immunogenicity of neoAgs**
- ✓ **Findings validate the precision and predictive power of the proprietary AI -Immunology™ platform**

1. EVX-01 manufacturing process and clinical study outline

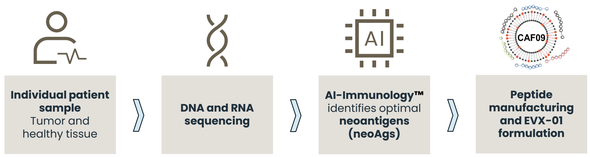

Figure 1. Manufacturing process of EVX-01 personalized vaccine. Illustration of the production process from tumor biopsy, prediction and selection of neoAgs, manufacturing and establishing of the final EVX-01 product with peptides and CAF®09b liposomal adjuvant.

Patient enrollment:
Treatment naive unresectable stage III or stage IV melanoma patients were enrolled in this clinical Phase 2 study.

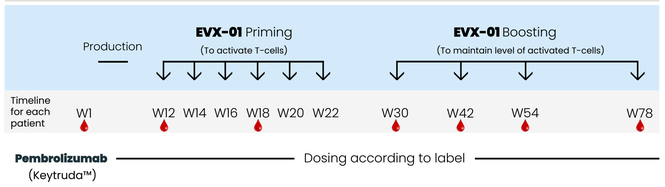

Figure 2. Clinical study design and blood sampling. The combination treatment consisted of anti-PD1 **Pembrolizumab** (Keytruda™, 400 mg Q6W) solution for infusion (IV) plus peptide neoAg vaccine **EVX-01** (2 mg peptide), adjuvanted with CAF09b (IM). EVX-01 was administered Q2W during priming vaccination followed by four booster vaccinations given at W30, W42, W54 and W78.

2. Patient demographics and EVX-01 safety profile

Patient demographics	
Sex, n (%)	
Male	10 (83)
Female	2 (17)
Age, n (%)	
<65	4 (33)
≥65	8 (67)
Ethnicity, n (%)	
Caucasian	12 (100)
ECOG performance-status score, n (%)	
0	12 (100)
Disease stage, n (%)	
Unresectable Stage IIIB	1 (8)
Unresectable Stage IV	9 (75)
Coding pending	2 (17)
Number of lesions, n mean (interval)	
Target	2.3 (1-5)
Non-target	1.6 (0-4)
BRAF mutation status, n (%)	
Positive	6 (50)
Negative	5 (42)
Unknown	1 (8)
Lactate dehydrogenase, n (%)	
Normal	9 (75)
Elevated	2 (17)
Missing	1 (8)

EVX-01 Safety Profile

EVX-01 was safe and well-tolerated, with primarily grade 1 and 2 ADRs related to EVX-01 even after boosting. The combination of EVX-01 and anti-PD1 also appeared safe and well tolerated.

Data cut-off:
14-Mar-2024.

3. EVX-01 induces neoAg-specific T-cell responses after priming

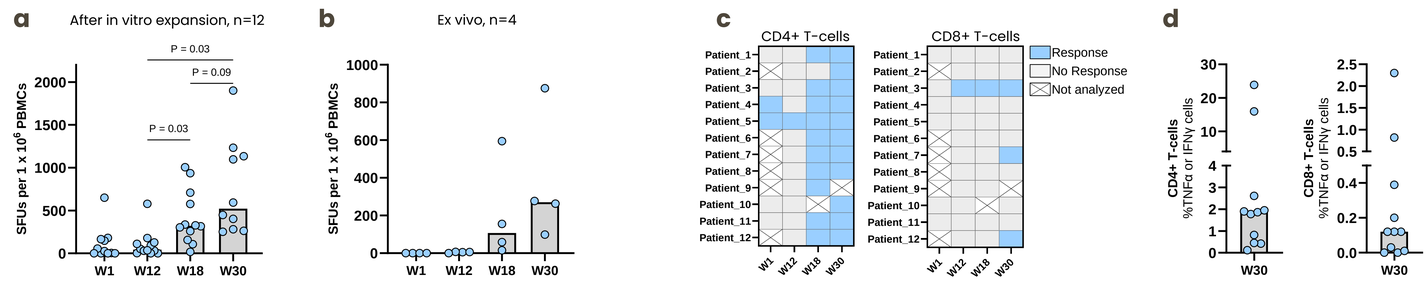

Figure 3 NeoAg-specific T-cell responses after EVX-01 priming. a) PBMCs from 12 patients were expanded in vitro against the EVX-01 peptide pool for 10 days and restimulated by the EVX-01 peptide pool in an IFNγ ELISpot assay. A significant induction of vaccine-specific T-cell responses was observed after three immunizations which tends to increase after six immunizations. Importantly, all patients had a vaccine-specific T-cell response after EVX-01 priming. **b)** In a subset of patients (n=4), PBMCs were analyzed in an ex vivo IFNγ ELISpot demonstrating that vaccine-specific T-cell responses can be detected directly in ex vivo PBMCs. **c)** Vaccine-specific CD4+ and CD8+ T-cells were analyzed by intracellular cytokine staining (ICS) and flow cytometry after in vitro expansion. T-cell responses were defined as **1.** %cytokine-positive$_{vaccine_pool\ STIMULATED}$ > 2.5 x %cytokine-positive$_{UNSTIMULATED}$ AND **2.** at least 0.1% of CD4/CD8. EVX-01 induced CD4+ neoAg T-cell responses in 10/11 patients after three immunizations and in all patients 11/11 after six vaccinations. One patient demonstrated neoAg-specific CD8+ T-cell responses after CPI treatment and after three vaccinations, while three patients demonstrated neoAg-specific CD8+ T-cell responses after six vaccinations. **d)** The magnitude of CD4+ and CD8+ T-cell responses demonstrates large variations among patients and the end of the EVX-01 priming phase (W30).

4. EVX-01 boosting sustains immune responses

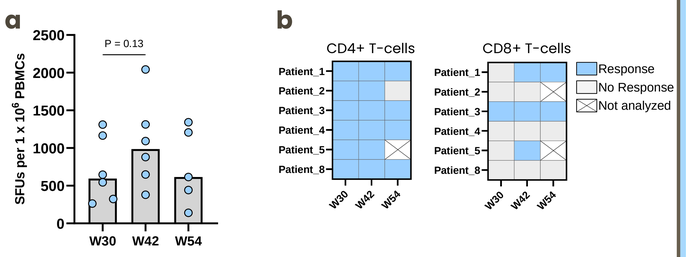

Figure 4. NeoAg-specific T-cell responses after EVX-01 boosting. PBMCs from six patients who received two EVX-01 booster immunizations were expanded in vitro and restimulated with the EVX-01 peptide pool in an **a)** IFNγ ELISpot and **b)** an ICS assay. There was a clear tendency of an increased neoAg-specific immune response after the 7th dose of EVX-01. Interestingly, the 7th dose induced CD8+ T-cell response in two patients (patient_1 and patient_5), which was not evident after the priming phase.

5. NeoAg quality correlates positively with T-cell responses

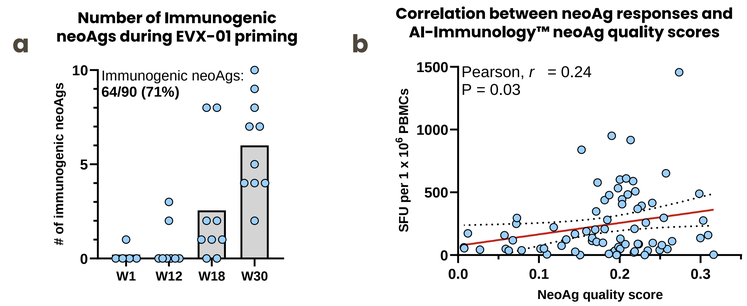

Figure 5. Immunogenicity of individual EVX-01 neoAgs and correlation with NeoAg quality score
a) Number of immunogenic neoAgs per patient at each sample timepoint during EVX-01 priming. Immunogenic neoAgs were determined in an IFNγ ELISpot assay using the criteria: [Mean SFU$_{neoAg\ STIMULATED}$] > 2 x [Mean SFU$_{UNSTIMULATED}$]+ 10 SFU. 64 out of 90 tested neoAgs were immunogenic. **b)** Correlation between IFNγ ELISpot responses and AI-Immunology™ neoAg quality scores assessed at week 30 after completion of EVX-01 priming (6x EVX-01) demonstrated a significant positive correlation between neoAg quality score and IFNγ responses.

Abstract no. 9561
ASCO 2024

Contact: ***Mads Lausen, PhD***
Senior Scientist
mla@evaxion-biotech.com

Acknowledgement: We wish to thank all the patients, family members and staff from all the units that participate in the study. This study is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA