Supplementary Figure 1 | Tumor response in patient 8 during treatment timeline. The patient was included after ten aPD-1 treatments. The patient was evaluated to have had SD for at least four months before inclusion. During vaccine production, the patient received additional three aPD1 treatments. Control scan placed after the first vaccination still showed SD. After additional six vaccinations and three aPD-1 treatments, the control scan showed tumor regression, which continued 11 months from the first vaccination. The scan showed complete regression in the target lesion but still visible non-target lesions, so we define the state as PR and not complete response (CR). Figure created with BioRender.com

Supplementary Figure 2 | EVX-01-specific PBMC derived T cell responses analyzed by Elispot. PBMC have been pre-stimulated with patient-specific vaccine-peptide pool, for 10-14 days prior to Elispot analysis. Each graph represents one patient, divided in following time points, T1; before ICI, T2; after ICI, before vaccine, T3; after vaccine, 3 IP injections, T4; after additional 3 IM vaccine injections, and FU; follow up samples. The x-axis shows the peptides used for re-stimulation, including single vaccine peptides (Peptide 1, Peptide 2 and so on), pooled vaccine peptides (pool) and an irrelevant peptide (Irr). Black bars represent significant responses, grey bars are not significant. The yellow and red dotted lines indicated the threshold value for a significant response; background(irrelevant peptide) plus 3xSD of the background (red) and at least 10 spots over background response (yellow). NB y-axis differs between patients.

Supplementary Figure 3 | information on vaccine peptides, and screened vaccine-related minimal peptides. Information on vaccine peptides; vaccpep number, intended vaccination dose, gene origin, and amino acid mutation (incl site) is noted above each vaccine peptide. The wild type sequence is shown in the top and the mutated (vaccine peptide) is shown below (black sequences). All screened vaccine-related, minimal peptides are aligned to each vaccine peptide. Minimal peptides are colored according to HLA-binding and mutated minimal peptides are marked in *italic*.

Supplementary Figure 4 | EVX-01 induced CD4+ and CD8+ T cell responses in PBMCs before and after vaccination. PBMC have been pre-stimulated with patient-specific vaccine-peptide pool, for 10-14 days prior to analysis. T cell responses showed after re-stimulation with pool vaccine peptides and stained intracellularly for IFN-γ, TNF-α, CD107a and CD137. The bars shows the percentage of cells, which are positive for at least two of above-mentioned markers. CD4+ T cells responses are shown to the left and CD8+ T cell responses to the right. Grey bars shows response after re-stimulation with irrelevant peptide, thus showing background response. Black bars shows response after re-stimulation with pooled vaccine peptides. A response exceeding background response is defined as a vaccine-specific response.

Supplementary Figure 5 | ICS flow cytometry dotplots. PBMC have been pre-stimulated with patient-specific vaccine-peptide pool, for 10-14 days prior to analysis. T cell responses showed after re-stimulation with pool vaccine peptides and stained intracellularly for IFN-γ, TNF-α, CD107a and CD137. Flow cytometry dotplots are shown for each patient. The top row per patient show responses to irrelevant peptide and the bottom shows responses towards peptide pool. Flow cytometry dotplots are shown for CD4+ and CD8+ T cell expression of INF-g and TNF-a. The red dotted line separate time points before and after EVX-01 vaccination.

Supplementary Figure 6 | EVX-01-specific T cell responses in SKILs (skin-test infiltrating lymphocytes) EVX-01-specific CD8+ and CD4+ T cells were identified by intracellular cytokine stain in the SKILs isolated from patients 6, 8 and 9.

Supplementary Figure 7 | Overview of EVX-01 prestimulated PBMCs derived CD8 T cells screened for EVX-01 CD8 epitopes. EVX-01 prestimulated PBMC were screened for EVX-01 specific CD8 T cells (VaccNARTs) using fluorochrome labelled pMHC-multimers. VaccNARTs are

gated in clusters with one or more specificities due to overlap cross reactivity between sequences. The frequency of VaccNART clusters detected in EVX-01 expanded PBMC from different time points. Each dot represent a cluster which are colored according to patient. The single clusters are connected with lines between time points to follow the single clusters dynamics. Boxplots summarize the distribution and median of clusters within single timepoints. The red triangles indicated before and after vaccination initiation.

Supplementary Figure 8 | VaccNART screening results per patient and cluster. EVX-01 prestimulated PBMC were screened for EVX-01 specific CD8 T cells (VaccNARTs) using fluorochrome labelled pMHC-multimers. VaccNARTs are gated in clusters with one or more specificities due to overlap cross reactivity between sequences. The frequency of VaccNART clusters detected in EVX-01 expanded PBMC from different time point. To the left, a flow plot showing the collected frequency per patient. Each color represents a single cluster. To the right, plot showing the frequency of single clusters, which are connected with lines between time point.

Figure 9 | Analysis of epitope spreading detected in *ex vivo* PBMCs, and expanded TILs and SKILs. Ex vivo PBMC and expanded TILs and SKILs were screened for neoepitope specific CD8+ T cells and virus specific CD8+ T cells (VARTs). Neoepitope specific CD8+ T cells were devided into two categories; CD8+ T cells recognizing EVX-01-embedded neopeptides, are referred to as VaccNARTs (also described in figure 4) and CD8+ T cells recognizing the remaining non-EVX-01-embedded neopeptides, are referred to as NARTs. **a)** The frequency of NARTs (Neo), VaccNART (Vaccine) and VARTs (Virus) detected in *ex vivo* PBMC from each time point and TILs from time point T1 and T2, and SKILs. Each dot represent a specific CD8+ T cells population which are colored according to patient. The single populations are connected with lines between time point to follow the dynamics. Boxplots summarize the distribution and median of clusters within single time points. The red triangles indicated before and after vaccination initiation. **b) T**he number of different responses detected by NARTs, VaccNARTs and VARTs within *ex vivo* PBMCs and expanded TILs and SKILs. The colors indicates each of the screened patients, to follow the flow in number of responses, within the patients.

Supplementary Figure 10 | Patient specific results for analysis of epitope spreading detected in *ex vivo* PBMCs, and expanded TILs and SKILs. *Ex vivo* PBMC and expanded TILs and SKILs were screened for neoepitope specific CD8+ T cells and virus specific CD8+ T cells (VARTs). Neoepitope specific CD8+ T cells were split into two categories; CD8+ T cells recognizing EVX-01-embedded neopeptides, are referred to as VaccNARTs and CD8+ T cells recognizing the remaining non-EVX-01-embedded neopeptides, are referred to as NARTs. The frequency of NARTs (neo), VaccNART (Vaccine) and VARTs (virus) populations detected in *ex vivo* PBMCs and expanded TILs is shown for each patient. To the left, a flow plot showing the collected frequency per patient. Each color represents a single specificity. To the right, plot showing the frequency of single specificities, which are connected with lines between time point.

Supplementary Figure 11 | Clinical responses reflected immune analyses and prediction scores. Patient clinical outcomes was grouped in good response; CR- Complete Response and PR - Partial Response, and bad response; SD -Stable Disease and PD – Progressive Disease. **a)** Delta spots detected by Elispot for peptides with T cell response (background stimulated with irrelevant peptide has been subtracted) compared to clinical responses. Immune responses detected before vaccination (Pre), during and after vaccination (Post) and in follow up samples are grouped. **b-c)** The frequency of functional CD4+ and CD8+ T cells responses towards EVX-01 vaccine detected by ICS was compared the two patient groups. **d-e)** The prediction scores (both

from PIONEER2 and PIONEER4) for immunogenic peptides compared between patients different clinical responses.

Supplementary Figure 12 – Patient overview a) Overview of patients in cohort A, b) overview of patients in cohort B: checkpoint inhibitor (CPI) initiation, baseline biopsy (day 0), vaccine treatment, and follow-up information of the twelve patients at three different dose levels. Small blue and green dots indicate either IP vaccinations or IM vaccinations, respectively. The depiction of disease condition and patient status are indicated in various colors. PD – Progressive Disease (red), PR; Partial Response (blue), MR; Mixed Response (salmon), SD; Stable Disease (purple), CR; Complete Response (green).