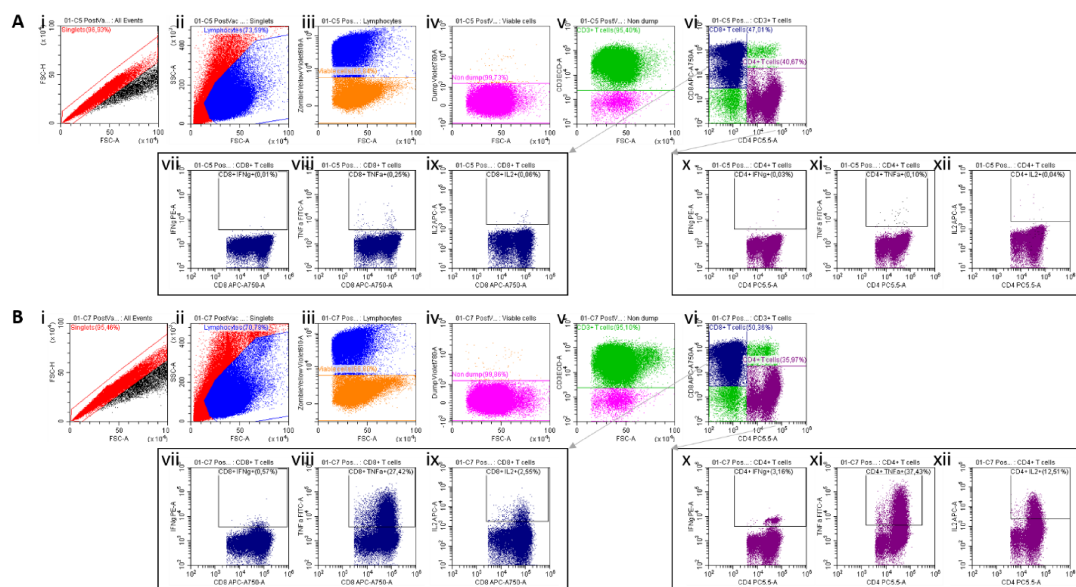# TriMix and tumor antigen mRNA electroporated dendritic cell vaccination plus ipilimumab: link between T-cell activation and clinical responses in advanced melanoma

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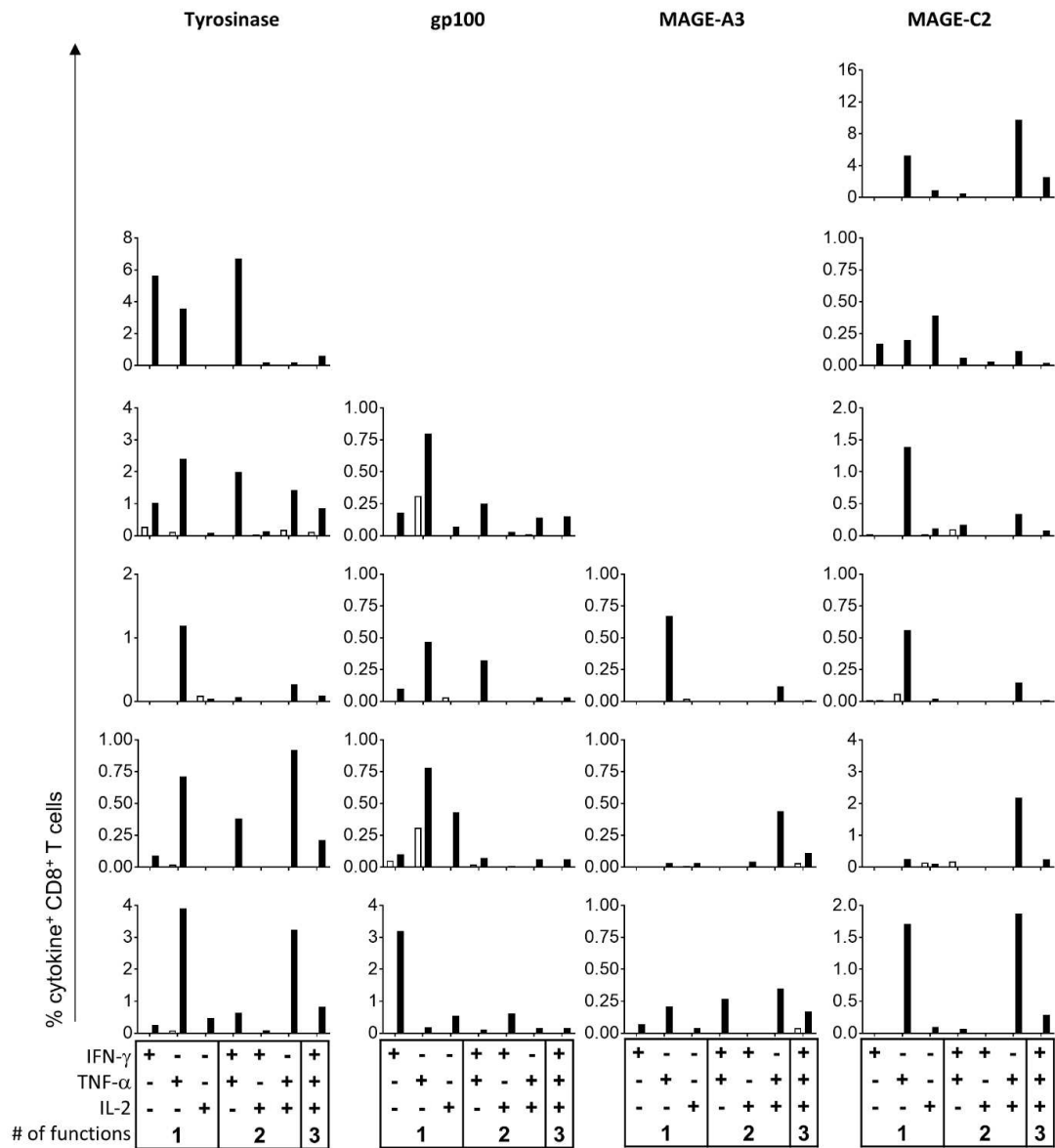
SUPPLEMENTARY DATA

Supplementary figure 1. ICS gating strategy

Dot plots from PostVac sample from patient 141 (A) cultured in absence of stimulus (negative control) and (B) stimulated with anti-CD3 and anti-CD28 coated microbeads (positive control). Plot i: gating on singlets based on forward scatter (FSC) area/FSC height plot); plot ii: within singlets, lymphocytes were gated based on FSC area/side scatter (SSC); plot iii: within lymphocyte population, viable cells were gated based on absence of positivity upon staining with Zombie Yellow; plot iv: within viable cells, CD14$^-$ CD19$^-$ cells were gated (dump channel); plot v: within viable lymphocytes, CD3$^+$ T cells were gated; plot iv: within CD3$^+$ T-cell population, CD8$^+$ and CD4$^+$ T cells were gated. Within the CD8$^+$ and CD4$^+$ T-cell populations, IFN-γ^+ (plots vii and x, respectively), TNF-α^+ (plots viii and xi, respectively) and IL-2$^+$ (plots ix and xii, respectively) cells were gated.
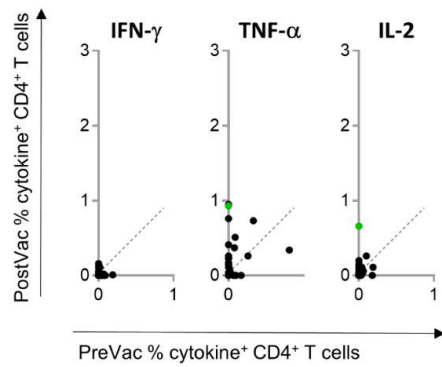
Supplementary figure 2. TriMixDC-MEL IPI T-cell responses are mainly mediated by CD8+ T cells

Each dot represents the PreVac and PostVac intracellular cytokine staining response measured for one patient for one TAA and one cytokine. T-cell responses that were considered as vaccine-specific immune responses are indicated in green. The line indicates an equal PreVac and PostVac response.

Supplementary figure 3. Multifunctional profile of CD8+ T-cell responses

Bar graphs show percentages of cytokine+ CD8+ T cells for one patient. Only responses that fulfilled the vaccine response criteria with IVS ICS are shown. White bars: PreVac, black bars: PostVac.
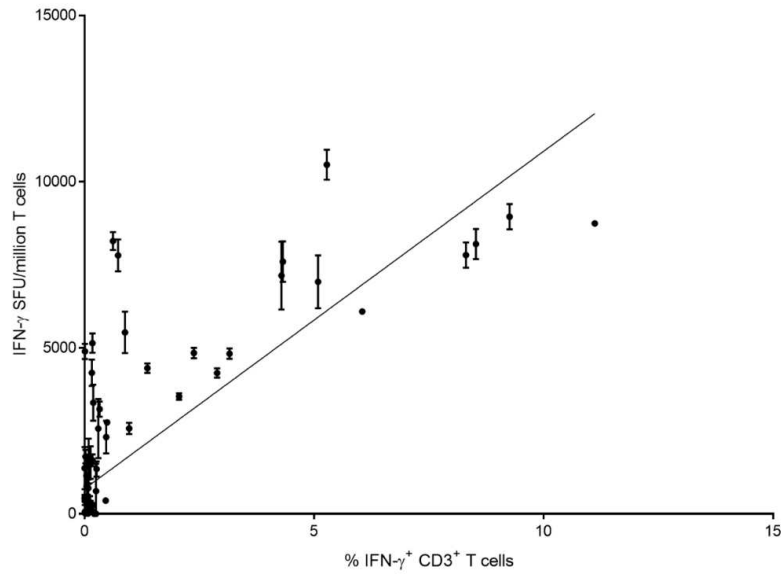
Supplementary figure 4. Comparison of T-cell responses measured by ELISPOT and ICS

Each dot represents the IFN-γ T-cell response as measured by ELISPOT (Y-axes) and ICS (X-axes), both performed after IVS. The error bars indicate the standard deviation of the ELISPOT responses. The line indicates the linear regression model, with an R^2 value of 0.629.
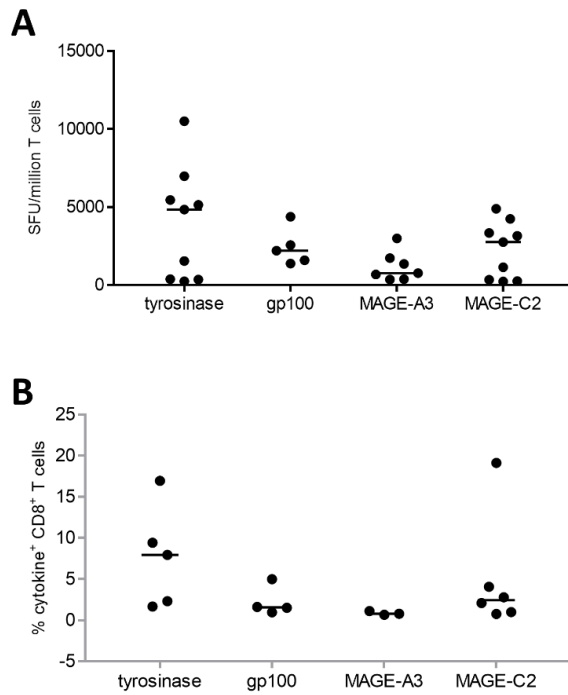
Supplementary figure 5. ELISPOT and ICS responses measured per TAA

(A) Responses obtained with IVS ELISPOT per TAA. ~~Each dot represents a vaccine-specific ELISPOT response for one patient for one TAA. The lines indicate the median.~~
(B) IVS ICS responses measured per TAA. (A-B) Each dot represents a vaccine-specific response for one patient for one TAA. The lines indicate the median.
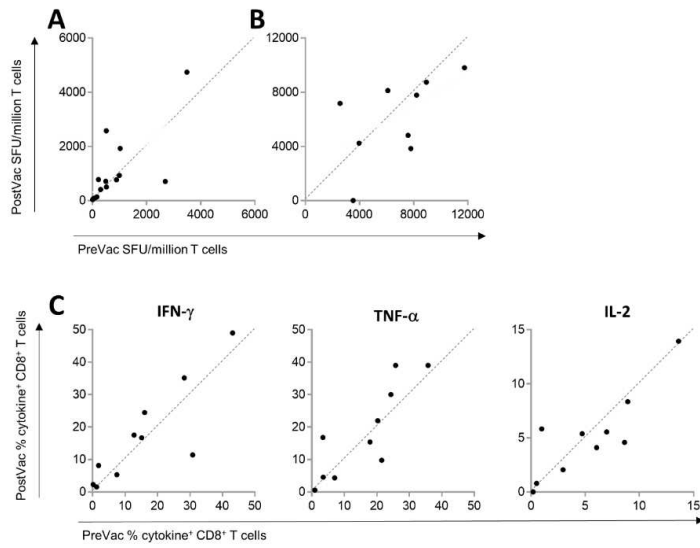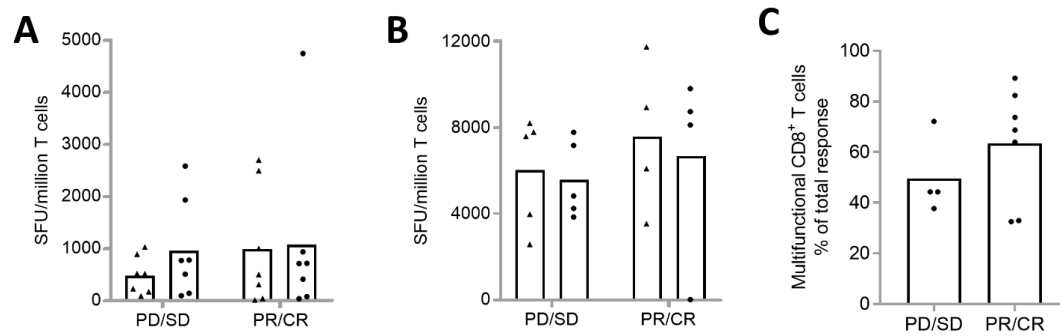
Supplementary figure 6. T-cell responses specific for viral recall antigens encoded by CEF mRNA remain stable upon treatment

(A) CEF-specific T cell responses as detected with (A) *ex vivo* ELISPOT, (B) IVS ELISPOT and (C) IVS ICS. Each dot represents the PreVac and PostVac response measured for one patient and one cytokine. The lines indicate an equal PreVac and PostVac response.
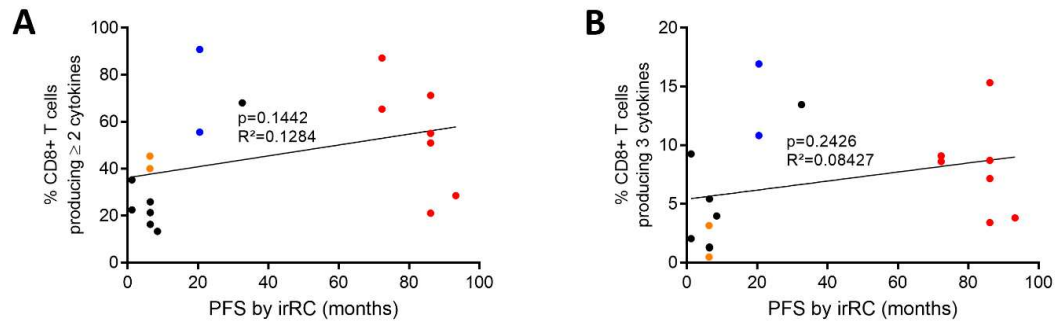
Supplementary figure 7. Insignificant trend towards longer PFS in patients with a high percentage of multifunctional TAA-specific T cells

Percentage of multifunctional T cells correlates with OS. Shown are the percentages of the total CD8+ T-cell IVS ICS response characterized by a multifunctional profile (A: at least 2 cytokines, B: 3 cytokines). Each dot represents one vaccine-specific IVS ICS response. A linear regression was performed to analyze the data. Red symbols indicate immune responses of patients that showed a CR to the TriMixDC-MEL IPI treatment which is still ongoing after >314 weeks. Blue symbols indicate immune responses of patients having experienced PD after respectively 6.77 and 20.45 months but that afterwards obtained CR to pembrolizumab treatment, which is currently still ongoing. Orange symbols indicate patients with a mixed response (complete regression of some of their metastases while others progressed).

Supplementary figure 8. **T-cell responses specific for viral recall antigens encoded by CEF mRNA are similar in patients showing PD/SD or PR/CR**

(A) CEF-specific *ex vivo* ELISPOT responses. (B) CEF-specific responses measured with IVS ELISPOT. Each data point represents ELISPOT response measured PreVac (triangles) or PostVac (dots) for one patient. The bars indicate the mean. (C) The graph shows the percentage of the total CD8$^+$ T-cell IVS ICS response characterized by a multifunctional profile (CD8$^+$ T cells producing at least 2 cytokines). Each dot represents one PostVac CEF-specific IVS ICS response. The bars indicate the mean. No significant differences between PD/SD and PR/CR groups were found; A: PreVac: p=0.6361 and PostVac: p=0.9759, B: PreVac: p=0.7226 and PostVac: p=0.8481; C: p=0.5273.