**Introduction**

- **TriMix** mRNA encodes immunomodulating proteins and is designed to unleash the full T cell stimulatory capacity of dendritic cells (DCs) (Fig. 1). TriMixDC-MEL co-electroporated with mRNA encoding tumor-associated antigens (TAA) have shown antitumor activity as a monotherapy in patients with advanced melanoma (TriMixDC-MEL)¹.

- The phase II TriMixDC-MEL-IPI study was performed to investigate the combination of TriMixDC-MEL with ipilimumab (IPI) in patients with pretreated advanced melanoma. Encouraging signs of clinical activity were previously published². Here, we report the vaccine-induced immune responses and we provide an update of clinical responses after long term follow-up.

![Figure 1: TriMixDC-MEL](image)

TriMix components are shown in purple, blue, and green.

**Methods**

- **TriMixDC-MEL vaccines** expressing tyrosinase, gp100, MAGE-A3 and MAGE-C2 were administered intradermally and intravenously together with IPI. For 18/39 patients, an additional vaccine was administered before the first IPI administration. Patients with no signs of progression could enter a IPI maintenance phase (Fig. 2).

![Figure 2: Study scheme](image)

- T cells isolated from PBMC collected before treatment (PreVac) and 1 week after the last TriMixDC-MEL vaccine (PostVac) were tested for vaccine-specific cytolytic secretion by IFN-γ ELISPOT and intracellular cytokine staining (ICS). Nanostar Pan Cancer Immune profiling was performed on a pre-treatment FFPE tumor samples.

**Results**

- **Durable clinical responses**: The 6-month disease control rate was 51% and the overall tumor response rate was 38%, including 8 complete responses (CR) and 7 partial responses (PR), as described previously². Now, after more than 5 year follow up, 7/8 patients that showed a CR at 6 months remain progression free (Fig. 3).

- **Robust immune responses**: Immune monitoring was performed for 15 patients with sufficient PreVac and PostVac PBMC available: 5/15 with progressive disease (PD), 2/15 with stable disease (SD), 4/15 with PR and 4/15 with CR. 12/15 patients were considered as vaccine responders (Figs. 4-5).

![Figure 3: Clinical responses](image)

Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS).

![Figure 4: TriMix-DC MEL specific immune responses](image)

Graphs show ELISPOT responses and ICS responses (cytokine in 7 T cells and <E2.5>). ***Statistically significant difference compared to the baseline.***

- **Correlation between clinical responses and vaccine-induced immune responses**: 8/8 patients with CR or PR showed ELISPOT responses directed against multiple antigens compared to only 2/7 patients with PD or SD (Fig. 6, left). Moreover, responses detected in clinical responders were characterized by higher ELISPOT numbers (Fig. 6, right) and a higher degree of polyfunctionality (Fig. 7).  

![Figure 5: Immune monitoring assays - example](image)

In vitro stimulated T cells were tested in ELISPOT and ICS. For the ELISPOT, 50,000 T cells were plated per well. 1 out of 4 replicate wells is shown per condition. The ICS plot shows IFN-γ, TNF-α and IL-2 production by viable CD8 T-cells.

![Figure 6: Broad immune responses detected in clinical responders](image)

- A number of TAA for which a vaccine-induced ELISPOT response was detected. B: magnitude of the BG-corrected ELISPOT response detected. Each dot represents a response directed against one vaccine antigen. Responses that were considered positive are indicated in green. **PreVac response; ● PostVac response; BG background**

![Figure 7: Polyfunctional responses detected in clinical responders](image)

Bar graphs indicate the PreVac BG-corrected percentage of CD8⁺ T cells expressing 1, 2 or 3 cytokines (IFN-γ, TNF-α and/or IL-2) upon TAA encounter as measured by ICS. Only responses considered as positive are shown. Numbers above the bars indicate the percentage of multifunctional T cells (2 or 3 functions) of the total response.

- **Expression of TAA in pre-treatment FFPE samples**: Preliminary results suggest a correlation between MAGE-A3 and MAGE-C2 expression and the presence of T cell responses specific for these antigens in the periphery. Since data from both Nanostar analysis as well as immune monitoring were only available for 5 patients, additional tests need to be performed to draw solid conclusions.

**Conclusions & perspectives**

- **TriMixDC-MEL treatment results in robust, broad and multifunctional CD8⁺ T-cell responses, mainly detected in patients with PR or CR.**

- Further research is needed to unravel the potential correlation between the mRNA expression levels of TAA by the tumor versus T-cell and clinical responses towards the treatment.

**Hungry for more?**

**References:**


**Short Talk Session Clinical Trials, 23.05.2019, 12:00 North Fayer Hall**

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