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Exploring biomarkers of response for combination therapy with pembrolizumab and lenvatinib in metastatic melanoma resistant to anti-PD1 inhibitor

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Introduction

❖ Immunotherapy with immune checkpoints inhibitors (ICIs) has shown remarkable clinical outcomes in metastatic melanoma (MM) patients, however most of them develop resistance and represent an expanding population with limited treatment options.

❖ Phase II LEAP-004 study of multikinase inhibitor Lenvatinib plus Pembrolizumab, for melanoma with confirmed progression on anti-PD1, demonstrated relative safety and durable efficacy of such experimental immunotherapy, thereby suggesting that it could be a valid treatment strategy to overcome resistance to anti-PD1.

❖ Here, using fresh tumor material, we developed organoids from 9 patients with MM, who progressed on anti-PD-1 (Pembrolizumab), which we treated with the single agent and the combination of Lenvatinib and Pembrolizumab to perform a translational study to investigate the effect of treatments on PDOs viability and apoptosis induction, and on the release of soluble mediators of immune suppression and immune response in the tumor.

Results

			PDOs viability (%)			
Pz	Therapy	BRAF status	CTRL	Lenva	Pembro	Combo
#1	anti-PD-1	wt	100	79.1±5.2	94.0±5.6	57.6±4.8 †,***
#2	anti-PD-1	wt	100	57.9±6.5	145.2±11.2	55.2±3.3 ***
#3	anti-PD-1	wt	100	101.9±9.2	83.0±6.6	51.7±5.1 †††,***
#4	anti-PD-1	wt	100	79.9±4.7	102.6±10.0	67.9±6.7 *
#5	anti-PD-1	wt	100	92.9±7.1	99.9±8.5	84.2±8.1 **
#6	anti-PD-1	wt	100	94.9±8.6	98.9±1.9	87.3±2.9 *
#7	1.Target 2. anti-PD-1	V600E	100	67.8±2.2	78.3±6.3	61.3±7.3 **
#8	1.Target 2. anti-PD-1	V600E	100	91.4±4.6	136.3±10.8	92.5±9.1 ***
#9	1.Target 2. anti-PD-1	V600E	100	67.2±5.3	114.6±12.6	58.5±6.9 ***

Table 1: Patients' clinical information and % of PDOs viability after treatment(s). Summary of therapy regimen (1.first line, 2. second line), BRAF status and data of PDOs' viability assay, performed after treatment(s) in presence of autologous T cells using CellTiter-Glo® 3D Cell Viability Assay (Promega), and expressed as percentage of untreated cells (CTRL). († significance respect to lenvatinib, *significance respect to pembrolizumab, †/* p<0.05, ††/** p<0.01, †††/*** p<0.001).

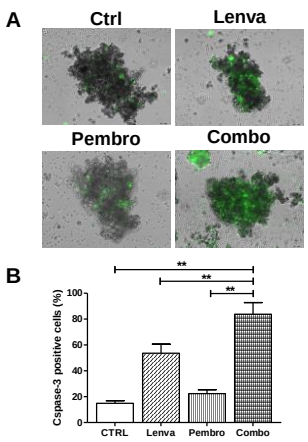

Figure 1: Apoptosis induction in PDOs from non-responders after treatment(s) A. Representative images showing caspase-3 activation in PDOs evaluated by NucView® 488 Caspase-3 Assay kit (Green: caspase-3 positive cells, scale bar 100 µm); B. histogram plot showing the quantification of caspase-3 positive cells (%) reported as mean±SD (** p<0.01).

According to clinical outcome, we found that organoids from almost all patients were resistant to anti-PD-1 pembrolizumab and showed variable sensitivity to lenvatinib (Table 1). Notably, the combined treatment resulted in a greater antitumor efficacy respect to single drug in almost all PDOs (Table 1). In agreement with cytotoxicity data, Lenvatinib and to a greater extent the combined treatment induced caspase 3-mediated apoptosis in PDOs (Fig. 1).

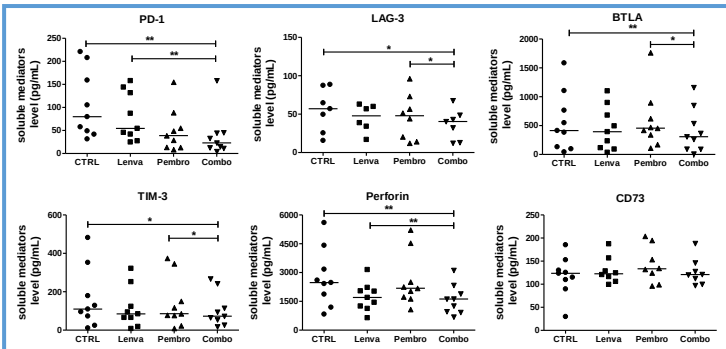

Figure 2: Dot plots showing quantity of soluble mediators in supernatants of co-culture of PDOs and autologous T cells by using Immune Checkpoint 37-Plex Human ProcartaPlex™ Panel (Invitrogen, Thermo Fisher Scientific) and analysed by Bio-Plex 200 system (BioRad Laboratories).

Among the soluble factors analysed (arginase-1, B7-H6, BTLA, OX40, CD137, CTLA4, CD27, B7-H3, CD28, IAP, BLAST-1, CD73, CD80, CD96, E-cadherin, GITR, HVEM, ICOS ligand, IDO, LAG-3, MICA, MICB, nectin-2, PD-1, PD-L1, PD-L2, perforin, PVR, S100A8/A9, siglec-7, siglec-9, TIM-3, TIMD-4, ULBP-1, ULBP-3, ULBP-4, VISTA), the combined treatment reduced the release of soluble immune checkpoints PD-1, LAG-3, BTLA, TIM-3. However we found also a significant reduction of perforin, suggesting a reduced toxicity of T cells. Noteworthy, no treatment(s) reduced the release of CD73 (Fig. 2).

Conclusions

- **Collectively, the results showed either a variable sensitivity of PDOs to treatments and a variable reduction of immunosuppression in the tumor microenvironment, potentially through reduction of T cells exhaustion.**
- **Further investigations are warranted to investigate the impact of treatments on the reduction of perforin and on the enzymatic activity of CD73.**

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