

# Pembrolizumab associated to Trastuzumab exerts strong cardiac pro-inflammatory effects mediated by the overexpression of NF- $\kappa$ B and LeukotrieneB4-related pathways

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## PURPOSE

The immunotherapy has revolutionized the world of oncology in the last decades with considerable advantages in terms of overall survival in cancer patients. A combination therapy based on the co-administration of Pembrolizumab (PEM) (an antibody against PD-1) and Trastuzumab (TRA) (the humanized anti-Her2 mAb) was recently proposed in clinical trials for the treatment of Trastuzumab-resistant advanced HER2-positive breast cancer. Although immunotherapies are frequently associated with a wide spectrum of immune-related adverse events, the cardiac toxicity has not been properly studied. We studied, for the first time, the putative cardiotoxic effects of PEM associated to TRA turning the light on the pro-inflammatory effects of this novel combined therapy

## METHODS

### Cellular experiments:

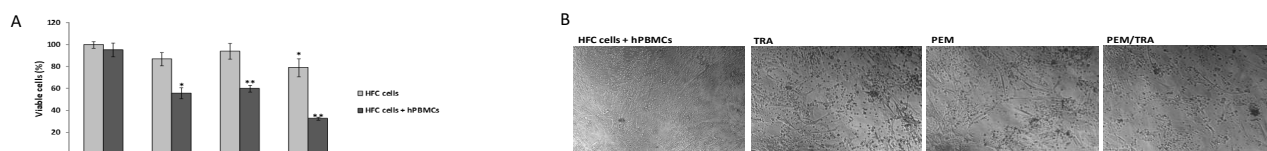
To test the effects of PEM and TRA combinatorial treatments on co-cultures of human fetal cardiomyocytes (HFC cells) with lymphocytes, the cells were plated in 96-well flat-bottom plates at the density of  $1.5 \times 10^4$  cells/well for 16 hours. Human Peripheral Blood Mononuclear Cells (hPBMCs) from healthy donors were added at effector: target ratio 5:1 in the absence or presence of mAbs, used alone or in combination (both at 200 nM), and incubated for 24 hours at 37°C. After treatments we studied cell viability and expression of pro-inflammatory markers.

### Preclinical study:

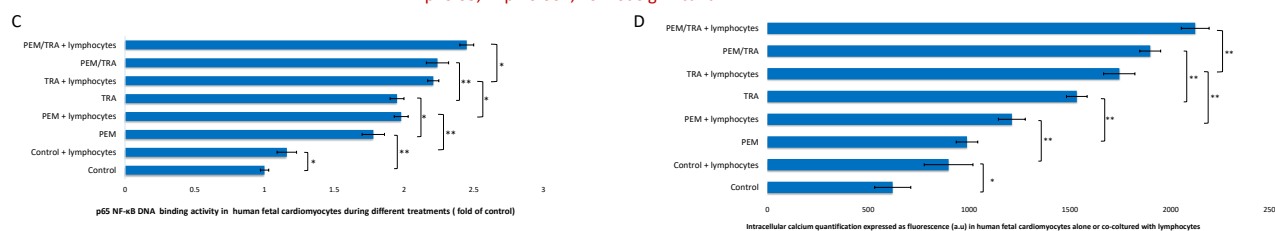
Briefly, twenty-four female C57Bl/6 mice were randomized for weight and enrolled in four treatment groups (6/each group). Mice were intraperitoneally administrated with PEM at 10 mg/Kg for the first dose, followed by 5 mg/Kg dose every 5 days until the study end point; in another group, mice were intraperitoneally administrated with TRA at 10 mg/kg/day; in another group mice were intraperitoneally administrated with both drugs in combination. Control mice (Sham) were given the same volume of saline solution for three weeks. The experimental procedures were performed in compliance with European Directive 63/2010 / EU and Italian Law (DL 26/2014, authorized by the Minister of Health, Italy). After treatments, heart tissues were processed to analyze the expression of Interleukin 1- $\beta$ , 8 and 6 (pg of interleukin/mg of heart tissue), Leukotriene B4 (pg/mg of tissue) and p65/NF- $\kappa$ B (ng/mg of protein). Moreover, hearts were excised and fixed in 10% neutral buffered solution, then the myocardial tissue was paraffin-embedded for morphometry. Six  $\mu$ m thick cross sections were deparaffinized and stained with hematoxylin-eosin for general morphology and with Picrosirius red to detect collagen fibres.

## RESULTS

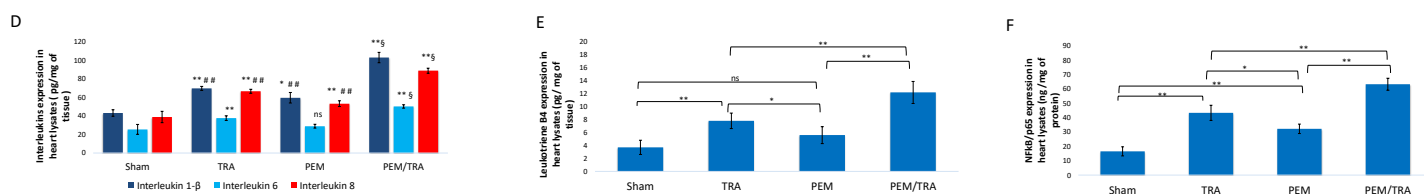
(A, B) Both drugs significantly affected the viability of HFC indicating cardiotoxic effects. The strongest cytotoxic effects were observed when the two antibodies were used in combination, as this led to an inhibition of about 60% of cardiac cell survival, when the mAbs were tested in combination in the presence of lymphocytes. \*p<0.05, \*\*p<0.001; ns: not significant.



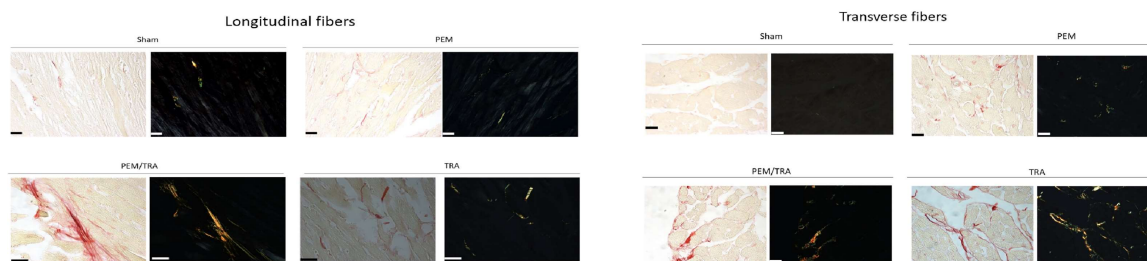
HFC cells, co-cultured with lymphocytes, exposed to PEM and TRA increased significantly the p65/NF- $\kappa$ B expression (C) and intracellular Ca<sup>2+</sup> accumulation (D), compared to the single treatments. \*p<0.05, \*\*p<0.001; ns: not significant.



The combination of PEM and TRA increased significantly the pro-inflammatory state in heart tissue, compared to single treatments. Interleukins (E), Leukotriene B4 (F) and p65/NF- $\kappa$ B (G) expressions were always significantly increased, also compared to untreated (Sham) mice, indicating a metabolic change in the cardiac microenvironment. \*p<0.05, \*\*p<0.001; ns: not significant; in Fig. E \* is the difference with the sham, # is the difference between PEM and TRA and § is the difference between PEM/TRA and TRA.



The histological analysis showed an increased amount of collagen fibers in TRA and PEM/TRA groups (H). While controls and PEM showed a typical intramyocardial collagen framework, with mainly type III collagen fibers in interstitial space, TRA and PEM/TRA groups showed a well defined reactive fibrosis rich in Type I collagen fibers. Scale Bar = 20  $\mu$ m.



## CONCLUSIONS

The overall picture of this study turns the light on the cardiotoxic effects of immune check-point inhibitors, especially when associated with Trastuzumab, providing preliminary scientific evidences for the urgent need of cardiovascular monitoring strategies and cardiotoxicity management in cancer patients.