Abstract: **P1537**

**Cardiotoxicity induced by the combinatorial treatment based on the immune checkpoint inhibitor pembrolizumab associated to trastuzumab**

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Background: 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 (an antibody against PD-1) and Trastuzumab (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.

Purpose: We studied, for the first time, the putative cardiotoxic effects of Pembrolizumab associated to Trastuzumab turning the light on the pro-inflammatory effects of this novel combined therapy

Methods: Cell viability, intracellular calcium quantification and pro-inflammatory assays (analyzing the production of Interleukin 1β, 6 and 8 as well as the expression of p65/NFκB and Leukotriene B4) were performed in human fetal cardiomyocytes in vitro. Preclinical studies were also performed in vivo on C57BL6 mice untreated (Sham) or treated with Pembrolizumab and Trastuzumab alone or in combination by analyzing (in cardiac tissue extracts) the same markers of inflammation used in cellular studies.

Results: Combination therapy leads to an increase of the intracellular calcium overload (more than 3 times compared to untreated cells) and to a reduction of the cardiomyocytes viability (of more than 65 and 20-25%, compared to untreated and Pembrolizumab or Trastuzumab treated cells, respectively) thus indicating cardiotoxic effects. Notably, combination therapy increases the inflammation of cardiomyocytes enhancing significantly the production of p65/NFκB and Interleukins. Moreover, in in vivo studies on mice, the association of Pembrolizumab and Trastuzumab shows pro-inflammatory effects in cardiac tissue by stimulating the Interleukin 1β, 8 and 6 expression of 40-50 % more than the single treatments; the expression of p65/NFκB and Leukotriene B4 was also increased indicating pro-inflammatory effects. Conclusion: Combination therapy based on Pembrolizumab associated to Trastuzumab leads to significant cardiac pro-inflammatory effects mediated by overexpression of NFκB/p65 and Leukotriene B4 related pathways