Dysregulation of immune checkpoint proteins in newly- diagnosed early breast cancer patients undergoing neoadjuvant chemotherapy. A comparison between TNBC and non-TNBC patients.

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Background: Checkpoint proteins regulate the immune system. Breast cancer is associated with up-regulation or down-regulation of these proteins to evade anti-tumor immune responses. Soluble forms of immune checkpoint molecules (sICMs) can be measured in human plasma. The study aimed to measure the systemic levels of a series of co-stimulatory and co-inhibitory ICMs at diagnosis, post-neo-adjuvant chemotherapy (NAC) and post-surgery in newly- diagnosed BC patients (pts) and compare the biological behaviour of these sICMs between TNBC and non-TNBC patients.

Methods: Soluble ICMs were measured using multiplex bead array technology in plasma from 72 BC pts and 45 healthy controls. Data were prospectively obtained, and levels were compared between pre-treatment, post-NAC, and post-surgery using non-parametric tests (Mann-Whitney & Kruskal-Wallis).

Results: Following NAC, the plasma levels of six soluble co-stimulatory checkpoints (CD28, CD40, ICOS, CD27, CD80, GITR), all involved in the activation of CD8+ cytotoxic T cells, were significantly increased ($p < 0.04$-$p < 0.00001$). Four of the soluble co-inhibitory checkpoints (LAG-3, PDL1, TIM-3 and HVEM) increased significantly post-NAC, reaching levels significantly greater than those of the control group. PD-1 remained unchanged, while BTLA and CTLA-4 decreased significantly ($p < 0.03$ and $p < 0.00001$, respectively). Normalization of soluble co-stimulatory immune checkpoints seemingly indicates a reversal of systemic immune dysregulation following the administration of NAC in early BC. In contrast, recovery of immune homeostasis may explain the increased levels of several negative checkpoint proteins, albeit with the exceptions of CTLA-4 and PD-1. No significant differences were detected between the changes of 16 sICMs levels at pre-NAC, post-NAC and post-surgery when comparing the TNBC and non-TNBC patients.

Conclusions: This study demonstrates low levels of co-stimulatory and co-inhibitory sICMs in newly-diagnosed, non-metastatic BC pts. Following treatment with NAC, the sICMs levels increase substantially, except CTLA-4. In the case of co-stimulatory sICMs, these novel findings are indicative of an immune-restorative mechanism. The pattern of co-inhibitory sICMs (elevated levels of PD-L1, LAG-3, TIM-3 and HVEM), might be indicative of immune-therapeutic resistance, underscoring the augmentative immune-therapeutic promise of targeting these molecules, either individually or in combination, to improve the outcome of pts with early BC. These differences were not significant between TNBC and non-TNBC patients.