For effective killing of cancer cells in an anticancer immune response, a series of events involving different immune cells needs to be initiated and allowed to proceed. The steps in the cancer immunology cycle start with the release of tumor cell antigens, which are recognized by and lead to the killing of cancer cells by cytotoxic T cells. This immune response is modulated by a variety of stimulatory and inhibitory factors.

1. Cells need two signals for activation: binding of the TCR (T-cell receptor) to the MHC (major histocompatibility complex) and activation of co-stimulatory molecules.
2. Immune checkpoints can stimulate or inhibit these events thereby regulating the functionality of immune cells.
3. Accordingly, checkpoints play important roles in the maintenance of immune homeostasis.

Examples of stimulatory molecules include TCR/MHC, CD137L/CD137 and OX40L/CD40, while CTLA-4/CD80 or CD86 and PD-1/PD-L1 are typical inhibitory checkpoints. Increasing numbers of novel regulatory receptors and ligands have been recently described and are summarized in Figure 1.

Recently, a series of soluble systemic immune checkpoints such as sCTLA-4 (soluble CTLA-4), sCD28 (soluble CD28), sCD86 (soluble CD86), sPD-1 (soluble PD-1) and others have been identified that can be measured in plasma.

Figure 1. Stimulatory and inhibitory immune checkpoint molecules.

### Methods

#### Aim

The circulating levels of 15 immune checkpoint-related proteins panel (BTLA, GITR, GITRL, ICOS, ICOSL, GITRL, GITRL, CD80, CD86, CD40, and OX40L) were measured in plasma from breast cancer patients and healthy controls. Immunohistochemistry was performed on formalin-fixed paraffin embedded tissue. The results of this study were compared with the published data using a paired t-test to identify statistically significant differences.

#### Lab Method

Plasma levels of immune-chemistry checkpoints, chemokines and cytokines were analyzed using Bio-Plex Suspension Biorad Array platforms (Bio-Plex). The methods were followed according to the manufacturer’s specifications.

Statistical Methods

The primary hypothesis was that there was a significant difference in the plasma levels of soluble immune checkpoints, cytokines and chemokines between breast cancer patients and healthy controls. Descriptive statistics were used to tabulate patient characteristics. The Mann-Whitney U test was used to compare the plasma levels of immune checkpoint proteins between breast cancer patients and healthy controls. Fisher’s exact or Chi-squared tests were used for the analysis of categorical variables. All analyses were performed using the R software (version 3.5.3).

### Results

The results indicate that early breast cancer is associated with a down-regulation of both stimulatory and inhibitory immune checkpoints. These results suggest that early breast cancer patients appear to have a generalized immune-negativity independent of estrogen and stage, which, to our knowledge, is the first study to intrinsically describe soluble immune checkpoints in early breast cancer patients.

### Conclusions

Lower levels of a number of soluble cytokines (sIFN-gamma) and co-inhibitory (sPD-1) increase in plasma. These results indicate that early breast cancer is associated with a down-regulation of both stimulatory and inhibitory immune checkpoint proteins. Newly diagnosed early breast cancer patients appear to have a generalized immune-negativity independent of estrogen and stage, which, to our knowledge, is the first study to intrinsically describe soluble immune checkpoints in early breast cancer patients.