



# NIVERSITEIT VAN PRETORIA



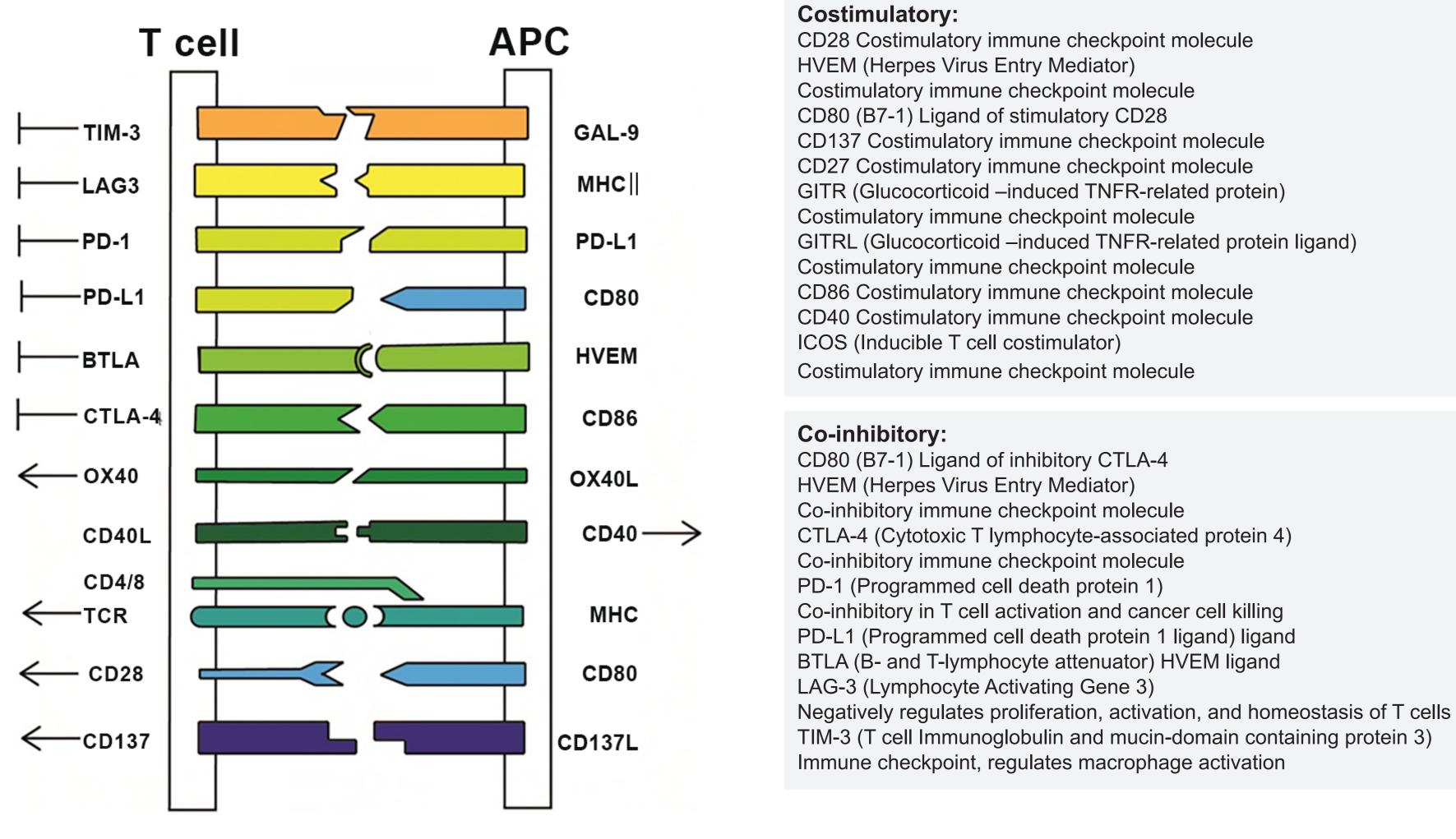


The Medical Oncology Centre of Rosebank Personalised Cancer Care

## Background

- > 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 immunity 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;
- > T 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;
- Immune checkpoints can stimulate or inhibit these events thereby regulating the functions of immune cells;
- 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 potent inhibitory checkpoints. Increasing numbers of novel regulatory receptors and ligands have recently been described and are summarized in figure 1;
- Recently, a series of soluble systemic immune checkpoints such as sCTLA-4 (soluble CTLA-4), sPD-1 (soluble PD-1) and others have been identified that can be measured in plasma.

Figure 1. Stimulatory and inhibitory immune checkpoint molecules.



#### Reference

Gu, D., Ao, X., Yang, Y. et al. Soluble immune checkpoints in cancer: production, function and biological significance. j. immunotherapy cancer 6, 132 (2018). https://doi.org/10.1186/s40425-018-0449-0

## Methods

> The circulating levels of 16 immune checkpoint-related proteins panel (BTLA, GITR, GITRL, HVEM, LAG-3, PD-1, PD-L1, TIM-3, CD27, CD28, CD80, CD86, CD40, CD137, ICOS, TLR-2 and CTLA-4), as well as chemokines (CXCL5, CCL26, CX3CL1, CXCL10, CXCL9, CCL23) and cytokines (IL2, IL4, IL6, IL8, IL10, IL16, IL17A, IL1RA, Interferon α, Interferon γ TGF β1) were profiled in 98 early breast cancer patients (patient characteristics are summarized in table 1) and compared to those of 45 healthy controls.

#### Lab Method

Plasma levels of immune-oncology checkpoints, chemokines and cytokines were assayed using Bio-Plex Suspension Bead Array platforms (Milliplex® or Bio-Rad® human magnetic bead panels). The methods were followed according to the manufacturers specifications and the data analysed using Bio-Plex Manager software 6.0 and results reported as either ng/mL or pg/mL. C-reactive protein (CRP) levels were determined by nephelometry using the CardioPhase® hsCRP assay (Siemens Healthcare Diagnostics). The method was followed according to the manufacturer's instructions. Results are reported as µg/mL.

#### **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 levels of the various test biomarkers between breast cancer patients and healthy controls. Fisher's exact or Chi-squared tests were used for the analysis of categorical variables. NCSS software version 11 for Windows (USA) was used for statistical analyses.

## **Dysregulation of immune checkpoint proteins in newly- diagnosed** early breast cancer patients

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### Results

breast cancer patients and healthy controls are shown in Table 2.

Table 1. Patient Characteristics.

Table 2. Immune Checkpoint Molecule

Age (n=98)							
Median Age	52						
Range	27-85						
Menopausal Status							
Peri-menopausal	2 (2%)						
Pre-menopausal	55 (56%)						
Post- menopausal	41 (42%)						
Biological Type							
Her2 positive	16 (16%)						
Luminal A	2 (2%)						
Luminal B	14 (14%)						
TNBC	66 (68%)						
Grade							
1	1 (1%)						
2	25 (26%)						
3	69 (70%)						

Stage									
1a	13 (13%)								
2a	40 (41%)								
2b	31 (32%)								
3a	7 (7%)								
3b	4 (4%)								
3c	3 (3%)								
Glands									
Negative	46 (47%)								
Positive	52 (53%)								
Estrogen Status									
Negative	67 (68%)								
Positive	31 (32%)								
Progesterone Status									
Negative	81 (82%)								
Positive	17 (17%)								
Her2 Status Status									
Negative	82 (84%)								
Positive	16 (16%)								
Ki-67 mean = 50% [6-100%]									
15 - 39%	31 (32%)								
≤ 14%	7 (7%)								
≥ 40%	58 (61%)								

Table 2. Immune Checkpoint Molecule.											
		Breast Cancer (n=98)			Controls (n=45)						
	Immune Checkpoint Molecule	Median	95% LCL of Median	95% UCL of Median	Median	95% LCL of Median	95% UCL of Median	p value			
	CD27 pg/mL	3131,285	2639,21	3568,54	4577,35	3391,13	5784,85	0,00041			
-stimulatory	CD28 pg/mL	32176,41	27889,65	3568,54	46135,18	27210,29	67544,1	0,00229			
	CD40 pg/mL	1464,685	1262,67	1620,9	1977,68	1404,82	2569,56	0,00054			
	ICOS pg/mL	14364,95	11122,68	15964,4	26506,65	15897,52	31725,99	0,0001			
Co-s	GITR pg/mL	5529,8	4868,15	6407,6	7151,12	5528,36	9878,41	0.00234			
0	GITRL pg/mL	5529,8	4868,15	6407,6	7151,12	5528,36	9878,41	0,00234			
ory	PD-1 pg/mL	11571,18	10147,12	13426,83	14917,48	7874,92	21795,02	0,11977			
	PD-L1 pg/mL	1580,695	1198,87	1978,97	3342,62	2628,64	4750,96	0.0000			
	CTLA-4 pg/mL	1585,73	1330,19	1790,69	2618,23	1578,44	3110,47	0,0006			
oito	CD80 pg/mL	1613,265	1317,61	1792,55	2329,77	1395,01	3042,87	0,00108			
-inhibit	CD86 pg/mL	11199,42	9447,21	12851,98	14297,09	9391,46	20525,14	0,011			
Co-i	TIM-3 pg/mL	3834,44	3436,22	4132,4	5046,87	4732,72	5958,87	0,00069			
U	LAG-3 pg/mL	120377,5	93854,44	138811,3	150416	94508,53	187997,2	0,11396			
	BTLA pg/mL	12907,97	11108,41	17084,76	12907,97	11108,41	17084,76	0,18276			
	ENA 78 CXCL5 pg/mL	535,58	250,7	763,49	2246,51	1540,24	3246,49	0,00002			
S	Eotaxin 3 CCL26 pg/mL	4,31	4,31	8,41	6,36	3,28	8,41	0,35476			
okines	Fractalkine CX3CL1 pg/ mL	445,13	399,04	489,3	397,12	366,07	431,69	0,00871			
Chemo	IP-10 CXCL10 pg/mL	485,82	426,98	607,59	543,33	498,35	638,22	0,86849			
Сh	MIG CXCL9 pg/mL	91,305	76,65	112,33	92,92	74,64	117,5	0,39967			
	CCL23 pg/mL	535,58	250,7	763,49	2246,51	1540,24	3246,49	0,00002			
	IL-2 pg/mL	9,01	8,19	10,37	9,81	7,29	11,5	0,32947			
	IL- 4 pg/mL	126,24	102,57	156,37	146,78	113,16	200,93	0,1878			
	IL-6 pg/mL	10,52	8,9	11,3	10,4	7,56	13,71	0,74975			
	IL-8 pg/mL	9,61	8,18	10,87	9,81	7,49	10,34	0,20475			
les	IL-16 pg/mL	1931,855	1569,84	2087,37	3535,39	2932,85	3813,73	0,00005			
kin	Interferon α pg/mL	174,275	152,73	192,52	199,64	176,29	214,94	0,00751			
Cytokines	IL-1 Ra pg/mL	418,735	346,33	466,68	503,33	448,08	625,49	0,01766			
U	Interferon y pg/mL	59,74	51,01	66,56	69,55	44,35	80,45	0,08564			
	IL-10 pg/mL	42,76	36,5	50,87	47,61	34,16	59,25	0,40669			
	IL-17A pg/mL	23,055	20,19	25,9	23,92	20,84	28,38	0,96121			
	TGF β1 pg/mL	20353,26	14180,32	24904,45	23785,83	16184,42	36390,72	0,98696			
Other	TLR-2 pg/mL	24059.42	20551,28	28354,07	30477.2	20928,44	50302,64	0.01406			
ot	HVEM pg/mL	1866,92	1674,84	2007,57	2290,19	2079,46	2618,44	0,00001			

Figure 2. GITR Breast Cancer vs. Control.

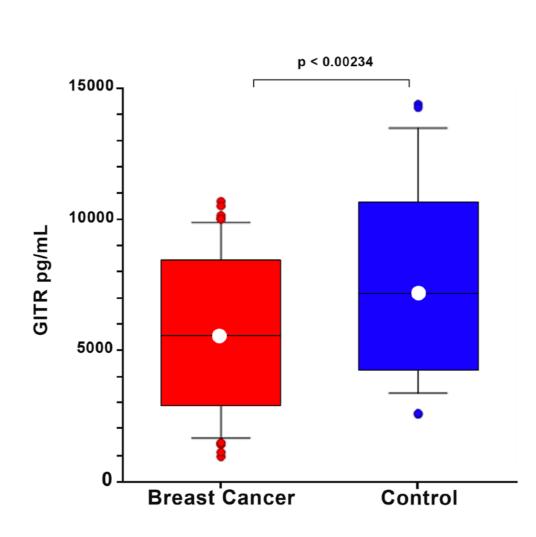
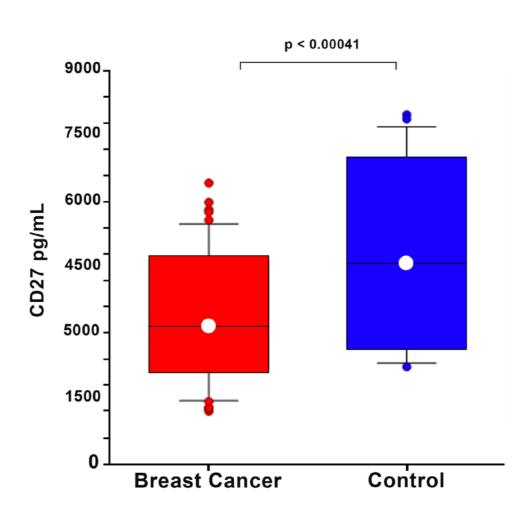


Figure 3. CD27 Breast Cancer vs.



Molecular Analysis for Personalised Therapy Congress 2020; October 8 - 10, 2020 Corresponding author: <u>bernardo.rapoport@up.ac.za</u>

Patient characteristics are shown in table 1. Comparison of plasma levels of immune checkpoints, chemokines, and cytokines between

Figure 4. CD28 Breast Cancer vs. Control.

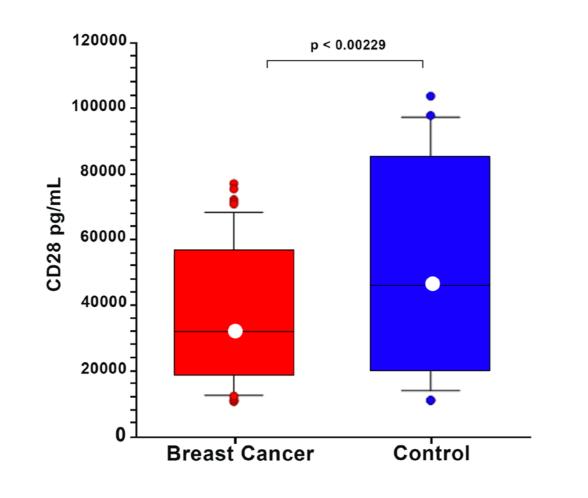


Figure 5. CD40 Breast Cancer vs Control

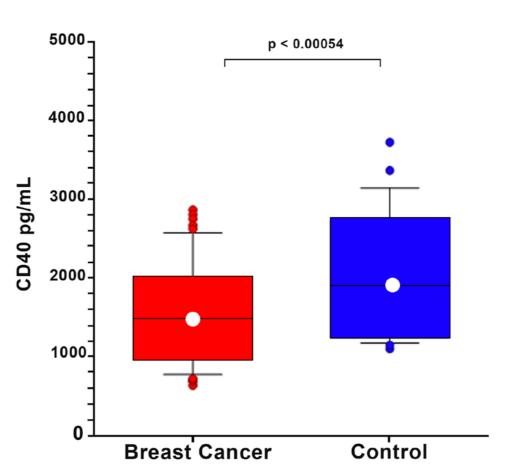
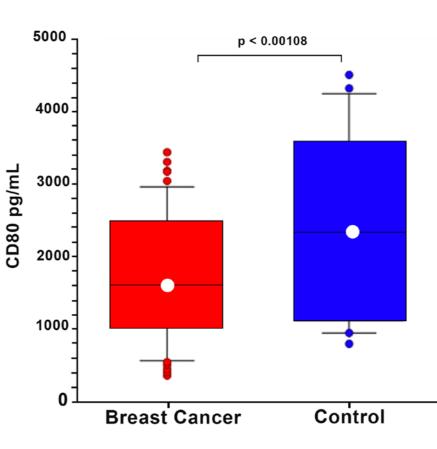


Figure 6. CD80 Breast Cancer vs





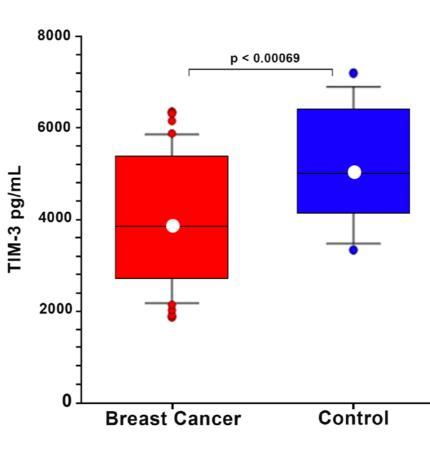


Figure 14. CCL23 Breast Cancer vs. Control

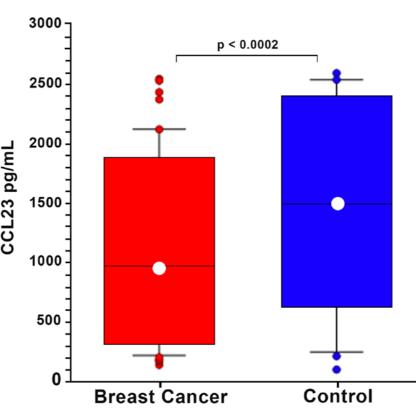
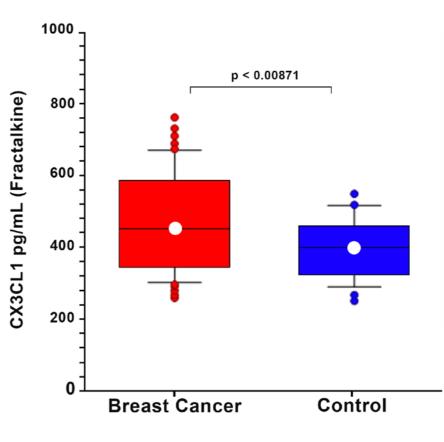


Figure 18. CX3CL1 Breast Cancer vs Control

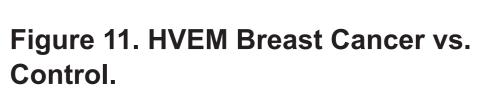


## **Conclusions**





Figure 7. ICOS Breast Cancer vs -



Control

Breast Cancer

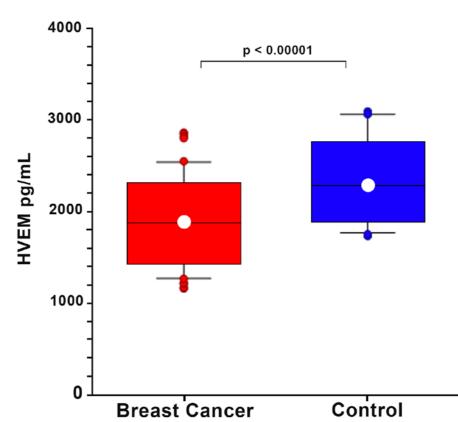


Figure 15. IL-16 Breast Cancer vs. Control.

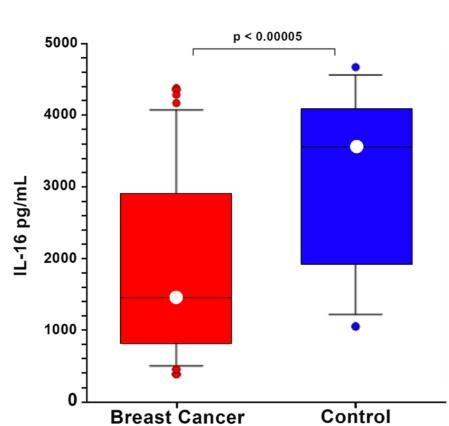


Figure 19. ENA 78 CXCL5 Breast Cancer vs. Control.

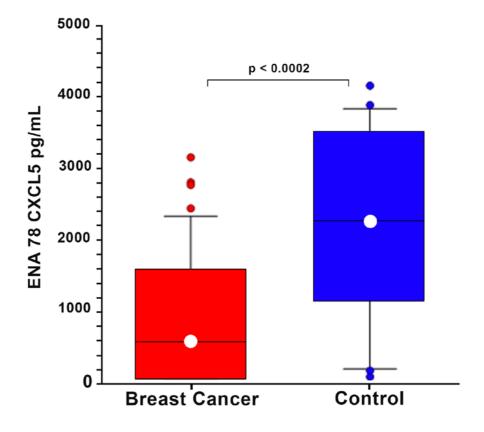
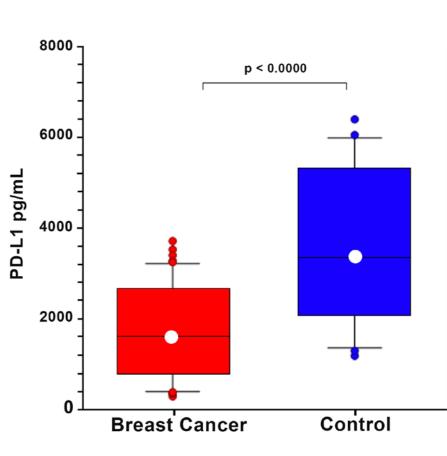
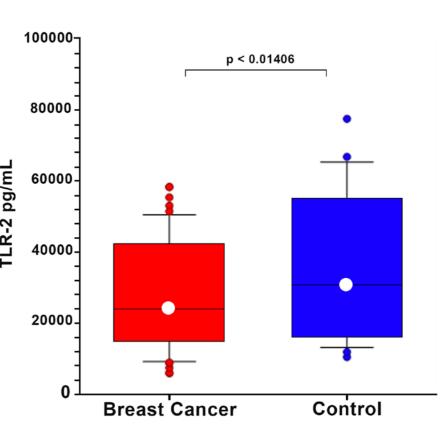


Figure 8. PD-L1 Breast Cancer vs. Control.









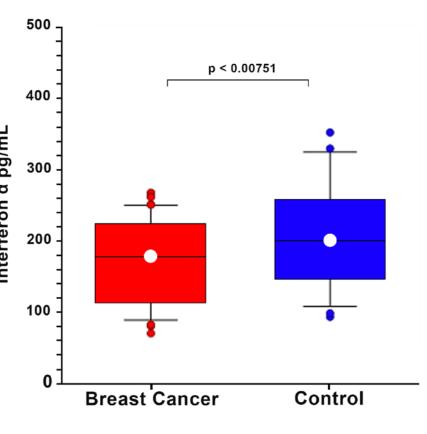


Figure 20. GITRL Breast Cancer vs. Control.

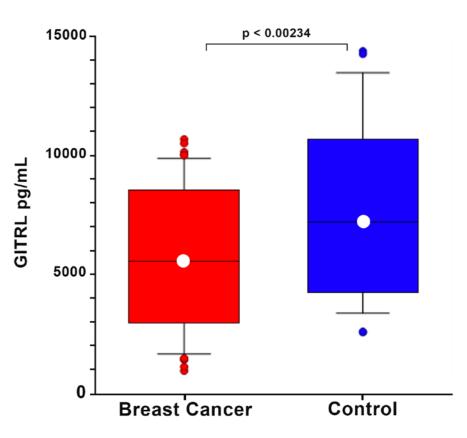


Figure 9. CTLA-4 Breast Cancer vs.

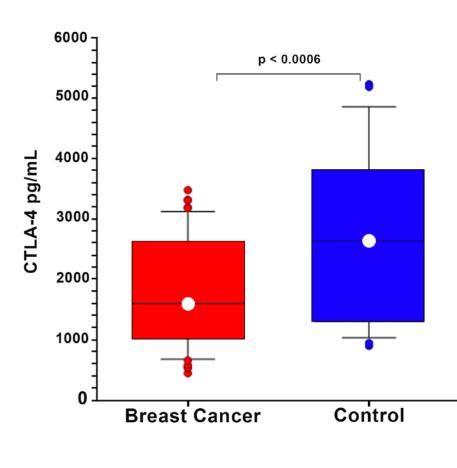


Figure 13. CXCL5 Breast Cancer vs.

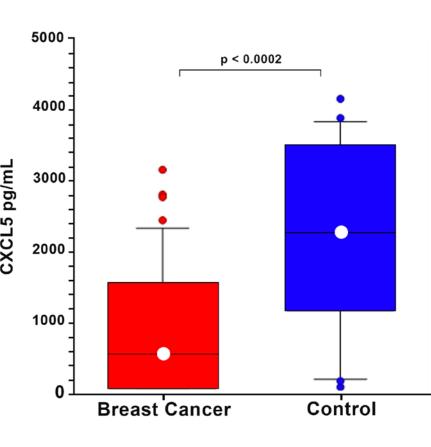


Figure 17. IL-1 Ra Breast Cancer vs. Control.

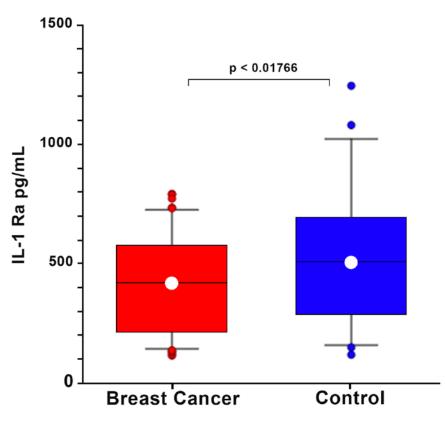
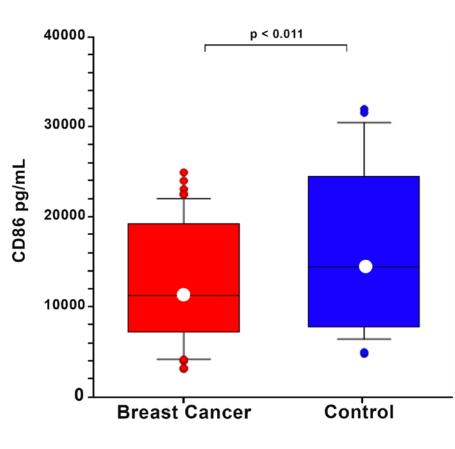


Figure 21. CD86 Breast Cancer vs.



Lower levels of a number of soluble costimulatory (n=6/6) and co-inhibitory (n=7/9) immune checkpoints, as well as chemokines (n=2/6) and cytokines (n=3/11), were identified in newly-diagnosed, non-metastatic breast cancer patients compared to healthy controls.

• These results indicate that early breast cancer is associated with a down-regulation of both stimulatory and inhibitory immune-checkpoint pathways. Newly-diagnosed early breast cancer patients appear to have a generalized immune-suppression independent of subtype and stage, which, to our knowledge, is the first study to simultaneously describe soluble immune checkpoints in early breast cancer patients.

> An analysis of these biomarkers comparing pre neo-adjuvant treatment, post neo-adjuvant treatment, as well as post surgery is underway.