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

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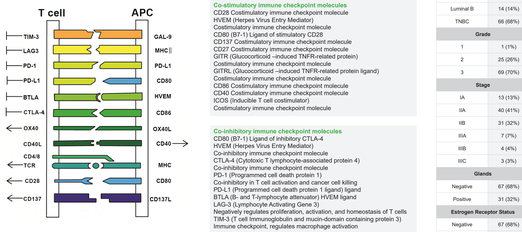
The Medical Oncology Centre of Rosebank
Personalised Cancer Care

BREAST CARE CENTRE

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.



Co-stimulatory immune checkpoint molecules
CD28 Costimulatory immune checkpoint molecule
HVEM (Herpes Virus Entry Mediator)
Costimulatory immune checkpoint molecule
CD80 (B7-1) Ligand of stimulatory CD28
CD137 Costimulatory immune checkpoint molecule
CD27 Costimulatory immune checkpoint molecule
GITR (Glucocorticoid –induced TNFR-related protein)
Costimulatory immune checkpoint molecule
GITRL (Glucocorticoid –induced TNFR-related protein ligand)
Costimulatory immune checkpoint molecule
CD86 Costimulatory immune checkpoint molecule
CD40 Costimulatory immune checkpoint molecule
ICOS (Inducible T cell costimulator)
Costimulatory immune checkpoint molecule

Co-inhibitory immune checkpoint molecules
CD80 (B7-1) Ligand of inhibitory CTLA-4
HVEM (Herpes Virus Entry Mediator)
Co-inhibitory immune checkpoint molecule
CTLA-4 (Cytotoxic T lymphocyte-associated protein 4)
Co-inhibitory immune checkpoint molecule
PD-1 (Programmed cell death protein 1)
Co-inhibitory in T cell activation and cancer cell killing
PD-L1 (Programmed cell death protein 1 ligand) ligand
BTLA (B- and T-lymphocyte attenuator) HVEM ligand
LAG-3 (Lymphocyte Activating Gene 3)
Negatively regulates proliferation, activation, and homeostasis of T cells
TIM-3 (T cell Immunoglobulin and mucin-domain containing protein 3)
Immune checkpoint, regulates macrophage activation

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).

Methods

Aim

- The circulating levels of 16 immune checkpoint-related proteins (BTLA, GITR, GITRL, HVEM, LAG-3, PD-1, PD-L1, TIM-3, CD27, CD28, CD80, CD86, CD40, CD137, ICOS, TLR-2 and CTLA-4), chemokines (CXCL5, CCL26, CX3CL1, CXCL10, CXCL9, CCL23), cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, IL-16, IL-17A, IL-1RA, Interferon α, Interferon γ, Transforming growth factor β1), as well as the following growth factors (VEGF, G-CSF, GM-CSF, M-CSF, FGF21, GDF15) 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, growth factors 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 pg/mL.

Statistical Methods

- The primary hypothesis was that that there was a significant difference in the plasma levels of soluble immune checkpoints, cytokines, chemokines and growth factors between early 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. The area under the ROC curve (AUC) was used as a measure of discriminatory ability for the biomarkers. The Youden index, a summary measure of the ROC curve, 23 was used as an agnostic method for choosing an optimal cut-off value on the biomarker value to illustrate potential clinical usefulness. P < 0.05 was considered statistically significant. NCSS software version 11 for Windows (USA) was used for statistical analyses.

Results

- Patient characteristics are shown in table 1. Comparison of plasma levels of immune checkpoints, chemokines, and cytokines between breast cancer patients and healthy controls are shown in Table 2.

Table 1. Patient Characteristics.

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	
IA	13 (13%)
IIA	40 (41%)
IIB	31 (32%)
IIIA	7 (7%)
IIIB	4 (4%)
IIIC	3 (3%)
Glands	
Negative	67 (68%)
Positive	31 (32%)
Estrogen Receptor Status	
Negative	67 (68%)
Positive	31 (32%)
Progesterone Receptor Status	
Negative	81 (82%)
Positive	17 (17%)
Her2 Status	
Negative	82 (84%)
Positive	16 (16%)
Ki-67 mean = 50% [5-100%]	
≤ 14%	7 (7%)
15 - 39%	31 (32%)
≥ 40%	58 (59%)
Not done	2 (2%)

Table 2. Immune Checkpoint Molecules, Chemokines, Cytokines and Growth Factors.

	Immune Checkpoint Molecule	Breast Cancer (n=98) Median pg/mL	95% LCL of Median	95% UCL of Median	Controls (n=45) Median pg/mL	95% LCL of Median	95% UCL of Median	p value
Co-stimulatory	CD27	3131.28	2639.21	3568.54	4577,35	3391,13	5784.85	0.00041
	CD28	32176,41	27889,65	3568,54	46135,18	27210,29	67544,1	0.00029
	CD40	1464.68	1262.67	1620.9	1977.68	1404.82	2569.56	0.00054
	ICOS	14364.95	11122.68	15964.4	26506.65	15897.52	31725.99	0.0001
	GITR	5529,8	4868,15	6407,6	7151,12	5528,36	9678,41	0.00234
	GITRL	5529,8	4868,15	6407,6	7151,12	5528,36	9678,41	0.00234
Co-inhibitory	PD-1	11571,18	10147,12	13426,83	14917,48	7874,92	21795,02	0.11977
	PD-L1	1580,69	1198,87	1978,97	3242,62	2628,64	4750,96	0.0000
	CTLA-4	1585,73	1330,19	1790,69	2618,23	1578,44	3110,47	0.0006
	CD80	1613,26	1317,61	1792,55	2329,77	1395,01	3042,87	0.00108
	CD86	11199,42	9447,21	12851,98	14297,09	9391,46	20525,14	0.011
	TIM-3	3834,44	3436,22	4132,4	5046,87	4732,72	5958,87	0.00069
	LAG-3	120377,5	93654,44	138811,3	150416	94508,53	187997,2	0.11388
	BTLA	12297,97	11106,41	17084,76	12907,97	11106,41	17084,76	0.18276
Chemokines	ENA 78 CXCL5	535,58	250,7	763,49	2246,51	1540,24	3246,49	0.00002
	Eotaxin 3 CCL26	4,31	4,31	8,41	6,36	3,28	8,41	0.35478
	Fractalkine CX3CL1	445,13	399,04	489,3	397,12	366,07	431,69	0.00871
	IP-10 CXCL10	485,82	426,98	607,59	543,33	498,35	638,22	0.36649
	MIG CXCL9	91,305	76,65	112,33	92,92	74,64	117,5	0.20992
	CCL23	535,58	250,7	763,49	2246,51	1540,24	3246,49	0.00002
Cytokines	IL-2	9,01	8,19	10,37	9,81	7,29	11,5	0.32947
	IL - 4	126,24	102,57	156,37	146,78	113,16	200,93	0.1878
	IL-6	10,52	8,9	11,3	10,4	7,56	13,71	0.74975
	IL-8	9,61	8,18	10,67	9,81	7,49	10,34	0.20475
	IL-16	1931,85	1599,84	2087,37	3535,39	2932,85	3813,73	0.00005
	Interferon α	174,275	152,73	192,52	199,64	178,29	214,94	0.00751
	IL-1 Ra	418,73	346,33	466,68	503,33	448,08	625,49	0.01766
	Interferon γ	59,74	51,01	66,56	69,55	44,35	80,45	0.08564
	IL-10	42,76	36,5	50,87	47,61	34,16	59,25	0.40699
	IL-17A	23,05	20,19	25,9	23,92	20,84	28,38	0.36121
	TGF β1	20353,26	14180,32	24904,45	23765,83	16184,42	36390,72	0.39596
Growth Factors	TLR-2	24059,42	20551,28	28354,07	30477,2	20926,44	50302,64	0.01406
	HVEM	1866,92	1674,84	2007,57	2290,19	2079,46	2618,44	0.00001
Other	FGF21 Breast cancer	24.36	8.64	33.8	8.64	8.64	8.64	0.001110
	GDF15 Breast cancer	806.82	741.37	879.48	430.03	368.72	467.45	0.000000
	M-CSF Breast cancer	84.41	65.74	88.98	13.34	13.34	13.34	0.000000
	GM-CSF Breast cancer	13.30	13.30	13.30	13.30	13.30	13.30	0.000000
	G-CSF Breast cancer	8.75	8.75	8.75	8.75	8.75	8.75	0.000000
	VEGF Breast cancer	9.14	2.4	13.64	8.66	2.87	13.64	0.566788

Figure 2. CD27 Breast Cancer vs. Control.

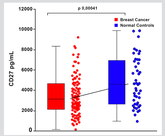


Figure 3. CD28 Breast Cancer vs. Control.

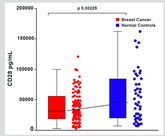


Figure 4. CD40 Breast Cancer vs. Control.

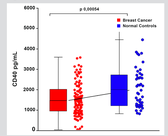


Figure 5. ICOS Breast Cancer vs. Control.

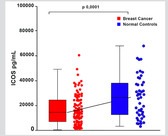


Figure 6. GITR Breast Cancer vs. Control.

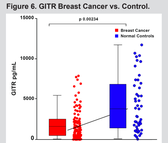


Figure 7. GITRL Breast Cancer vs. Control.

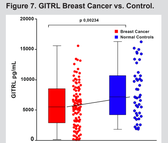


Figure 8. PD1 Breast Cancer vs. Control.

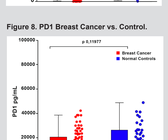


Figure 9. PDL1 Breast Cancer vs. Control.

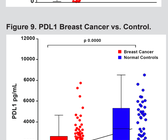


Figure 10. CTLA-4 Breast Cancer vs. Control.

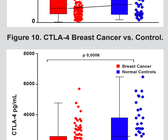


Figure 11. CD80 Breast Cancer vs. Control.

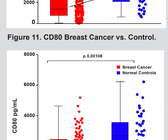


Figure 12. CD86 Breast Cancer vs. Control.

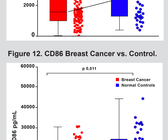


Figure 13. TIM3 Breast Cancer vs. Control.

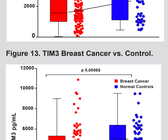


Figure 14. LAG3 Breast Cancer vs. Control.

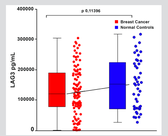


Figure 15. BTLA Breast Cancer vs. Control.

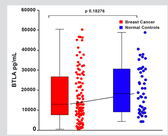


Figure 16. TLR-2 Breast Cancer vs. Control.

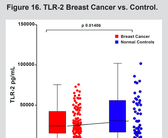


Figure 17. HVEM Breast Cancer vs. Control.

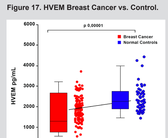


Figure 18. M-CSF ROC Curve.

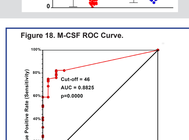


Figure 19. M-CSF Breast Cancer vs. Control.

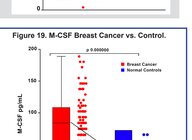


Figure 20. FGF21 ROC Curve.

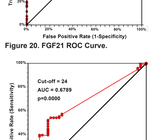


Figure 21. FGF21 Breast Cancer vs. Control.

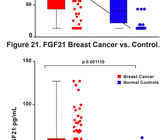


Figure 22. GDF15 ROC Curve.

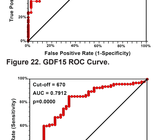


Figure 23. GDF15 Breast Cancer vs. Control.

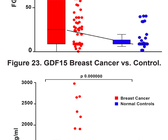


Figure 24. Matrix correlation of M-CSF, GDF15 and FGF21 vs cytokines and chemokines.

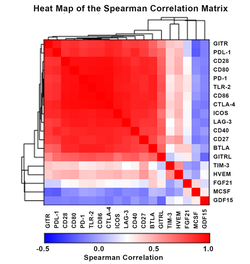


Figure 25. Matrix correlation of M-CSF, GDF15 and FGF21 vs Immune checkpoint molecules.

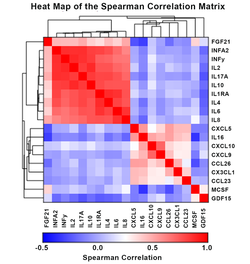


Table 4. Matrix correlation of M-CSF, GDF15 and FGF21 vs Immune checkpoint molecules.

	Variables	GITR	CD27	CD28	CD40	CD80	ICOS	PD-1	PD-L1
M-CSF	Spearman Correlation	-0.2216	-0.1407	-0.0962	-0.1078	-0.0912	-0.1414	-0.0211	-0.2392
	Spearman P-Value	0.0441	0.5387	0.7572	0.9704	0.8784	0.6987	0.4019	0.03
GDF15	Spearman Correlation	-0.116	-0.0947	-0.1095	+0.0507	-0.1317	-0.0893	-0.1006	-0.1261
	Spearman P-Value	0.0913	0.3234	0.1765	0.3098	0.1144	0.1943	0.1411	0.0948
FGF21	Spearman Correlation	0.1081	0.17	0.2565	0.1998	0.2527	0.2114	0.3197	0.0967
	Spearman P-Value	0.9674	0.4113	0.0933	0.2863	0.0798	0.1703	0.0082	0.8551

	Variables	CTLA-4	TIM-3	HVEM	TLR-2	LAG-3	GITRL	BTLA	CD86
M-CSF	Spearman Correlation	-0.1207	-0.0708	0.0047	-0.0368	-0.1011	0.0225	0.049	-0.0635
	Spearman P-Value	0.6542	0.3284	0.2274	0.6063	0.8283	0.2976	0.1241	0.793
GDF15	Spearman Correlation	-0.1332	0.1754	0.227	-0.1048	-0.1363	-0.1944	-0.036	-0.1149
	Spearman P-Value	0.0742	0.0053	0.0581	0.1352	0.1183	0.0228	0.2295	0.1205
FGF21	Spearman Correlation	0.2674	-0.0374	0.0682	0.3076	0.2497	0.2982	0.3904	0.2913
	Spearman P-Value	0.0814	0.8972	0.936	0.0124	0.0269	0.0032	0.0006	0.0212

Figure 26. Entire Group.

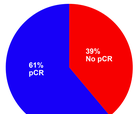


Figure 27. Her2 Positive.

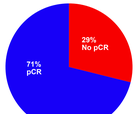


Figure 27. Luminal Tumors.

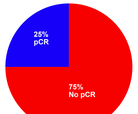


Figure 28. TNBC.

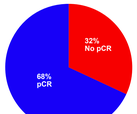


Table 5. Matrix correlation of M-CSF, GDF15 and FGF21 vs cytokines and chemokines.

	Variables	IL16	INFA2	IL1RA	INFγ	IL6	IL2	IL8	IL10
M-CSF	Spearman Correlation	-0.3512	0.0343	-0.0398	0.0433	0.1258	0.1161	0.227	0.0028
	Spearman P-Value	0.0013	0.7626	0.7112	0.619	0.1414	0.0968	0.0257	0.7639
GDF15	Spearman Correlation	-0.0999	-0.1879	-0.2327	-0.1169	-0.1865	-0.1925	-0.0215	-0.1806
	Spearman P-Value	0.2309	0.0134	0.1006	0.7035	0.671	0.0518	0.681	0.1292
FGF21	Spearman Correlation	-0.3184	0.457	0.3484	0.449	0.382	0.4445	0.5031	0.3862
	Spearman P-Value	0.0018	0.0004	0.0008	0.0013	0.0004	0.0157	0.0008	0.0832

	Variables	IL17A	IL4	CXCL5	CCL23	CCL26	CX3CL1	CXCL10	CXCL9
M-CSF	Spearman Correlation	0.0697	0.0948	-0.1306	0.0416	-0.2734	-0.0816	-0.0358	-0.1372
	Spearman P-Value	0.6225	0.1308	0.0245	0.8008	0.0483	0.3881	0.5841	0.3281
GDF15	Spearman Correlation	-0.253	-0.2155	-0.1364	0.0179	-0.0983	-0.0689	0.0893	0.1585
	Spearman P-Value	0.0074	0.0075	0.0494	0.8252	0.4484	0.6311	0.2253	0.3354
FGF21	Spearman Correlation	0.4904	0.4419	-0.2577	-0.0881	-0.1762	-0.0673	0.0843	-0.0223
	Spearman P-Value	0.0243	0.0004	0.0427	0.7541	0.4195	0.5669	0.7127	0.8839

Response to neo-adjuvant chemotherapy treatment.

- Patients received neo-adjuvant therapy including TAC, AC & taxane, taxane, TC, AC, taxane & Doxorubicin, AC & taxane & Trastuzumab, or taxane & Trastuzumab.
- Pathological complete response (pCR) was defined as the complete disappearance of the invasive cancer in the breast and absence of tumor in the axillary lymph nodes.
- No biomarker (soluble immune checkpoint, chemokine, cytokine or growth factor) was associated with pCR in this patient cohort.

Conclusions

- Lower levels of a number of soluble co-stimulatory (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.
- Additionally, higher levels of M-CSF, FGF21, and GDF15 compared to healthy controls were also identified in this patient population.
- M-CSF, FGF21, and GDF15 may play a significant role in the pathogenesis of breast cancer
- Several positive and negative correlations were identified between the growth factors and soluble immune checkpoints molecules, chemokines, cytokines.
- 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 and immune-evasion independent of subtype and stage, which, to our knowledge, is the first study to describe soluble immune checkpoints in early breast cancer patients simultaneously.
- An analysis of these biomarkers comparing pre-neoadjuvant treatment, post-neoadjuvant treatment, as well as post-surgery is underway.

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